Size-dependent endocytosis of gold nanoparticles studied by three-dimensional mapping of plasmonic scattering images

Sheng-Hann Wang\textsuperscript{1,2}, Chia-Wei Lee\textsuperscript{1,3}, Arthur Chiou\textsuperscript{2}, Pei-Kuen Wei\textsuperscript{1,2}

\textsuperscript{1}Research Center for Applied Sciences, Academia Sinica, 128, section 2, Academia Road, Nankang, Taipei 11529, Taiwan. \textsuperscript{2}Institute of Biophotonics Engineering, National Yang Ming University, Taipei, 112, Taiwan. \textsuperscript{3}Department of Material Science and Engineering, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei, Taiwan 10617.

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Kamalesh
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Introduction

- Understanding the endocytosis process of AuNPs is important for the drug delivery and photodynamic therapy applications.
- The fluorescent labeling suffers from photobleaching and no long term observation is possible.
- AuNPs have large optical scatterings at 550-600 nm wavelengths due to localized surface plasmon resonances.
- Using an enhanced contrast between yellow and blue CCD images, AuNPs can be well distinguished from cellular organelles.
- Targeting can be achieved with the help of AuNPs coated with aptamers for surface mucin glycoprotein.
Identifying AuNPs in scattering images

- The scattering cross-section of a nanoparticle is usually described by the Mie scattering theory. According to which scattering depends on,

\[
C_s(\lambda) = \frac{32\pi^4}{3\lambda^4} r^4 n^4 \frac{[\varepsilon_r(\lambda) - n^2]^2 + \varepsilon_i^2(\lambda)}{[\varepsilon_r(\lambda) + 2n^2]^2 + \varepsilon_i^2(\lambda)}
\]

\(r\) - radius of the nanoparticle,
\(\lambda\) - incident wavelength,
\(n\) - refractive index of environmental medium
\(\varepsilon_r\) and \(\varepsilon_i\) the real and imaginary parts of the dielectric constant of the nanoparticle, respectively.
Identifying AuNPs in scattering images

- The AuNP has a negative dielectric constant.
- On the other hand, the dielectric constant of cellular organelles is positive.
- Single 50 nm AuNP shows as yellow and the dielectric sphere shows as blue.
- For AuNP inside vesicle scattering image is visualised as an orange centre with a blue periphery.
Identifying AuNPs in scattering images
Dark-field optical sectioning microscopy

dark-field CCD image for a HeLa cell without any AuNPs.
Experimental setup

- Experiment was done in a chamber maintained at 37°C humidified atmosphere.
- The light source was a 60 W metal halide lamp.
- In dark-field sectioning microscopy, it made a 16 μm movement from the focal position.
- Interaction of AuNPs (Citrate reduced) was studied with two kinds of cancer cells, non-small lung cancer cells (CL1-0) and HeLa cells.
- AuNPs were modified with aptamer for cellular surface mucin glycoprotein (MUC1) which is over-expressed in the extracellular matrix of cancer cells.

SH-(CH2)10-GCAGTTGATCCTTTTGGATACCCCTGGG
SEM images for 13 nm, 45 nm, 70 nm and 110 nm AuNPs on glass substrates.
Zeta potential analysis to confirm aptamer immobilization on AuNP surface

<table>
<thead>
<tr>
<th>Size of gold nanoparticles (nm)</th>
<th>Zeta potential before ssDNA conjugated (mV)</th>
<th>Zeta potential after ssDNA conjugated (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 ± 2.6</td>
<td>-13.99 ± 1.75</td>
<td>-27.27 ± 1.03</td>
</tr>
<tr>
<td>45 ± 3.1</td>
<td>-17.83 ± 1.31</td>
<td>-28.69 ± 1.07</td>
</tr>
<tr>
<td>70 ± 4.9</td>
<td>-19.14 ± 1.48</td>
<td>-24.66 ± 1.88</td>
</tr>
<tr>
<td>110 ± 5.1</td>
<td>-10.25 ± 0.80</td>
<td>-19.48 ± 0.97</td>
</tr>
</tbody>
</table>
Cell-nanoparticle interactions

Lung cancer cells were incubated for 10 mins with AuNPs and then washed.

Images were recorded with 5 min interval and 100 ms exposure time for 1.5 hours.
Cell-nanoparticle interactions
Interaction of 70 nm AuNPs
Interaction of 110 nm AuNPs

120 mins
Interaction of 45 nm AuNPs

$\Delta z = 0 \mu m$
Interaction of 45 nm AuNPs after 120 mins
Image processing

- Image was divided into the colours red (R), green (G) and blue (B).
- The (G+R)/2 image yielded a yellow image (Y) which had a stronger scattering intensity for AuNPs.
- On the other hand, the organelles were brighter in the blue image.
- Image process of (Y-B), gives a grey image which is positive for AuNPs and negative for organelles.
- In grey image central position of every bright spot was recorded as the x-y position (xp, yp) of AuNPs.
- The z position (zp) for each AuNP was determined by finding the maximum scattering intensity.
Image processing

(a) Color
(b) Yellow
(c) Blue
(d) (Y-B)
3D distribution of AuNPs

45 nm

70 nm
Relation between the scattering optical intensity and #(AuNPs) in the vesicle.

- 500-nm-diameter holes were prepared in a transparent film to mimic the vesicles and coated on a glass substrate.
- The sample was dipped in the AuNP solution for six and then washed to measure the scattering images in water.
- Then dried sample was observed by the SEM to identify the number of AuNPs in each hole.
Relation between the scattering optical intensity and \#(AuNPs) in the vesicle.
Quantitative calculation of the endocytosis

- The scattering intensity has increased with the AuNP number in the aggregate.
- Using the scattering curve and the measured scattering intensity $I(x_p, y_p, z_p)$ for the AuNP aggregates, we can quantitatively estimate the AuNP numbers at each $(x_p, y_p, z_p)$ position.
Quantitative calculation of the endocytosis

Amount of Au-NPs in/on Lung Cancer Cell (CL1-0)

- 1376 (n=60) on Cell
- 1817 in Cell
- 370 (n=4) on Cell
- 283 in Cell
- 143 (n=41) on Cell
- 4 in Cell
Quantitative calculation of the endocytosis

Amount of Au-NPs in/on HeLa Cell

- On Cell
- In Cell

Amount of AuNPs per cell (HeLa)

45 nm:
- On Cell: 836 (n=48)
- In Cell: 1331

70 nm:
- On Cell: 441 (n=38)
- In Cell: 128

110 nm:
- On Cell: 103 (n=26)
- In Cell: 5
Conclusions

- The total amount is consistent with the result measured by using inductively coupled plasma atomic emission spectroscopy and transmission electron microscopy.
- The optimal diameter for AuNPs falls in the range of 40-60 nm for reasonable values of membrane bending rigidity and ligand-receptor binding energy.
- The proposed 3D scattering method is suited only for medium-sized AuNPs.
- This proposed method is very useful for long-term tracking of the process of endocytosis without any labelling.
- Particle size of 45 nm has the highest efficiency for drug delivery by AuNPs.
- Large AuNPs which remain bound to the cell membrane can be used to reconstruct the morphology of the cell.
Thank you