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Conference Abstract

Surface Derivatized Silica-Sol Particles as Immunospecific Markers for High Resolution Electron Microscopy*

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INTRODUCTION

Thanks to methods developed in the forties and early fifties, highly concentrated, extremely stable silica sols are produced in tonnage quantities and are incorporated into such everyday products as floor waxes, textiles and plastics¹⁻⁵. The most widely used technique to make such sols is controlled acidification or the sodium removal from sodium silicate solutions with cation exchange resins. At a first glance the technique appears quite simple. In practice, however, the essential parameters, pH, concentration, temperature, have to be precisely manipulated to obtain satisfactory products. Examples of the particle sizes of commercial sols are shown in Figure 1. Commercial grades range from 4 to 70 nanometer particle diameter, but the techniques described in the literature allow extension of this range in both directions. For comparison the figure shows also some other spherical sols such as latexes.

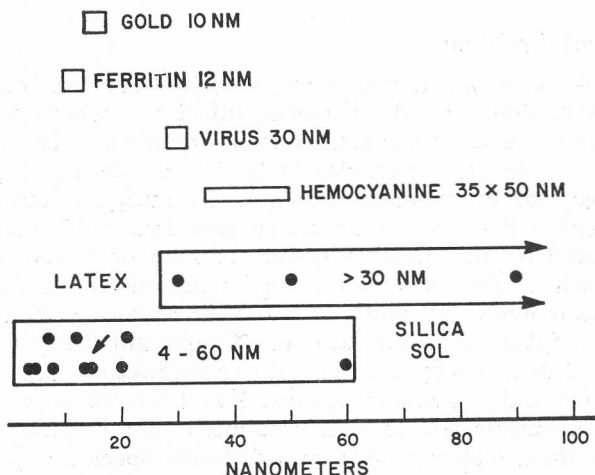


Figure 1. Particle sizes of silica-sols, latexes and previous immunospecific EM-markers.

* Based on an invited lecture presented at the 5th »Ruder Bošković« Institute's International Summer Conference *Chemistry of Solid/Liquid Interfaces*, Cavtat/Dubrovnik, Croatia, Yugoslavia, June 1979.

The perfect roundness, high density, electron contrast, good size uniformity and wide range of sizes should make silica sol particles good resolution and size standards for electron microscopy. Compared with high polymer latexes, they do not change size on drying and are available in smaller sizes which is becoming increasingly important as the resolution of electron microscopes are improved.

In many biological or biochemical investigation one views cells or structures in the electron microscope. To enhance information, such objects are frequently marked, stained or labeled. This is analogous to the application of dyes in light microscopy, except that a wider variety of contrast criteria is desired. Of particular interest are immunological differentiations where one wishes to discriminate, for instance, between infected and non-infected parts of a cell or tissue. Such immunological labeling requires a surface specific antibody which adsorbs itself at a specific site of a cell surface. Since the antibody molecule, however, is generally too small and too indistinguishable from other features in the specimen one attaches to it some other particle which has a unique shape to make it clearly recognizable in the electron microscope. In Figure 1. particles previously used as markers are listed.⁶⁻¹²

In the light of what I just described it appeared very challenging to employ the dense, perfectly round silica sphere of a sol as a marker. In order to do this, however, it was necessary to modify the silica surface. We were greatly aided in this by the experience which we had gained in past years when preparing surface-derivatized controlled-pore glass.^{13,14} Such glass is being used in permeation-, affinity-, adsorption-, and ion exchange-chromatography as well as in enzyme reactors and radio-immuno assays. By contrast, however, derivatizing of sols is complicated by their tendency to coagulate due to charge and pH shifts. In the following it will be illustrated in an example how one can overcome this.

The Biochemical Problem

An electron-microscopical marker was desired for the labeling of virus infected regions of tissue. Unlike bacterial infections, which are always characterized by the presence of the transmitting organism, only at certain stages of virus infections are virus particles to be found. More often, there are no obvious microscopical or chemical changes. To recognize such regions, one can, however, enlist the help of naturally produced antibodies, which have been manufactured by the immuno system of man or animal in response to an infection. Such antibodies continue to be present in the blood after the infection has been overcome and are the part of the plasma which confer immunity against future infection. The antibodies fight the invading organism in various ways. One of it is by adsorbing and precipitating the invader. Specific antibodies are not only produced against live bacteria or viruses, but also against certain chemicals and foreign proteins, as for instance against tissue, proteins, and antibodies stemming from a different species, e. g., sheep against rabbit.

In our particular case we used human fibroblast cells (HeLa) grown in tissue culture as the infected tissue and influenza (A_0PR_s) as the infecting virus.¹⁵

Rather than binding anti-influenza antibodies to the silica-spheres and letting them recognize the influenza-infected parts of the cell, we use the so called indirect technique. The indirect techniques has the advantage of economy, inasmuch as only one type of marker has to be produced. It is however still necessary to produce the disease-specific antibody by itself, without bonding it to the marker.

Preparation of Silica Sphere Marker

We used commercially available silica sol (Ludox HS40)* of 13–14 nm diameter as cores of the marker. Since all antibody molecules have amino-groups, we followed a derivatization scheme which could be used for the covalent bonding of any amino-carrying ligand.

The derivatization scheme is shown in Figure 2. and Figure 3B.

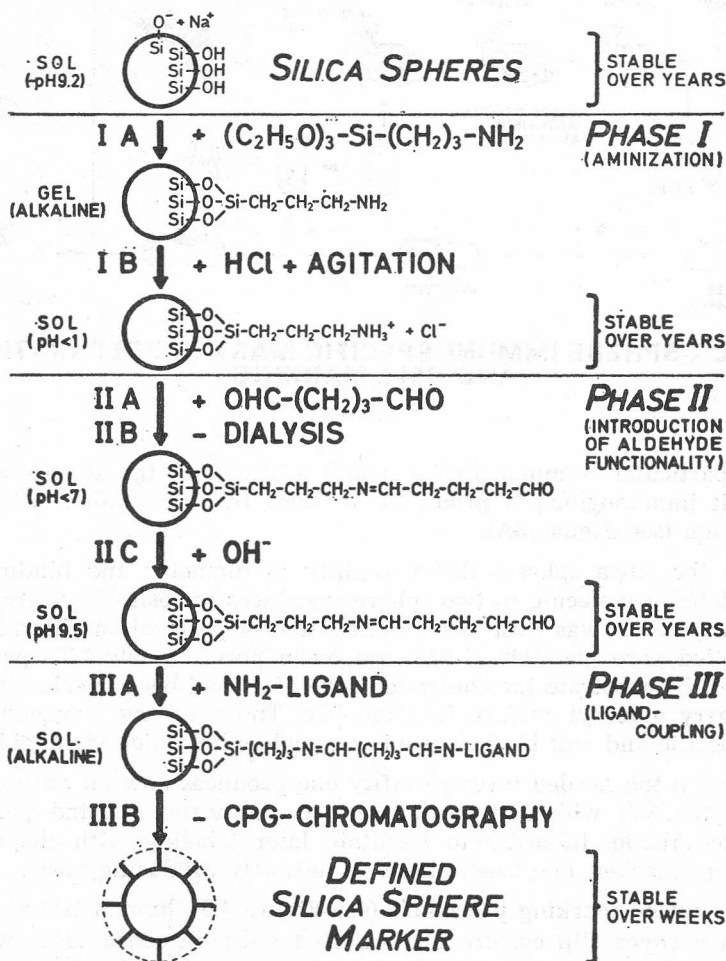


Figure 2. Schematic of derivatization steps to make silica sphere markers.

* Mentioning of trade names or suppliers does not constitute an endorsement the author or his institution.

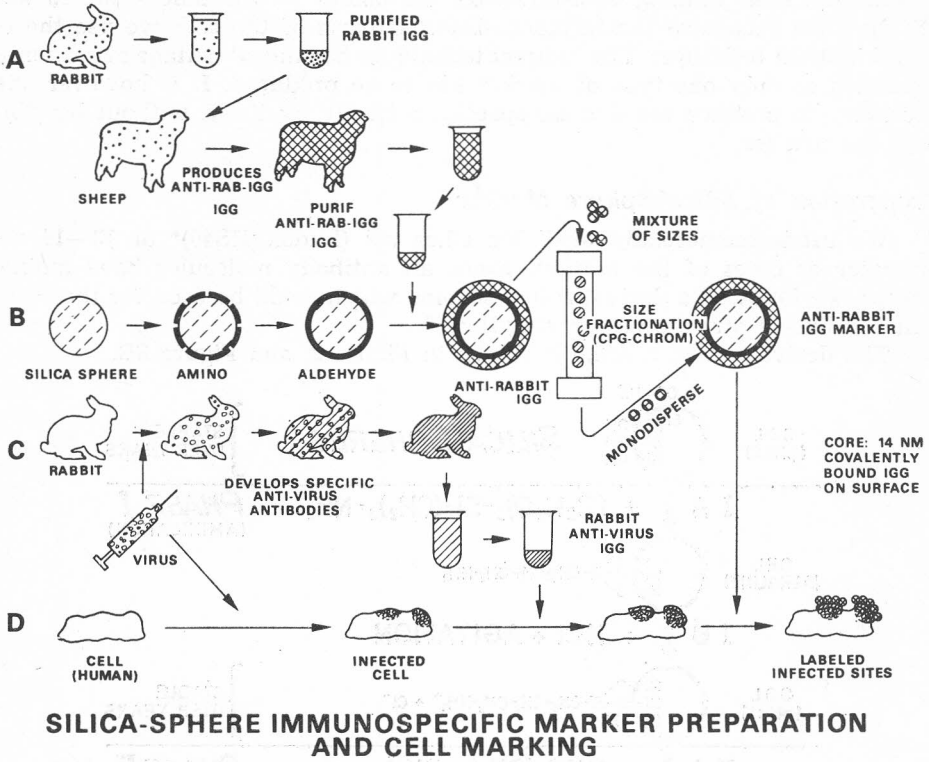


Figure 3.

The particular immunospecific ligand attached to the sphere was sheep anti-rabbit immunoglobulin produced in sheep by inoculation with purified rabbit serum (see Figure 3A).

Since the silica spheres differ slightly in diameter and binding of one immunoglobulin molecule to two spheres produces undesirable aggregates, the marker suspension was first size fractionated by permeation chromatography on controlled pore glass^{13,16} of 51.9 nm mean pore diameter. To prevent adsorption to the substrate the controlled pore glass had been covalently derivatized to carry glyceryl surface functionality. The resulting markers are anti-rabbit specific and will bind to any immunoglobulin which is of rabbit origin.

To obtain the needed virus-specificity one produces now an anti-virus antibody (Figure 3C) which attaches itself to the virus infected part of the investigated tissue. In order to facilitate later labeling with the anti-rabbit silica sphere marker, one uses rabbits as antibody producing hosts.

In the actual marking procedure (see Figure 4D), human HeLa cells were grown on a cover slip culture and infected with influenza virus which had been cultured in embryonated duck eggs. The virus infected cells were exposed to the rabbit anti-influenza antibodies which adsorb to the virus infected regions of the cells. After washing, the anti-rabbit silica sphere suspension

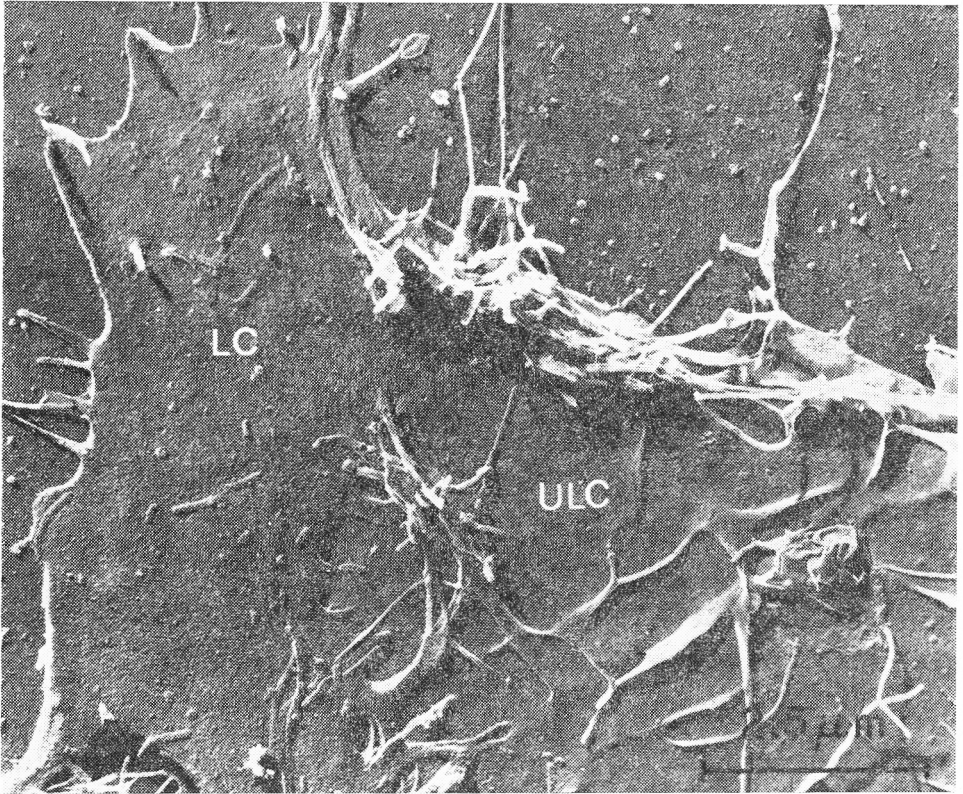


Figure 4. EM-replica. Influenza-Infected HeLa cell. Prestabilized. LC = Labeled surface.
ULC = Unlabeled surface.

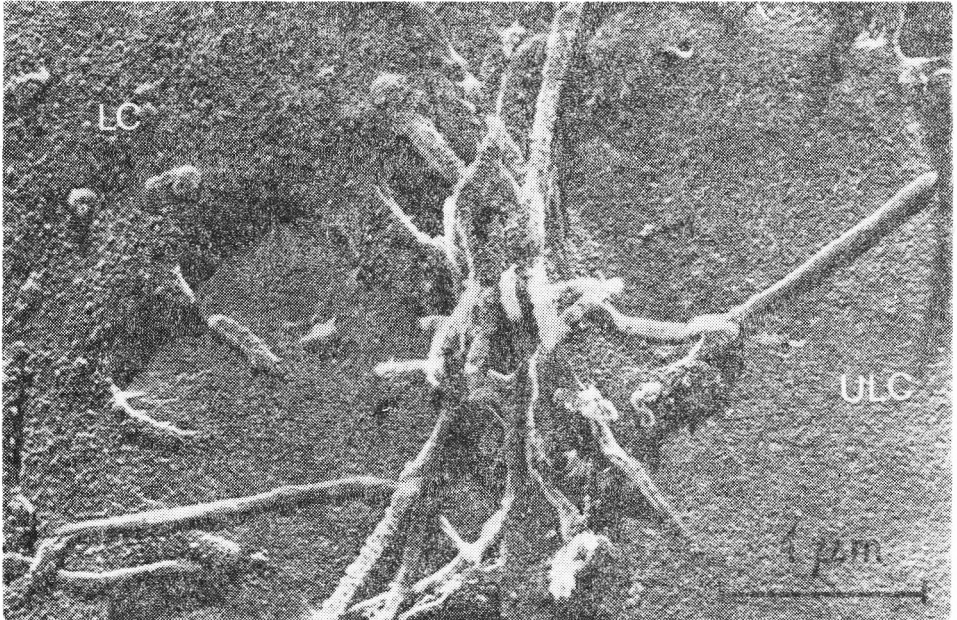


Figure 5. Details of Figure 4.

(10^{14} markers/cm³) is applied. The markers recognize the rabbit globulin and attach themselves to it.

The Figures show electromicrographs of the marked cells in replicas (Figures 4, 5) and thin-section (Figure 6) preparations.

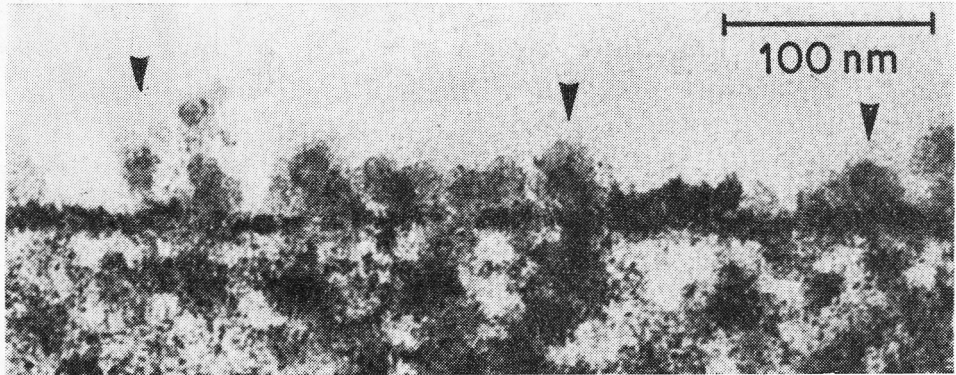


Figure 6. Marked cell surface. Thin section in TEM

Histological discussions of micrographs and details of electron microscopic preparations can be found in reference 15, from which some of the figures were taken. The lecturer wishes to acknowledge the collaboration of K. R. Peters, G. Rutter, H. H. Gschwender and the help of Mrs. Gisela Schönmath-Kölln and Mr. C. L. Baigent.

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SAŽETAK

Čestice silike sa derivatiziranom površinom kao imunospecifični obilježivači za elektronsku mikroskopiju visokog razlučivanja

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Opisan je postupak za površinsku derivatizaciju komercijalnog silika-sola (LUDOX HS 40) u nosač za specifični ovčji imunoglobulin protiv kunića. Taj obilježivač bio je potreban za specifično obilježavanje dijelova tkiva koji su bili zaraženi virusom. Opisani je produkt idealno sfernih čestica visoke gustoće i uske raspodjele veličina čestica koje sušenjem ne mijenjaju dimenzije. Pokazane su elektronske mikrofografije stanica zaraženih virusima.

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