Monoclonal Antibody Against *Helicobacter pylori* Protein Labelled the Surface Microvesicles: A Preliminary Report of Immuno-Scanning Electron Microscopy

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Microvesicles on the surface of *Helicobacter pylori* (*H. pylori*) is suspected to be related to the release of bacterial toxins or the autolytic processes of *H. pylori* during the exponential growth condition. The molecular nature has not been known yet. In this study, we tested 2 commercially available monoclonal antibodies to test the hypothesis that the microvesicular domain has unique molecular composition.

Because the microvesicles are seldom observed by the conventional transmission electron microscopy, we adapted the immuno scanning electron microscopy. *H. pylori* 26695 strain obtained from the ATCC were cultured on Mueller Hinton agar plate supplemented with 10% FBS (Fetal Bovine Serum) in a 10% CO_2, 37 ℃ environment for 2.5 days. Cultured bacteria were spread onto each of the 0.5% gelain-coated Thermanox coverslips, cultured for 10 minutes. Immunolabelling was done either before fixation or after fixation. Fixation was performed by immersing coverslips in 4% paraformaldehyde buffered with 1M phosphate buffer, pH 7.4, for 15 minutes.

Monoclonal antibodies MAB921 and MAB922 were purchased from the Chemicon, Inc. and used for this experiment. *H. pylrori* were incubated with primary antibodies for 1 hour at room temperature, washed, and labelled with 15 nm gold-conjugated protein A, and freezing dry (ES-2030, Hitachi) and carbon coating by evaporator (HUS-5GB, Hitachi)

Gold signals were detected at an acceleration voltage of either 3kV or 15kV with a BSE (backscattered electron) mode[1] following the operation manual of field emission scanning electron microscope (Hitachi S-4700). For proper observation of gold signals along with the prevention of charge-up and deformation, low acceleration voltage had benefits over higher voltage. We adapted carbon coating because of the possible masking of BSE signals of commonly used coating materials such as gold and platinum, however, precise coating condition with carbon needs to be verified.

Immunolabelling of unfixed *H. pylori* was not a proper process because it failed not only to show the labelled gold but also to preserve good morphology of *H. pylori*. Most of *H. pylori* were damaged and bursted during the immunolabelling process. Pre-fixed H. pylori showed several gold particles, in the form of glistening white particles, localized on the surface of *H. pylori*, especially at the microvesicular domains, even that the *H. pylori* morphology was not completely preserved. The molecules against monoclonal antibodies raised are not known at the time of the experiment. Only the molecular weight was identified as 87kD, with minor bands at 60, and 116kDs. Precise identification of the molecule using proteome technology would be necessary for further study.

The above data showed that microvesicles often observed on the H. pylori surface had unique molecular components. Some technical refinements are necessary for further studies.

Reference

1. Kuniaki Takara et al. Colloida Gold Labed Observed with a High Resolution Backscattered Electron Imaging in Mouse Lymphocytes. J Electron Microsc **37(6)**, 346-350 (1988)

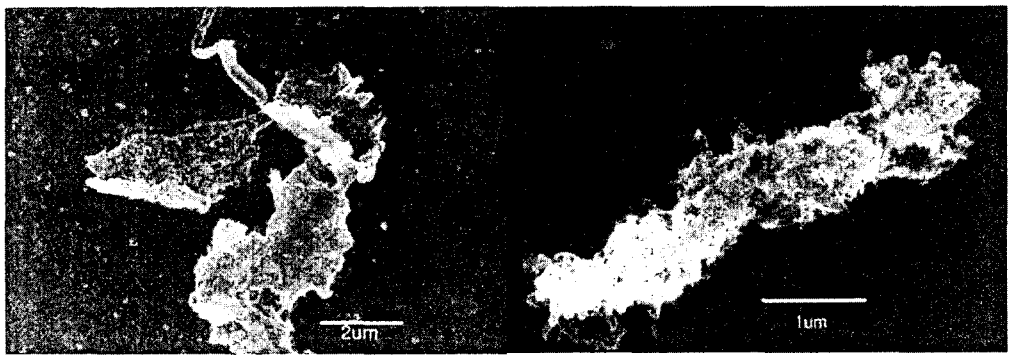

Fig. 1. Post fixed *Helicobacter pylori*. it was bursted and unable to observe gold particle.

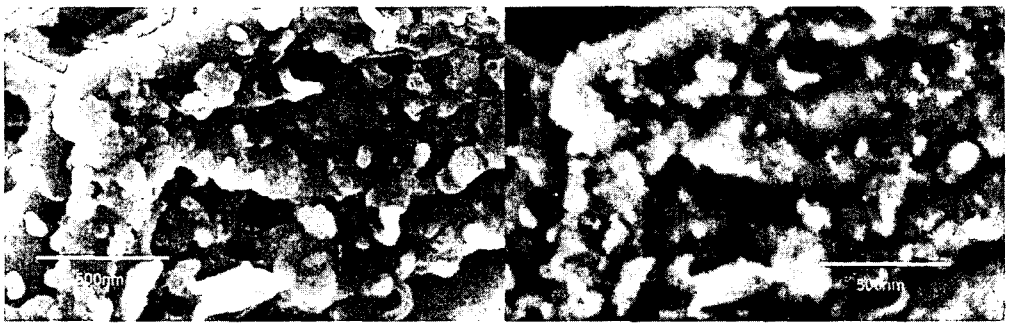

Fig. 2. It show3 kV upper detector BSE image, Pre-fixed group. It shows almost similar size of gold particles and gold particles was distinct from *Helicobacter pylori*.

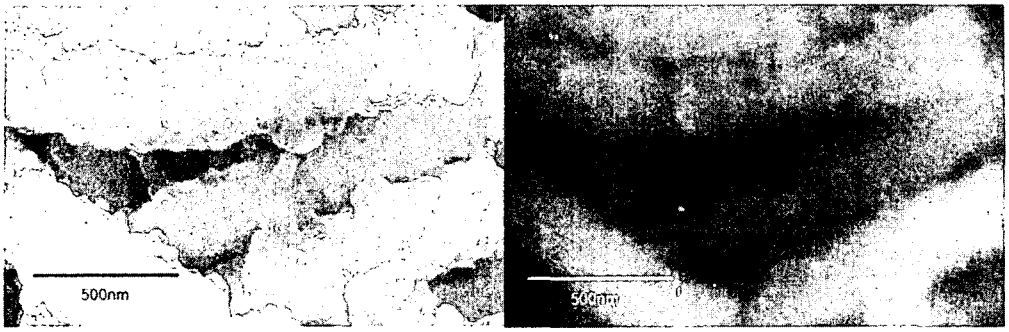

Fig. 3. It show15^{kV} lower detector BSE image and it's Pre-fixed group. This pictures shows scattered gold particles to whole *Helicobacter pylori* image.