Mechanisms of laser nanoparticle-based techniques for gene transfection—a calculation study

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Abstract Cell plasma membranes can be transiently permeabilized to uptake exogenous molecules with high efficiency using a laser nanoparticle-based gene transfection technique. In combination with experimental results, a theoretical model is set up to calculate the temperature distribution and variance around the nanoparticles. This paper also provides a thorough discussion of the underlying mechanisms of cell permeabilization. We find that, rapid heating of the particles and the accompanying extreme temperature rise can lead to microbubble formation around laser-heated particles, which is the origin of photoacoustic effects and other nonlinear optical responses. This transient heat is also capable of causing protein denaturation through thermal inactivation and photochemistry. Furthermore, the dynamic mode that involves the overlapping of bubbles is presented. This mode can significantly increase the plasma membrane permeability of the cells without affecting their viability.

Keywords Laser · Nanoparticle · Gene transfection · Bubble formation

1 Introduction

Gene transfection means the introduction of exogenous genes into the cytoplasm of living cells, about which the elevation of plasma membrane permeability is one of the most important issues. It has been reported that the plasma membrane of mammalian cells can be transiently permeabilized by optical means, such as focusing a laser directly and tightly on the cells for optoinjection, laser-induced stress waves (LISW), photochemical internalization, and irradiation following selective cell targeting with light-absorbing particles [1]. Optoinjection and LISW are laser-based methods capable of loading exogenous molecules

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into selected cells. In both methods, a pulsed laser beam is used to transiently permeabilize cells. Mechanisms of laser irradiation and laser-generated stress transiently perturbing cell membranes are still under investigation. Photochemical internalization is easier to operate *in vivo*, but the toxicity of photosensitizers is another problem. Selective targeting methods by particles are becoming promising transfection approaches. With the LISW, the stresses that increase membrane permeability are not limited to the plasma membrane, whereas the selective method shows no adverse effects to inner parts of the cells. In addition, the effects are localized to particle contact sites, limiting the size or extent of plasma membrane damage. Thereby, this method has the potential to be used for *in vivo* applications if the DNA can be transferred into the host cells [2].

Among all the selective targeting absorbing particles, gold nanoparticles with unique optical properties and appropriate size scale are generating much attention in gene transfection. Gold nanoparticles are photostable, nontoxic, and can be easily and selectively conjugated to antibodies or proteins. Furthermore, it is feasible to tune the optical properties gradually with particle shape and size. These advantages have made gold nanoparticles more attractive than the organic dye molecules.

In our laboratory, Yao and co-workers have demonstrated through experimental results that the plasma membrane can be transiently permeabilized with high efficiency when 30-nm gold nanoparticles are bound to membrane proteins and irradiated with nanosecond pulses [3]. Based on this technique, they have successfully transferred small exogenous molecules into cells. The elevation of plasma membrane permeability is based both on the creation of high peak temperatures and microscopic mechanical disruption that is localized at the cellular level when energy is selectively deposited into the particles. Here, we first discuss 30-nm gold nanoparticle absorption spectroscopy, based on which we can then calculate the temperature distribution and variance around the particles. Finally, we will discuss the mechanisms of laser nanoparticle-based techniques for cell permeabilization since little has been studied in this field.

2 Thirty-nanometer gold nanoparticle surface plasmon absorption spectroscopy

Gold nanoparticles have a distinct absorption peak near 520 nm that is size-dependent, as described by Mie in his theory of absorption and scattering of light by small particles [4]. Mie explained the origin of the phenomenon by solving Maxwell's electromagnetic equation for the interaction of light with a spherical particle. For a spherical nanoparticle much smaller than the wavelength of light (diameter $d \ll \lambda$), an electromagnetic field at a certain frequency can induce a resonant, coherent oscillation of the metal-free electrons across the nanoparticle, which is known as surface plasmon resonance (SPR) and is the cause of surface plasmon absorption [5].

For 30-nm nanoparticles, we obtained the following expression for the absorption cross-section σ_{abs} [6–8]:

$$\sigma_{\text{abs}}(\omega) = 9 \frac{\omega}{c} (\alpha \varepsilon_m)^{3/2} V \frac{\varepsilon_2(\omega)}{[\varepsilon_1(\omega) + 2\varepsilon_m]^2 + \varepsilon_2(\omega)^2}, \quad (1)$$

where V is the particle volume, ω is the angular frequency of the exciting light, c is the speed of light, α is the adjusting factor, and ε_m and $\varepsilon(\omega) = \varepsilon_1(\omega) + i\varepsilon_2(\omega)$ are the dielectric functions of the surrounding medium and the material itself, respectively. According to

Drude's model, the real and imaginary parts of the material dielectric function may be written [9]:

$$\varepsilon_1(\omega) = \beta\varepsilon^\infty - \omega_p^2 / (\omega^2 + \omega_d^2) \quad (2)$$

and

$$\varepsilon_2(\omega) = \omega_p^2 \omega_d / \omega (\omega^2 + \omega_d^2), \quad (3)$$

where β is the adjusting factor, ε^∞ is the high frequency dielectric constant due to interband and core transitions, and ω_p is the bulk plasma frequency

$$\omega_p^2 = Ne^2 / m\varepsilon_0, \quad (4)$$

where N is the concentration of free electrons in the metal and m is the effective mass of the electron. The damping frequency ω_d is related to the mean free path of the conduction electron, R_{bulk} , and the velocity of electrons at the Fermi energy, v_f , by

$$\omega_d = v_f / R_{\text{bulk}}. \quad (5)$$

When the particle radius R , is smaller than the mean free path in the bulk metal, which is the situation for 30-nm nanoparticles [10], conduction electrons are additionally scattered by the surface, and the mean free path R_{eff} , becomes size-dependent with

$$1/R_{\text{eff}} = 1/R + 1/R_{\text{bulk}}. \quad (6)$$

In Fig. 1, the typical surface plasmon absorption band of 30-nm Au nanoparticles was calculated with $c = 3 \times 10^8 \text{ m/s}$, $\alpha\varepsilon_m = 0.27$, $\beta\varepsilon^\infty = 14.8$, $\omega_p = 1.39 \times 10^{16} \text{ s}^{-1}$, $v_f = 1.39 \times 10^6 \text{ m/s}$, $R_{\text{bulk}} = 50 \text{ nm}$ [6, 8, 9]. The spectrum is shown in terms of efficiency, which is the ratio of the calculated optical absorption cross-section of a nanoparticle to its actual geometrical cross-section, and wavelength λ , which is related to the angular frequency ω by $\lambda = \frac{2\pi c}{\omega}$. The result shows that the absorption spectrum reaches its maximum at around 530 nm. This agrees with the studies of Link and El Sayed [6] and Jain et al. [11].

Fig. 1 Calculated spectrum of the efficiency of absorption for 30-nm gold nanoparticle

3 Temperature calculation

The local temperature rise at the surface and in the surroundings of 30-nm gold nanoparticles after laser heating was calculated on the basis of a heat transfer model developed by Goldenberg and Tranter (1952) of a uniformly heated homogeneous sphere embedded in an infinite homogeneous medium [12]. For a homogeneous sphere of radius R , with power density A of absorbed light, the heat transfer equations are

$$\frac{1}{k_1} \left(\frac{\partial}{\partial t} T_1 \right) = \frac{1}{r^2} \left(\frac{\partial}{\partial r} \left[r^2 \left\{ \frac{\partial}{\partial r} T_1 \right\} \right] \right) + \frac{A}{K_1} \text{ for } 0 \leq r < R \text{ and } t > 0, \quad (7)$$

$$\frac{1}{k_2} \left(\frac{\partial}{\partial t} T_2 \right) = \frac{1}{r^2} \left(\frac{\partial}{\partial r} \left[r^2 \left\{ \frac{\partial}{\partial r} T_2 \right\} \right] \right) \text{ for } r > R \text{ and } t > 0. \quad (8)$$

The boundary conditions are $T_1 = T_2 = 0$ at $t = 0$, $T_1 = T_2$ and $K_1 \left(\frac{\partial}{\partial r} T_1 \right) = K_2 \left(\frac{\partial}{\partial r} T_2 \right)$ at $r = R$, T_1 is finite as $r \rightarrow 0$ and T_2 is finite as $r \rightarrow \infty$, where r is the distance from the center of the sphere, and T_1 , T_2 , K_1 , K_2 , and k_1 , k_1 are the temperature, thermal conductivity, and diffusivity of the particle and the medium, respectively. The power density A of absorbed light inside a particle, which is irradiated with the irradiance I , can be calculated with the absorption cross-section σ_{abs} obtained above:

$$A = I \frac{\sigma_{\text{abs}} \pi R^2}{4/3\pi R^3}. \quad (9)$$

The solutions for the temperature distribution in and around the heated sphere are:

$$T_1(t, r) = \frac{R^2 A}{K_1} \left[\frac{1}{3} \frac{K_1}{K_2} + \frac{1}{6} \left(1 - \frac{r^2}{R^2} \right) - \frac{2Rb}{\pi} \int_0^\infty \frac{\exp\left(-\frac{y^2 t}{\gamma_1}\right)}{y^2} \frac{(\sin y - y \cos y) \sin\left(\frac{ry}{R}\right)}{[(c \sin y - y \cos y)^2 + b^2 y^2 \sin^2 y]} dy \right] \quad (10)$$

$$T_2(t, r) = \frac{R^3 A}{r K_1} \left[\frac{1}{3} \frac{K_1}{K_2} - \frac{2}{\pi} \int_0^\infty \frac{\exp\left(-\frac{y^2 t}{\gamma_1}\right)}{y^3} \times \frac{(\sin y - y \cos y)[by \sin y \cos \sigma y - (c \sin y - y \cos y) \sin \sigma y]}{[(c \sin y - y \cos y)^2 + b^2 y^2 \sin^2 y]} dy \right] \quad (11)$$

with $b = \frac{K_2}{K_1} \sqrt{\frac{\kappa_1}{\kappa_2}}$, $c = 1 - \frac{K_2}{K_1}$, $\sigma = \left(\frac{r}{R} - 1\right) \sqrt{\frac{\kappa_1}{\kappa_2}}$, $\gamma_1 = \frac{R^2}{\kappa_1}$.

Equations (10) and (11) give the time-dependent temperature for an unlimited long pulse. Since the differential equations for heat diffusion are linear in time, a solution for a rectangular laser pulse width τ can be constructed by subtracting two solutions $T(t, r)$, which are separated in time [13, 14]:

$$\Delta T = T(r, t) - T(r, t - \tau). \quad (12)$$

Yao's study indicates that high transfection efficiency can be achieved when 30-nm gold nanoparticles are irradiated by five 6-ns pulses at 532 nm with 15-mJ pulse energy.

Fig. 2 Temperature distribution and variation inside and around the 30-nm nanoparticle after the beginning of the irradiation. **a** Temperature distributions at times 6 ns, 7 ns, and 8 ns after the beginning of the irradiation. **b** Temperature variation at 15-nm, 20-nm, and 25-nm distances from the center of the nanoparticle

The single pulse radiant exposure in the center of the beam is 450 mJ/cm^2 and the pulse frequency is of the order of 20 Hz [3]. Based on the theoretical model mentioned above, we calculated the temperature distribution and variation inside and in the surroundings of the particle under these circumstances (Fig. 2). As the pulse frequency is only of the order of 20 Hz, we only calculated the temperature distribution and variance of single pulse. Multiple pulses will cause this process to be repeated.

4 Results and discussion

Figure 2a shows that the heated volume is strictly localized to the vicinity of the particle. Short laser pulses ensure that the absorbed energy does not have time to diffuse away from the particles during the laser pulse [14, 15]. On the other hand, as the thermal diffusivity of gold is nearly 900 times larger than that of water, the temperature inside the particles stays

constant across the radius during cooling while the temperature decreases quite rapidly in the surroundings of the particle. The temperature of the fluid falls to $1/e$ of the surface temperature at a distance of approximately one particle radius. These effects ensure that the damage is highly localized in those cells that have particles (internal or on the surface), which is of great significance for elevation of transfection efficiency without affecting cell viability.

Figure 2b shows that the gold temperature maximum is of the order of 6,600 K. The physical property changes of gold and of the medium (water) due to temperature increase are not considered here. As gold absorption efficiency will decrease to 25% when the temperature is above the gold particle's melting point (1,063 K), and the energy consumption of the state transformation (63 J/g) also needs to be considered, we estimated the real maximum temperature enhancement should be around 2,000 K [13, 16, 17]. This transient heat is capable of causing physical damage to the cell membrane and protein denaturation, both of which we will discuss in detail below [18, 19].

Membrane physical damage is caused through mechanical destruction due to shock waves or evaporation of the water. Nonlinear phenomena such as bubble formation has been the subject of many studies [20–23]. Rapid heating of the particles and the extreme temperature rise can lead to vaporization of a thin layer of fluid surrounding the particle, producing microscopic underwater explosion and cavitation bubble formation, as the initial high vapor pressure overcomes the surface tension of the fluid. Kelly et al. show that bubbles nucleate heterogeneously at a temperature of $T_{\text{nuc}} = 150^\circ\text{C}$ on the particle surface, and then, within 1 ns, the heterogeneous nucleated bubbles coalesce to a vapor blanket around the particle; the formation of a vapor bubble around each irradiated particle becomes more and more evident. A pressure front can be seen as a ring around the bubble, which detaches and propagates away from the bubble after 1 ns, i.e., bubble expansion [24]. An upper estimate for the front velocity can be obtained from inertia-limited bubble growth [21, 23]:

$$\frac{dR}{dt}(T) = \sqrt{\frac{2}{3} \frac{p_{\text{sat}}(T) - p_{\infty}}{\rho(T_{\infty})}}, \quad (13)$$

where $p_{\infty} = 101$ kPa is ambient pressure, $p_{\text{sat}}(T)$ is saturated vapor pressure at ambient temperature, and $\rho(T_{\infty}) = 1,000$ kg/m³ is water density. In the expansion phase, the pressure of the vaporized water drives bubble growth, which will generate dynamic shear stress by fluid displacement. Since the heat conductivity of vapor is small compared to water, the vapor isolates the particle thermally, and only the energy of the surrounding water can be transferred into the bubble by vaporization at the bubble interface. This erosion of the bubble interface consumes latent heat of evaporation, causing cooling of the thermal boundary layer around the bubble. Additionally, the thermal boundary layer of the bubble thins out, due to the enlargement of its surface during bubble expansion. After the thermal boundary layer is cooled down, the vapor inside the bubble condenses at the vapor/water interface and the bubble collapses with a maximal expansion diameter of a few micrometers (depending on laser fluence). Neumann et al. obtained a linear relationship between bubble size and lifetime τ_{bubble} [23]:

$$\tau_{\text{bubble}} = 0.915 \cdot \sqrt{\frac{\rho(T_{\infty})}{p_{\infty} - p_{\text{sat}}(T_{\infty})}} \cdot d_{\text{bubble}}^{\text{max}} \quad (14)$$

in which $d_{\text{bubble}}^{\text{max}}$ is maximum bubble diameter. While bubble expansion may play a major role in physical membrane damage, high pressure accompanied by acoustic, mechanical, and chemical phenomena such as shock, acoustic waves, or the generation of radicals can also arise during the final stage of bubble implosion and contribute to the damage caused by cavitation.

For protein denaturation caused through thermal inactivation and photochemistry, it is important to discuss the denaturation rate. The Arrhenius equation is used to describe the thermal inactivation rate k_{TD} [6]:

$$k_{TD} = A_0 e^{-\frac{E_a}{RT}} \quad (15)$$

in which $A_0 = 1.85 \times 10^{23}$ is a frequency factor acquired from experiments, $E_a = 161$ kJ/mol is activation energy, and R is the universal gas constant. For photochemistry, two different mechanisms are Possible. First, electrons may be injected from the gold particles into water to oxidize the amino acids of the proteins when irradiated by the nanosecond pulses. The second photochemical mechanism is the direct two-photon excitation of the protein, in which case inactivation rate k_{PD} can be estimated with the following equation:

$$k_{PD} = N\eta_{PD}\sigma_{2ph} \left(\frac{E}{\tau \frac{hc_0}{\lambda}} \right)^2 \quad (16)$$

where $\eta_{PD} = 5\%$ is a quantum efficiency, $\sigma_{2ph} = 10^{50}$ cm²/photons is the two-photon absorption cross-section, τ is the pulse width and E is the radiant exposure. In our case of gene transfection, as the temperature peak and the radiant exposure reached around 2,000 K and 450 mJ/cm², we calculated the thermal inactivation rate maximum and the two-photon excitation inactivation rate being 1.51×10^{19} /s and 1.93×10^5 /s, respectively.

Another mechanism of laser nanoparticle gene transfection is the effect of cell-to-particle ratio on transient plasma membrane permeabilization. In Yao's study, particle load is 1.1×10^5 /cell, which, according to Zharov, means that the particles are located close enough so that the optical, thermal, acoustic, and bubble-formation phenomena around each particle initiated by laser irradiation can be well overlapped and create some synergistic effects [25]. This may explain why the fraction of successfully permeabilized cells depends on the gold concentration in a form that resembles a normal distribution [3]. The characteristic radii for these overlapping effects can be defined by the thermal diffusion length, $R_T = 2(k t_p)^{1/2}$, the sound transfer distance, $R_{ac} = c_s t_p$, and the microbubble radius, $R_{\text{bubble}} = v_b t$, where k is the heat diffusion coefficient, c_s is the speed of sound, v_b is the bubble growth velocity, and t_p is the laser pulse duration [22, 25]. The laser-induced overlapping thermal fields may lead to a dramatic increase in thermal and accompanying effects. Moreover, the close location of nanoparticles may be considered in certain conditions to result in a single nucleation center. The interaction of acoustic waves may significantly change the local refractive index and may change the threshold of nucleation. The interaction of growing bubbles may allow a decrease in the bubble formation threshold and provide for creation of one larger bubble with longer lifetime that finally leads to a substantial increase in membrane cell damage.

One thing interesting to note is that the localized stresses alter the plasma membrane permeability of the cells without affecting their viability [3]. As is mentioned above, short laser pulses ensure that the absorbed energy does not have time to diffuse away from the particles during the laser pulse and therefore do not produce enough damage to kill the cells. When going to small structures, the main problem is the reduction of the laser pulse

width, which is necessary to maintain the thermal confinement (shorter than 10 ns) so as to decrease the duration of increased temperature [14, 15]. Figure 2b shows that the particle rapidly cools down within tens of nanoseconds after the end of the laser pulse. Since thermal damage depends on temperature and time, the reduction in time has to be compensated for by an increased temperature. Therefore, the bubbles cause little structural damage within a cell and do not affect cell viability.

5 Conclusion

We have presented a theoretical model to study the mechanism of laser nanoparticle-based gene transfection. It is calculated that the maximum temperature around the particle is of the order of 2,000 K, providing transient heat capable of causing physical damage to a cell membrane and protein denaturation through thermal inactivation, mechanical destruction, and photochemistry. At present, no time-resolved investigations of the laser nanoparticle-based gene transfection procedure are yet available, but laser-induced cell poration and lysis by the dynamics of bubble formation have already been studied sufficiently [22, 23, 26, 27]. These effects are of interest in the context of transient membrane permeabilization of cells for the transfer of genes or other substances.

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References

1. Umebayashi, Y., Miyamoto, Y., Wakita, M., Kobayashi, A., Nishisaka, T.: Elevation of plasma membrane permeability on laser irradiation of extracellular latex particles. *J. Biochem.* **134**, 219–244 (2003). doi:[10.1093/jb/mvg132](https://doi.org/10.1093/jb/mvg132)
2. Yao, C.P., Zhang, Z.X., Rahmanzadeh, R., Huettmann, G.: Laser-based gene transfection and gene therapy. *IEEE Trans. Nanobioscience* **7**, 111–119 (2008). doi:[10.1109/TNB.2008.2000742](https://doi.org/10.1109/TNB.2008.2000742)
3. Yao, C.P., Rahmanzadeh, R., Endl, E., Zhang, Z.X., Gerdes, J., Huttman, G.: Elevation of plasma membrane permeability by laser irradiation of selectively bound nanoparticles. *J. Biomed. Opt.* **10**, 64012–64019 (2005). doi:[10.1117/1.2137321](https://doi.org/10.1117/1.2137321)
4. Mie, G.: Optical properties of colloidal gold solutions. *Ann. Phys.* **25**, 377–445 (1908). doi:[10.1002/andp.19083300302](https://doi.org/10.1002/andp.19083300302)
5. Jain, P.K., El-Sayed, I.H., El-Sayed, M.A.: In: Au nanoparticles target cancer. *Nanotoday*. <http://www.nanotoday.com/archive/2007/02-01/february> (2007). Cited 6 February 2007
6. Link, S., El-Sayed, M.A.: Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals. *Int. Rev. Phys. Chem.* **19**, 409–453 (2000). doi:[10.1080/01442350050034180](https://doi.org/10.1080/01442350050034180)
7. Huang, X.H., Jain, P.K., El-Sayed, I.H., El-Sayed, M.A.: Determination of the minimum temperature required for selective photothermal destruction of cancer cells with the use of immunotargeted gold nanoparticles. *Photochem. Photobiol.* **82**, 412–417 (2006). doi:[10.1562/2005-12-14-RA-754](https://doi.org/10.1562/2005-12-14-RA-754)
8. Mulvaney, P.: Surface plasmon spectroscopy of nanosized metal particles. *Langmuir* **12**, 788–800 (1996). doi:[10.1021/la9502711](https://doi.org/10.1021/la9502711)
9. Kittel, C.: *Introduction to Solid State Physics*. Wiley, New York (1956)
10. Link, S., El-Sayed, M.A.: Optical properties and ultrafast dynamics of metallic nanocrystals. *Annu. Rev. Phys. Chem.* **54**, 331–366 (2003). doi:[10.1146/annurev.physchem.54.011002.103759](https://doi.org/10.1146/annurev.physchem.54.011002.103759)
11. Jain, P.K., Lee, K.S., El-Sayed, I.H., El-Sayed, M.A.: Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *J. Phys. Chem. B* **110**, 7238–7248 (2006). doi:[10.1021/jp057170o](https://doi.org/10.1021/jp057170o)
12. Goldenberg, H., Tranter, C.J.: Heat flow in an infinite medium heated by a sphere. *Br. J. Appl. Phys.* **3**, 296–298 (1952). doi:[10.1088/0508-3443/3/9/307](https://doi.org/10.1088/0508-3443/3/9/307)

13. Huttmann, G., Serbin, J., Radt, B., Lange, B.I., Birngruber, R.: Model system for investigating laser-induced subcellular microeffects. *Proc. SPIE* **4257**, 398–409 (2001). doi:[10.1117/12.434739](https://doi.org/10.1117/12.434739)
14. Pitsillides, C.M., Joe, E.K., Wei, X.B., Anderson, R.R., Lin, C.P.: Selective cell targeting with light-absorbing microparticles and nanoparticles. *Biophys. J.* **84**, 4023–4032 (2003). doi:[10.1016/S0006-3495\(03\)75128-5](https://doi.org/10.1016/S0006-3495(03)75128-5)
15. Garwe, F., Bauerschäfer, U., Csaki A., Steinbrück, A., Ritter, K., Bochmann, A., Bergmann, J., Weise, A., Akimov, D., Maubach, G., König, K., Hüttmann, G., Paa, W., Popp, J., Fritzsche, W.: Optical controlled thermal management on the nanometer length scale. *Nanotechnology* **19**, 055207–055218 (2008). doi:[10.1088/0957-4484/19/05/055207](https://doi.org/10.1088/0957-4484/19/05/055207)
16. Kreibig, U., Vollmer, M.: *Optical Properties of Metal Clusters*. Springer, Berlin (1995)
17. Doremus, R.H.: Optical properties of small gold particles. *J. Chem. Phys.* **40**, 2389–2396 (1964). doi:[10.1063/1.1725519](https://doi.org/10.1063/1.1725519)
18. Zharov, V.P., Mercer, K.E., Galitovskaya, E.N., Smeltzer, M.S.: Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. *Biophys. J.* **90**, 619–627 (2006). doi:[10.1529/biophysj.105.061895](https://doi.org/10.1529/biophysj.105.061895)
19. Huttmann, G., Birngruber, R.: On the possibility of high-precision photothermal microeffects and the measurement of fast thermal denaturation of proteins. *IEEE J. Sel. Top. Quant. Electron* **5**, 954–962 (1999). doi:[10.1109/2944.796317](https://doi.org/10.1109/2944.796317)
20. Vogel, A., Noack, J., Huttman, G., Paltauf, G.: Mechanisms of femtosecond laser nanosurgery of cells and tissues. *Appl. Phys. B* **81**, 1015–1047 (2005). doi:[10.1007/s00340-005-2036-6](https://doi.org/10.1007/s00340-005-2036-6)
21. Lin, C.P., Kelly, M.W.: Cavitation and acoustic emission around laser-heated microparticles. *Appl. Phys. Lett.* **72**, 2800–2802 (1998). doi:[10.1063/1.121462](https://doi.org/10.1063/1.121462)
22. Zharov, V.P., Galitovskaya, E.N., Johnson, C., Kelly, T.: Synergistic enhancement of selective nanophotothermolysis with gold nanoclusters: potential for cancer therapy. *Lasers Surg. Med.* **37**, 219–226 (2005). doi:[10.1002/lsm.20223](https://doi.org/10.1002/lsm.20223)
23. Neumann, J., Brinkmann, R.: Microbubble dynamics around laser heated microparticles. *Proc. SPIE* **5124**, 82–87 (2003). doi:[10.1117/12.499944](https://doi.org/10.1117/12.499944)
24. Kelly, M.W.: *Intracellular Cavitation as a Mechanism of Short-Pulse Laser Injury to the Retinal Pigment Epithelium*. Dissertation, Tufts University (1997)
25. Zharov, V.P., Letfullin, R.R., Galitovskaya, E.N.: Microbubbles-overlapping mode for laser killing of cancer cells with absorbing nanoparticle clusters. *J. Phys. D Appl. Phys.* **38**, 2571–2581 (2005). doi:[10.1088/0022-3727/38/15/007](https://doi.org/10.1088/0022-3727/38/15/007)
26. Lapotko, D.O., Zharov, V.P.: Spectral evaluation of laser-induced cell damage with photothermal microscopy. *Lasers Surg. Med.* **36**, 22–30 (2005). doi:[10.1002/lsm.20119](https://doi.org/10.1002/lsm.20119)
27. Venugopalan, V., Guerra, A., III, Nahen, K., Vogel, A.: Role of laser-induced plasma formation in pulsed cellular microsurgery and micromanipulation. *Phys. Rev. Lett.* **88**, 078103 (2002). doi:[10.1103/PhysRevLett.88.078103](https://doi.org/10.1103/PhysRevLett.88.078103)