Nanoscopic characterization of the membrane surface of the HeLa cancer cells in the presence of the gold nanoparticles: an AFM study

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Recent interest in the nanomedicine, especially the nanoparticles treatment, and theirs use as the deliver vials for drugs to the cancer cells, demands development of new and more specific tools and methodologies for the cell morphology characterization. In particular is interesting to study interaction and of the nanoparticles with cells membrane. Here in this work, the Atomic Force Microscopy (AFM) was used to study interaction and distribution of the gold nanoparticles (diameter of 40±5 nm) within the plasmatic membrane of the cancer cells of cervicouterino (HeLa). The study was based on the collection of the high resolution AFM images, to reveal the morphology characteristics at the nanometric scale. Imaging was performed in the AFM tapping mode, under standard “ex-situ” procedure. HeLa cells were previously cultivated on the gold films as a substrate. During the cell culture, the HeLa cells were exposed to the gold nanoparticles at 15 minutes, 4, 8, 16 and 24 hours. Gold nanoparticles and specific details of the HeLa cell morphology were successfully identified. Following, the RMS\(_{Rq}\) value, as a general factor of the surface roughness, were determinate, for each sample. This quantitative analysis helps us to understand nanoparticle distribution after the contact with HeLa cells. Our results show that RMS\(_{Rq}\) is increasing up to 3 times in the first 4 hours of treatment, from 27.8 nm to 69.13 nm, due to accumulation of gold nanoparticles in the region of the cell membrane (surface). For longer treatments, a decrease of the RMS\(_{Rq}\) value was observed, back to 24, 86 nm. It indicates on the process of the nanoparticles incorporation into cell interior. The obtained results could be of significant help to better understanding dynamic of the nanoparticles incorporation into cancer cells, which could be a new approach to the Nanomedicine development.

Keywords: Nanoparticles; AFM; HeLa; membrane surface.

El reciente interés en la nanomedicina, particularmente en el desarrollo de tratamientos con nanopartículas y sus aplicaciones en el transporte específico de drogas a las células cancerosas, exige el desarrollo de nuevas herramientas que sean más específica y nuevas metodologías para la caracterización de la morfología celular. En particular se busca estudiar a fondo la interacción de las nanopartículas con la membrana celular. En este trabajo la AFM fue empleada en el estudio de la interacción y la distribución de nanopartículas de oro de 40±5 nm de diámetro, las cuales fueron introducidas a la membrana plasmática de la línea celular de cáncer cervicouterino llamada HeLa. El presente estudio se basa en la obtención de imágenes de alta resolución por AFM, que nos permitan revelar las características morfológicas a escala nanométrica. Las imágenes se obtuvieron por AFM en modo tapping. Las células HeLa fueron previamente cultivadas sobre placas de vidrio recubiertas con películas de oro. Durante el cultivo celular se realizó la incorporación de nanopartículas a 15 minutos, 4, 8, 16 y 24 horas. Las imágenes de AFM muestran la presencia de nanopartículas de oro y detalles de la morfología celular de HeLa. Posteriormente se midió el factor de rugosidad en superficie (RMS\(_{Rq}\)) para determinar la distribución de las nanopartículas presentes en HeLa. Los resultados muestran que los valores de RMS\(_{Rq}\) se incrementan al triple durante las primeras 4 horas (27.8 nm a 69.13 nm) debido a la acumulación de nanopartículas en la superficie de la membrana plasmática. En tratamientos prolongados el valor de RMS\(_{Rq}\) decrece (24.86 nm), lo que sugiere que las nanopartículas se han incorporado en la célula. Los resultados obtenidos, proporcionan información acerca de la dinámica de la incorporación de nano partículas en células cancerosas, datos que podrán ser utilizados en el desarrollo de nuevas terapias de tratamiento contra el cáncer dentro de la Nanomedicina.

Descriptores: Nanopartículas; AFM; HeLa; superficie de membrana.

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1. Introduction

The nanomedicine, as a new discipline, has very much focus on to cancer related investigations. In particular, the target is development of the new cancer treatments, where nanoparticles could interactions specifically with the cancer cells. In majority of this work, nanoparticles of gold are main subject of investigations. Despite to numerous data so far presented, to determinate a mechanism how one or other cancer cell react to presence of nanoparticles is still very difficult and possibly this will be a long term goal of many research groups [1-5]. As described in the literature, nanoparticles are very convenient material to use in nanomedicine, due to high biocompatibility, absence of interactions with molecules in the organism (noble material) and because of relatively simple preparation in different shape and size [2,5]. In many studies so far, we have seen use of nanoparticles of 10 to 80 nm in diameter. In majority cases to determine presence of such nanoparticles into cell interior, a powerful SEM
and TEM microscopes were used. When interest ship to the cell surface and processes of incorporation via plasmatic membrane [4], the most direct and powerful tool, becomes the Atomic Force Microscopy (AFM) [6,7].

Here we present our study of characterization of the HeLa cell morphology in the presence of gold nanoparticles (AuNP) of 40±5 nm diameter, by AFM. HeLa is one of the most investigated cancer cell, and in many studies serves as a model cellular system, as was in our work, too. Successfully we identify presence of AuNP on the cell surface and easily determinate the particle distributions. Using advantage to have 3D images, we estimate the surface roughness of the HeLa cell plasmatic membrane before and after the exposure to AuNP. The obtained results clearly indicate on several steps during the AuNP incorporation into cell. This result could in any ways clarify the incorporation mechanism previously observed by other techniques.

2. Experimental details

The HeLa cell line was cultured at the gold fil with Au (111) surface in the presence of 2.5% DMEM and SFB. During the culture cell were exposed to gold nanoparticles (AuNP), with specific diameter of 40±5 nm, previously synthesized from solution of HAuCl₄ in ascorbic acid solution and N-vinyl-2-pyrrolidone, at the ambient temperature [3]. The HeLa cells were exposed to AuNP during a different period of time: 15 min., 4, 8, 16 and 24 h. After all, cellular material was fixed to the gold substrate by series of alcohol solutions, which allows better visualization by AFM. The commercial models: Nanoscope IIIa and MultiModeTMIV, (SPM, Vecco) operating in the tapping mode, with ultra sharp Si tips were used for the cell visualization, in the “ex-situ” mode. Images were interpreted in the quantitative and qualitative mode, with especial focus on the surface roughness analysis, which could be direct indicator of the HeLa cell-AuNP interactions.

3. Results

All images presented in our work are “the height mode images”, which means directly related to the surface morphology. In order to estimate quantitatively influence of the AuNP incorporation on the cell morphology, the RMS [Rq] factor'12 (the surface roughness factor) was estimated for the cell membrane surface after each AuNP treatment. First we show the high resolution images of the AuNP (40±5 nm diameter) dispersed on the Au (111) substrate (Fig. 1), which clearly demonstrate that such particle could be easily identified. The AuNP appeared as individual particles or sometimes as dimmers (ca. 70 nm). Figure 2 shows one of typical images of Au (111) surface covered by AuNP.

As a following we show set of images of the HeLa membrane surface without (Fig 3a) and in the presence of the AuNP (Fig.3 b-f). In a series of images B-F, a change in the cell morphology due to exposure to AuNP at different period of time: t₀=15 min. (B), t₁=4 h (C), t₅=8 h (D), t₁₀=16 h (E) and t24 =24 h (F). All were collected at the same conditions and same size of scanning: 5 × 5 µm.

![Figure 1](image1.png)  
**Figure 1.** The AFM image of the complete HeLa cell on the flat gold substrate shows numerous structural details. Image size: 42 × 42 µm, with z scale: 0-284.1 nm.

![Figure 2](image2.png)  
**Figure 2.** AuNP (diameter 40±5 nm) on the Au(111) surface. The image size: 4.45 × 4.45 µm, with z scale: 89.8 nm. Images are presented in show 2D (A) and 3D (B) view.
3-A) HeLa at Au(111) without AuNP
Image size: 5.4 x 5.4 µm, z=150 nm.

3-B) HeLa and AuNP after t0 = 15 min.
Image size: 2 x 2 µm, z= 150 nm.

3-C) HeLa and AuNP after t4 = 4 h.
Image size: 2 x 2 µm, z= 150 nm.

3-D) HeLa and AuNP after t8 = 8 h.
Image size: 2 x 2 µm z= 150 nm.

3-E) HeLa and AuNP after t16 = 16 h.
Image size: 5.7 x 5.7 µm, z= 150 nm.

3-F) HeLa and AuNP after t24 = 24 h.
Image size: 3 x 3 µm, z= 150 nm.

Figure 3. Set of the high resolution AFM images show nanometric details of the HeLa plasmatic membrane surface before (A) and after (B-F) the exposure to AuNP. Images and different period of the exposure time: t0 = 15 min. (B), t4 = 4h (C), t8 = 8h (D), t16 = 16h (E) and t24 = 24h (F) are related, respectively. The image size in all cases is 5 x 5µm, with z scale: 0 to 150 nm.
TABLE I. Values of RMS$_{\langle Rq \rangle}$ for the HeLa cells in the presence of AuNP.

<table>
<thead>
<tr>
<th>EXPOSURE TIME</th>
<th>RMS$_{\langle Rq \rangle}$ (nm)</th>
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<tbody>
<tr>
<td>$t_0 = 15$ min.</td>
<td>27.80</td>
</tr>
<tr>
<td>$t_4 = 4$ h</td>
<td>69.13</td>
</tr>
<tr>
<td>$t_8 = 8$ h</td>
<td>65.18</td>
</tr>
<tr>
<td>$t_{16} = 16$ h</td>
<td>58.39</td>
</tr>
<tr>
<td>$t_{24} = 24$ h</td>
<td>24.86</td>
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Figure 4. The surface roughness factor RMS$_{\langle Rq \rangle}$ in the function of the exposure time of HeLa cells to AuNP.

4. Discussion

Our results, based on collection of AFM images and estimation and monitoring of the RMS$_{\langle Rq \rangle}$ value indicate that process of incorporation of the AuNP into HeLa could be three step process (Table I. and Fig 4). At the first step, AuNP is concentrating at the cell surface, consequently increasing the cell surface roughness, significantly (three times) and relatively fast ($t_0$ and $t_4$). Thus, during a much slower step including: $t_4$, $t_8$ and $t_{16}$, the AuNP starts incorporation into cell. As a consequence, the RMS$_{\langle Rq \rangle}$ values, slowly and gradually decrease. During the last step, the HeLa surface will again have the same morphology characteristics and RMS$_{\langle Rq \rangle}$ value as at the beginning of the treatment with AuNP (compare images B and F in Fig.3). At this point we believe that incorporation of AuNP into cell interior is finished and AuNP again beginning to saturate the cell surface. Note that the cell surface morphology for $t_0$ and $t_{24}$ are very similar, with typical 40±5 nm particles.

5. Conclusions

In this work we clearly demonstrate that AFM is an excellent tool for monitoring process of incorporation of the gold nanoparticles into cellular material. Our study was focused on changes in the surface morphology of the cellular plasmatic membrane (HeLa cancer cells), during the exposure to AuNP. The observed changes were expressed via differentiation between membrane surface roughnesses (RMS$_{\langle Rq \rangle}$ factor).

The AFM analysis indicates on three steps of the AuNP incorporation into HeLa cell, with significantly different RMS$_{\langle Rq \rangle}$ value. Interestingly, we found that AuNP incorporation process could take more than 16 h., to be completed. Also the most drastically changes in the surface morphology occurs during the first 4 h., of the cell exposure to AuNP. This is the first rapid step, when AuNP accumulate at the cell surface. The second step is much slower, when AuNP from the surface migrating into the cell interior. Consequently the RMS$_{\langle Rq \rangle}$ values gradually decrease. In the last step the cell surface again recover the same surface morphology as at the beginning of treatment, with surface probably completely saturated by AuNP. We believe that results obtained in our study could be of especial importance for better understanding of the mechanism of nanoparticles interaction with a cellular material, which in general will increase our ability to develop new methodologies to fight cancer with Nanomedicine concept.

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