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Therapy with gold nanoparticles and lasers: what really kills the cells?

"...any therapy-related study should be based on a well-justified mechanism of laser-GNP-cell interactions. Otherwise photothermal nanomedicine may never get from the lab to the clinic."

Two articles were published recently in *Nanomedicine*: "Enhanced imaging and accelerated photothermal ablation of A549 human lung cancer cells by gold nanospheres" by Liu *et al.* [1] and "LANTCET: elimination of solid tumor cells with photothermal bubbles generated around clusters of gold nanoparticles" by Hleb *et al.* [2]. These two articles were published simultaneously and share many similarities: they aim to use gold nanoparticles (GNPs) and laser radiation for the detection and destruction of cancer cells, they use the same model cells (A549) and nanoparticles (gold spheres), and similar *in vitro* incubation methods of the cells with GNP and similar continuous 633-nm laser radiation is used. Reported results are also comparable: cell damage was observed after GNP and laser treatment. However, closer examination and comparison of these two studies has yielded several significant differences in the experimental methods, models and interpretation of the results. We have presented some of these differences in TABLE 1 assuming that the same cells and the same GNP were studied.

The key idea of photothermal therapy is that GNPs locally convert optical energy into thermal energy through their unique mechanism of plasmon resonance. This idea is the basis behind all published work on GNP-based photothermal methods. The two articles under consideration report almost opposite approaches and results in using 633-nm laser radiation: Liu *et al.* [1] employs 633 nm c.w. radiation for inducing NP-mediated damage to the cells, while Hleb *et al.* [2] uses the same radiation (with possibly lower power) for noninvasive optical monitoring of the same cells and employs different wavelengths of laser radiation (532 nm) for inducing NP-mediated damage to the cells. We later discuss this apparent contradiction hoping that such discussion may be helpful to the *Nanomedicine* readership for understanding and optimizing the GNP-based diagnostic and therapeutic methods.

Experimental approach & results by Lui *et al.*

Gold nanoparticles are suggested for cancer optical diagnostics and treatment for their excellent scattering and photothermal properties. Lung cancer cells (A549) were incubated with 40-nm gold spheres conjugated with IgG and were then treated with 633-nm continuous laser radiation. Cell viability was monitored through laser-induced fluorescence of a standard violet dye. The authors have already recently published approximately 20 papers related to the synthesis, modifications and biomedical applications of GNPs.

■ Targeting of the cells with 40-nm GNP-IgG conjugates: incubation with A549 cells for 24–26 h at 37°C

- Experimental results obtained by several independent researchers [3–13] show that IgG conjugates do not bind well with A549 when being applied directly;
- A549 expresses EGF receptor (EGFR) and therefore should be targeted with an anti-EGFR antibody. This was experimentally proved for other types of cancerous cells [3–12,14,15];
- IgG can be used for GNP targeting, but such targeting would require a sandwich method by sequential application to EGFR-positive cells of the primary antibody (anti-EGFR) and then the IgG conjugate. We have verified this method for targeting GNP to cancer cells previously [9–11];
- IgG, during the incubation time of 24–26 h, may kill the cells and therefore is cytotoxic;
- An incubation time of 24–26 h is too long and may cause exocytosis of GNP.
- The described conditions of the incubation are likely to cause the initial endocytosis of GNPs that would remove those GNPs from the cell membrane.

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Table 1. Comparison of the experimental designs for Liu and Hleb.

Subject	Liu <i>et al.</i> [1]	Hleb <i>et al.</i> [2]
633-nm continuous laser radiation	Used to heat GNP through plasmon resonance optical absorbance and thus damage cancerous cells	Used to noninvasively probe the cells as does not thermally influence GNP
GNP delivery to the cells	IgG-based membrane binding of GNP–IgG conjugates during their incubation with the cells for 24–26 h at 37°C	Nonspecific endocytosis by incubating the cell with bare GNP for 0.5–1 h at 37°C
Mechanism of cell damage	Thermolysis due to laser-induced temperature rise	Mechanical damage due to intracellular micro-bubbles

GNP: Gold nanoparticle.

■ Laser treatment

Laser treatment of living cells was performed for 58 s at 633 nm (0.5–5.0 mW, 1 W/cm² maximal intensity) and 405 nm (0.5 mW, intensity unknown) continuous lasers in the presence of GNP and fluorescent violet dye. Under such conditions there are two heat sources: the dye and GNP. In addition, there are at least several cell damaging factors, for example:

- Photothermolysis caused by GNPs heated by the laser radiation at 633 nm
- Photothermolysis caused by GNPs heated by the laser radiation at 405 nm
- Photothermolysis caused by the dye heated by 405 nm laser
- Photochemical and photobiological processes activated with 405 nm light
- Toxic action of IgG

■ Results

The viability of the cells was measured by Liu *et al.* [1] as the function of time and of 633-nm laser radiation power, and a drop of cell viability down to 8% has been reported.

The points indicated above cause the main concerns and contradict with the approaches reported by Hleb *et al.* [2]:

- All five above mechanisms are involved in cell damage, although Liu *et al.* [1] attribute this mainly to the first point;
- For the first mechanism described in the first one it is well established that the level of cell damage correlates to optical energy/power: it increases with the increase of optical power [4–7,9–11]. However, this does not happen (Figure 5 from [1,101]); Liu *et al.* observed very little dependence of cell death level upon 633-nm power;
- The experience obtained by us and other researchers is that low-power 633-nm laser radiation does not influence cell viability in the

presence of spherical GNPs [9–11,16–18]. In addition, several other groups have shown that 633-nm laser radiation does not heat GNPs significantly [19–21];

- Laser- and GNP-induced thermolysis was successfully realized at the wavelengths of the laser radiation that are close to the absorbance peak of spherical GNPs (520–540 nm) [3–7,9–11,15,22,23];
- An estimate of the laser- and GNP-induced temperature rise under the conditions that the authors report yields values below 1 K, which cannot cause thermolysis of the cells. For example, the minimal power and exposure time in other similar models of cell photothermolysis [6] were found to be 150 mW at 4 min for the laser wavelength of 514 nm with the corresponding optical absorbance of 40-nm GNPs being 3–4-times higher at this wavelength compared with that at 633 nm.

Therefore, continuous optical excitation at 633 nm of the spherical GNP (under described parameters) is not likely to cause thermolysis of the cells and can be used for noninvasive optical probing of the cells.

■ Several other issues that were reported by Liu *et al.*

- Liu *et al.* claim the red-shift of the optical absorbance peak of GNP clusters is relative to the optical absorbance peak wavelength for single GNPs. However, the clusterization of GNP broadens their optical absorbance spectrum but does not shift it significantly into the red region. It can be easily checked by measuring extinction spectrum of the GNP clusters versus that for unclustered GNPs (and was demonstrated in our work). Therefore, GNP clusters still absorb much better at 532 nm than at 633 nm.
- Imaging methods could be employed to provide a quantitative measure of GNP clusterization in the cells through the analysis of the

image pixel amplitudes of optical-scattering images. However, this was not done and authors qualitatively concluded about GNP binding to cellular membranes.

- Cell viability was not analyzed after GNP–cell interaction and without application of laser radiation. Application of 405-nm laser radiation for the viability measurements is incorrect because this radiation itself does influence cell viability as was found by the authors (see also the mechanisms earlier). Thus, the authors bypassed a very important stage in any cell–nanoparticle model: dark cytotoxicity of GNP conjugates was not measured.
- The viability of the intact cells after laser irradiation (see FIGURES 4 & 5 in [1,101]) was not good (60%). This compromises the selectivity and safety of GNP photothermal therapy, which are assumed to be the main advantages of this new method over the established ones.

Laser- and GNP-induced photothermalysis was shown by us and other researchers as the method that provides good selectivity, safety and efficacy of cell treatment [4–11,15,22–24]. Those key features of nanoparticle phototherapy significantly depend upon laser-induced physical and biological processes in and around intracellular GNPs. Therefore, any therapy and/or diagnostic-related study should be based on a well-justified mechanism of laser–GNP–cell interactions. Otherwise photothermal nanomedicine may never get from the lab to the clinic.

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