Plasmon nanoparticle-generated photothermal bubbles as universal biomedical agents

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ABSTRACT

We report the concept and preliminary experimental evaluation of the new biomedical optical agent, whose properties provide tunable effect from non-invasive sensing to selective mechanical damage at nano-scale and in living tissues and cells. Such agent uses the energy of light and heat at nanoscale as the plasmonic nanoparticle-generated photothermal vapor bubble (PTB). PTB of nano-size acts as optical probe by scattering the light and as therapeutic agent by local disruptive mechanical impact. We report cell level studies of the PTBs that have proved more than 1000 fold amplification of optical scattering by the PTBs without the damage to host cells and selective damage to target cells by the bigger PTBs though without collateral damage.

Keywords: nanoparticle, laser, bubble, photothermal, scattering

Biomedical applications of plasmon nanoparticles (NP) have demonstrated their biocompatibility, excellent optical scattering and photothermal (PT) properties and high photo- and thermal stability comparing to any molecular optical absorbers. The combination of PT sensing techniques with plasmonic properties of the NPs has shown very promising results with a detection limit of several nanometers. However, the sensitivity of the PT sensing requires increasing the laser-induced temperature, which may cause thermal damage to cells and tissues. Laser induced PT phenomena include the initial thermalization of NP that, in turn, rapidly causes environmental thermal processes: heating of the surrounding media (due to thermal diffusion), its vaporization (if the temperature exceeds vaporization threshold), and generation of acoustic and shock waves. Too long pulses (or continuous optical activation) cause large spatial spread of thermal field (many orders of magnitude of NP size) due to thermal diffusion. This limits the selectivity and safety of NP-based PT diagnostics and therapy. Ultra-short laser pulses concentrate the thermal field within the NP but generate pressure (and shock) waves that also spread over a large volume and may cause uncontrollable damage. The sensitivity, safety and specificity of the NP methods are limited at cell and molecular levels by strong scattering background of highly heterogeneous bio-environment and also by incidental (non-specific) accumulation of the NPs in normal cells and tissues. Therefore despite apparent advantages of nanomaterials their biomedical applications did not yet bring a significant gain on the established methods.

Laser-induced vaporization and cavitation around NPs still remain the most under-recognized phenomena among the PT effects of optical excitation of plasmon nanoparticles. Optical generation and detection of the tissue-generated vapor bubbles was studied at macro- and micro-scales for the different biomedical applications. However, the studies of the bubble generation around laser-heated NPs and at nano-scale are rather limited. The properties of NP-generated nano-bubbles are quite different from their macro-analogs. For example, for macro-bubbles the generation threshold laser fluence increases with an increase of the size of optical absorber (bubble source) while for the NP-generated bubbles this rule is an opposite: the bigger is the size of plasmonic NPs the lower is the laser fluence threshold for bubble generation [1-3]. The bubbles are also used as ultrasound-induced therapeutic and imaging extracellular agents. However, acoustic methods have low selectivity and poor temporal and spatial resolution of the bubble generation (well above cell damage level), which preclude intracellular applications. High-intensity focused ultrasound techniques were developed for therapy but besides low selectivity they require pro-longed treatment time, are associated with significant adverse effects, and require additional guidance during the procedure. Also, artificially engineered microparticles with gas inside can are employed with ultrasound for hyperthermia and imaging. Those exogenous bubbles are too big for intracellular targeting comparing to gold NPs and do not allow to generate local thermo-mechanical effects.

We may conclude that the optical and acoustic activation of diagnostic and therapeutic processes lacks the selectivity and therefore still have limited efficacy and safety. We may outline the several general problems that influence the development of diagnostic and therapeutic methods and agents:

1. Sensing (diagnostics) and actuation (treatment) as a rule are performed separately thus increasing the treatment time and the load on the target or organism. Ability to use one method, devise and agent for sensing and actuation would

ultimately make the treatment faster, safer and more efficient.
2. The methods that succeeded at the research stage often experience slow transfer to clinic due to the safety problems.
3. General trends in the development of biomedical technologies are aimed at the compatibility with living cells and tissues and at the increase of their sensitivity and selectivity so to provide cell- and molecule-level diagnostics and treatment. For many conventional methods these trends create principal limitations (stability and safety of fluorescent probes, selectivity of chemo-, thermo- , photo- and acoustic therapies).

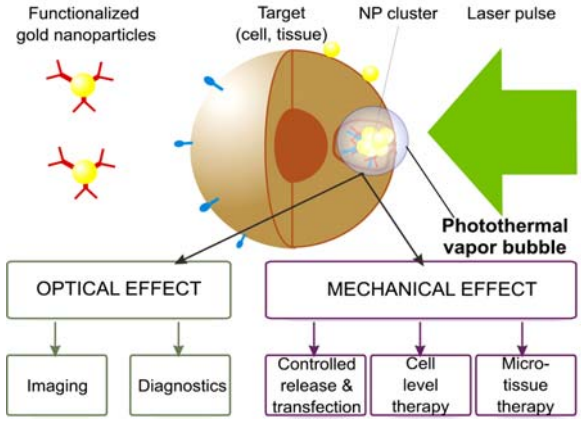

Figure 1: Functionalized gold nanoparticles form the cluster around the target molecule and when excited with short laser pulse act as a heat source thus generating intracellular photothermal vapor bubble. Optical and mechanical effects of the bubble can be controlled through the laser parameters so to tune the bubble to diagnostic or therapeutic task.

Our approach addresses these problems through the development of the tunable nano-scale imaging, diagnostic and therapeutic agent/process that would employ optical generation and detection of nano-bubbles around gold NPs that are selectively delivered to specific targets (Fig. 1). NP-generated vapor bubbles may concentrate around NP the laser-induced thermal field and mechanical (pressure) impact with their characteristic size and duration being determined by the bubble diameter (tunable in the range of 50 – 1000 nm) and lifetime (tunable in the range of 5 – 500 ns), respectively. Also the bubbles possess excellent optical scattering properties that may help in their detection at nano-scale and may improve NP-based optical sensing and imaging through the amplification of the scattered light by the orders of magnitude relatively to that of NP. Despite basically disruptive nature of the bubbles it has been experimentally determined that optically or acoustically generated bubbles of small size may not damage the cells [4]. Target-specific generation of the bubbles can be realized through the delivery of small functionalized NPs (which can enter the cell while big NP cannot) and intracellular formation of their clusters. The NP cluster, selectively formed around the target molecules, will act as the bubble source (Fig. 1), while no bubble would be generated around single non-specifically coupled NPs at specific level of laser fluence. Thus we will selectively generate the bubble as optical probe (acting through optical scattering) or/and therapeutic agent (acting through mechanical impact) in the right place (target-linked NP cluster) and in the right time (single short optical pulse). To distinguish the optical and thermal origin of such bubbles we define them as the photothermal bubbles (PTB). It also should be emphasized the PTB generation and detection do not require chemical agents other than relatively safe gold NPs. Safe and universal nature of the gold nanoparticle-generated PTB may shorten the transfer of PTB-based technologies from the research stage to clinic.

Imaging potential of the NP-generated PTBs was evaluated for A549 (carcinoma) cells that were incubated with 50 nm gold NP-C225 (anti-EGFR antibody) conjugates. Side scattering images where obtained for individual living cells before and during the pump with a time-delayed pulsed probe pulse (690 nm, 0.5 ns). The first image was formed mainly due to the scattering by intracellular NPs (Fig. 2a). The second image was registered with 9 ns time delay and was used for the PTB detection (Fig. 2b). Influence of the PT bubbles on optical scattering signal is illustrated with Fig. 2. The images show two A549 living cells that were exposed to a single pump pulses with the fluencies 0.64 J/cm^2 (cell 1, top panel of Fig. 2) and 0.3 J/cm^2 (cell 2, bottom panel of Fig. 2b), respectively.

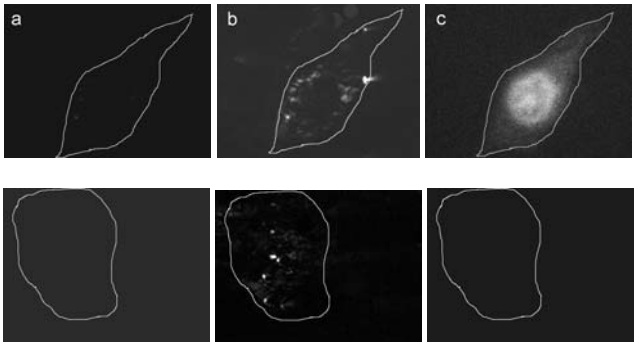

Figure 2: Images of A549 cancerous cells, treated with the gold nanoparticle-antiEGFR antibody conjugates and exposed to a single laser pulse: a) optical scattering image of the cell before exposure to laser pulse, b) optical scattering image of the same cell 9 ns after the laser pulse (bright spots show the photothermal bubbles), c) fluorescent image of the same cell (taken 60 s after the laser pulse) shows Ethidium Bromide fluorescence in a case of damage to cellular membrane. Top panel: the cell has survived laser pulse (532 nm, 0.5 ns, 0.3 J/cm^2), bottom panel: the cell was damaged with a single laser pulse (532 nm, 0.5 ns, 0.64 J/cm^2).

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According to the scattering images (Fig. 2b) the both cells have yielded the intracellular PTBs of sub-micrometers size. Specifically, the amplification coefficient at higher laser fluence (top cell 1 at Fig. 2b) was 2886, while at the lower laser fluence (bottom cell 2 at Fig. 2b) it reached 1779. Analysis of fluorescent has shown that the cell 1 was damaged by the PTB because its fluorescence has increased (Fig. 2c, top) and it shape significantly changed. Under the lower pump laser fluence the cell 2 has survived the pump pulse and PTB: no increase of the fluorescence (Fig. 2c, bottom) and no change in the cell shape were detected. PTB lifetime in survived cell was shorter than that for a damaged cell and this difference shows that the maximal diameter of the PTB in survived cell was smaller than that for a damaged cell. We may conclude that the bubble size, not NP size, is important for the amplification of optical scattering. Cluster-bubble mechanism has provided amplification of optical scattering in living cells by more than 1000 times (comparing to the scattering by gold NPs), and without the damage to the cell at the specific laser pulse fluencies (Fig. 3). Therefore the NP-generated intracellular PTBs may be considered as non-invasive and high-sensitive optical probes.

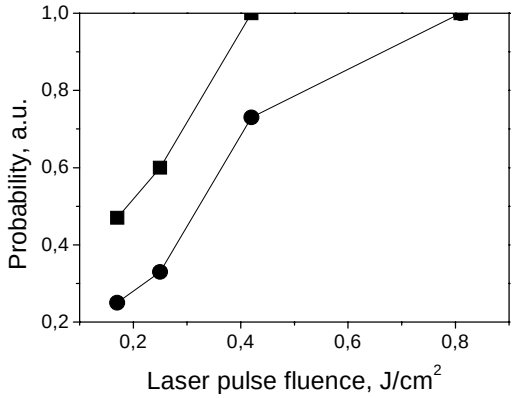

Figure 3: The probabilities of the generation of the photothermal bubbles in the cell (■) and of the cell damage with the bubbles (●) obtained for A 549 cells after their treatment witn gold nanoparticles and a single laser pulse (532 nm, 0.5 ns) vs laser pulse fluence.

We have evaluated therapeutic effect of the PTBs with the suspensions of the human leukemic and normal stem cells. Maximal damage level of 100% was achieved at this laser fluence when the combination of the two antibodies – CD 19 and CD22 – was used for cell targeting. It should be emphasized that the PTBs have damaged only those cells where the PTBs were generated and have never damaged collateral cells that did not produce the PTBs. The size of laser-induced PTBs with lifetime of 0.5 – 2 μs may be comparable with the cell dimensions. Such large PTBs mechanically damage the cell membrane causing their blebbing and lysis. Single cell level of the damage selectivity can be hardly achieved with the photothermal methods based on the thermal mechanisms and with relatively long exposures to excitation optical source because the temperature field cannot be localized within the single cell. Therefore the cluster-bubble mechanism may be efficiently applied for the elimination of the residual and therapy-resistant cancer cells including those in the grafts (transplants) of the blood and bone marrow so to improve the autologous bone marrow (and stem cell) transplantation.

The development of molecularly targeted therapy and gold NPs, which can be optically activated to generate the PTB suggests that coupling decease-specific molecular (or cellular) targets with NPs for activation of intracellular PTB could serve as an effective strategy for treatment at cell level. NP cluster-bubble mechanism may combine diagnostics, treatment and guidance in one devise/process (Fig. 4): (1) clusters of NPs can be selectively formed in specific tumor cells for detection of the decease-specific cells and tissues through NP and PTB optical scattering of low-energy laser pulse (small PTBs), (2) local mechanical destruction of the detected cells/tissues by generation of intracellular PTB with the diameter above cell damage threshold, and (3) real-time optical guidance of the damage at cell level through optical detection of large "therapeutic" PTB. Such combination of 3 stages in one process and devise should significantly improve the efficacy and selectivity of the treatment and also does not involve chemotherapy. We defined described method as LANTCET (laser-activated nano-thermolysis as cell elimination technology).

The LANTCET may be applied at cell and tissue levels [4-9]. Cell level technique allows selective elimination of specific target cells without the damage to collateral normal cells. This can be an efficient solution for cancer treatment: leukemia (detection and elimination of residual cancer cells in the blood and bone marrow grafts) and solid superficial tumors that can be topically accessed with NPs and laser radiation with fiber optical probe. Tissue level application may be employed for the re-canalization of the arteries blocked with thrombi and plaques that cannot be removed chemically and mechanically. The main difference of the LANTCET from the nanoparticle-, laser- and hyperthermia-based methods of diagnostics and therapy is associated with the use of the principally new type of the agent - the PTB. The PTB is *not the nanoparticle but physical phenomenon (bubble)* that can is generated and controlled with laser pulse and around plasmonic nanoparticle at specific moment and site.

At the current stage we can outline several general properties of the NP-generated photothermal bubbles:

1. The unique features of the PTB mechanism provide the generation of the bubbles in specific location (NP or NP cluster), with controllable diameter and lifetime, and without thermal load to micro-environment outside the PTB.
2. PTBs can be selectively generated around specific

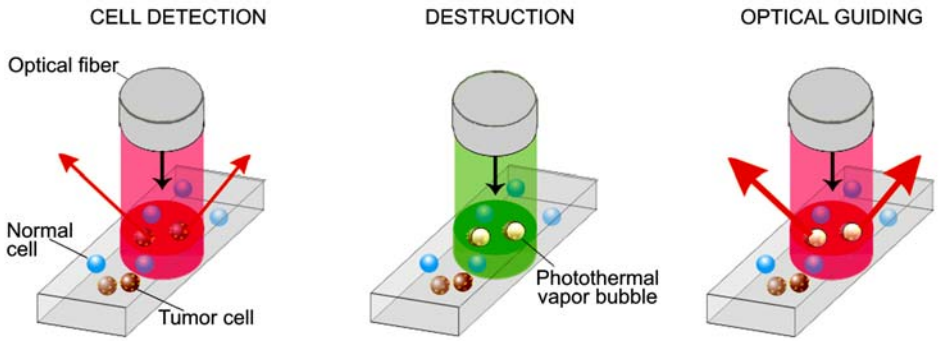

Figure 4: LANTCET stages: light-scattering detection of nanoparticle clusters in individual targeted cells with low-intensity probe laser beam (shown as pink column at the left panel); damage of targeted cells with photothermal bubbles that are induced by a short laser pulse at the wavelength of NP plasmon resonance(shown as a green column at the middle panel); guidance of cell damage through optical detection of photothermal bubbles with additional probe pulse (shown as a pink column at the right panel).

targets such at cell level through the cluster-bubble mechanism.

PTBs are the new class of high-sensitive and potentially minimally invasive optical probes for cell and molecule detection.

3. Biomedical effect of the PTB can be precisely tuned from non-invasive imaging to the localized damage and elimination of the target by varying the fluence and other parameters of excitation laser pulse.

4. The mechanisms of the PTB generation and detection provide the basis for the integration of the diagnostic, therapy and therapy guidance into one process that may be supported by one device.

Development of the PTB methods and agents will result in a new scientific platform that will generate technologies with a superior imaging, diagnostic, and therapeutic potential. Combination of the biological safety of gold NPs with the on-demand nature of nano-scale PTBs will significantly improve the safety, sensitivity, and selectivity of the research (molecular imaging, living cell analysis and detection); diagnostics (detection of disease-specific tissue, cells, bacteria with a great sensitivity); therapy and surgery (selective elimination of target tissues and cells such as tumors, atherosclerotic plaques, cell level hyperthermia, and PTB-enhanced drug and probe intracellular delivery by means of the membrane transfection, endosome disruption) and biomedical instruments (optical microscopy, endoscopy, flow cytometry, laser surgery and angioplasty). Thus, the PTB-based technology will improve the parameters of such tools as: optical microscopy, endoscopy, flow cytometry, laser surgery, as well as of angioplasty instruments, and will affect research, engineering, and clinical communities. The ability of PTBs to intensify heat-transfer at micro-scale level may also find various applications beyond the medical field. Being the non-chemical agents, the PTBs utilize at nano-scale the phenomena of light and heat that are natural and essential for all living systems.

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