Method of laser activated nano-thermolysis for elimination of tumor cells

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Summary

We describe novel ex vivo method for elimination of tumor cells from cell suspension, Laser Activated Nanothermolysis and propose this method for purging of bone marrow and blood transplants. K562 and human lympholeukemia cells were eliminated in experiments by laser-induced micro-bubbles that emerge inside individual target cells around selectively formed clusters of light-absorbing gold nanoparticles. Pretreatment of tumor cells with specific monoclonal antibodies and Ig-conjugated 30-nm gold particles allowed the formation of clusters of 10–20 on the surface of cell membrane. Electron microscopy found the nanoparticulate clusters inside the cells. Total (100%) elimination of K562 cells targeted with specific antibodies was achieved with single laser pulses with optical fluence of 5 J/cm² at the wavelength of 532 nm without damage to the same cells targeted without specific antibodies. Total elimination of human lymphoblasts from suspension of normal stem cells was achieved by a single laser pulse with the optical fluence of 1.7 J/cm², while the damage level of normal cells was 16%.

Keywords: Laser; Bubble; Nanoparticle; Purging; Residual cells; Thermal; Leukemia

1. Introduction

Efficient treatment of leukemia with modern protocols is limited by the persistence of residual tumor cells. Contamination of transplants (grafts) with residual tumor cells seriously limits the efficacy of autologous transplantation. Residual cells may be removed from graft with purging methods that were recently developed for in vivo and ex vivo applications by using pharmacological, magnetic, photochemical negative and positive selection of cells [1]. Current methods for purging demonstrated their potential in increasing patient survival rate by several times [2,3] but still no method provides sufficient clinical efficacy and safety [1–7]. Therefore the development of new approaches for purging with high efficacy and safety
is one of the major challenges of hematology. Laser ablation (destruction) of leukemic cells may be applied for elimination leukemic cells from the graft. Several laser methods were already developed for selective cell killing. They employ several general approaches: (i) thermal damage through absorption of continuous wave laser radiation by target cells [8], (ii) thermomechanical damage through generation of bubbles and other pulsed ablative events [10], and (iii) biochemical damage through low-power laser interactions [9]. A method for selective cell damage based on short laser pulses was introduced more than 20 years ago as selective photothermolysis [11]. This method provides localized thermal damage of light-absorbing structures but avoids thermal damage to surrounding tissue. In other method localized thermal damage was achieved with laser-induced cavitation microbubbles [12] or an ultrafast micro-explosion [13]. Slow heating can also be used for selective damage of cells, however, due to thermal diffusion requires much stronger optical contrast to achieve specificity [14]. As an alternative to thermal damage of target cells, photodynamic therapy employs low-intensity laser irradiation to produce necrosis and apoptosis through photochemical reactions [9]. All considered methods require that the target cells contain sufficient amount of endogenous or exogenous light-absorbing agents. Such endogenous cellular agents are limited by hem-proteins and melanin so that only blood and retinal-pigmented epithelium can be treated without a contrast agent [15,16]. For tumor cells with low endogenous light absorbance, however, an exogenous contrast agent must be used to provide sufficient thermomechanical effect. Exogenous molecular dyes strongly absorbing red laser pulses were applied for the chromophore-assisted laser inactivation (CALI) of specific proteins in cellular membranes [17]. Mechanisms of laser-induced bubbles that emerge around light-absorbing melanosomes and externally administered microparticles have been studied theoretically and experimentally to achieve selective destruction of abnormal cells [18,19]. Application of monoclonal antibodies and other molecular targeting vectors improves specificity of targeting tumor cells [20,21]. Metal (gold or silver) nanoparticles (NPs) have been recently introduced as contrast agents for detection [21–24] and thermal damage [24–26] of tumor cells. Metal NPs may be designed to absorb any desirable color of visible and near-infrared radiation by changing their shape or structure [24,27]. Gold NPs absorb light much stronger than any organic dyes, which makes NP a superior agent for tumor cells [21,25]. NP-assisted laser inactivation of CD8-specific white blood cells was recently demonstrated employing targeting of NPs to cells and superheating them with laser pulses [28]. The threshold of a vapor bubble formation around nanoparticles is significantly higher and defined by the surface tension and dynamic viscosity of surrounding water in contrast to purely thermal diffusion driven generation of bubbles around microparticles [29]. Thus, special efforts are required for reduction of the bubble generation threshold to achieve selective destruction of target cells with no or minimal damage to normal cells. We propose to target tumor cells specifically using small nanoparticles (a procedure not possible with microparticles) and produce clusters of nanoparticles to convert them effectively into microparticles.

Any purging method should satisfy the two main criteria: efficacy and safety. Efficacy of the method is defined by its ability to destroy as many target cells (ideally, all of them) as possible. The safety of the method is determined by its capability to keep all normal cells viable. Therefore any laser method for purging must employ highly selective mechanisms of cell elimination.

All currently employed methods do not possess efficacy and safety sufficient for clinical needs. Here, we introduce a novel chemical-free purging method referred to as Laser Activated Nano-Thermolysis Cell Elimination Technology (LANTCET) that selectively damage tumor cells through generation of laser-induced micro-bubbles around clusters of light-absorbing nanoparticles targeted to tumor-specific receptors in the cells. The goal of reported work was to evaluate the feasibility of LANTCET for ex vivo purging of leukemia cells. To achieve better experimental efficacy we employed a combination of primary tumor-specific monoclonal antibodies (MAB) and a secondary MAB conjugated to gold nanoparticles capable of aggregation around primary MAB, a technique that improved clusterization and uptake of NPs by tumor cells. This in turn resulted in efficient generation of micro-bubbles in target cells. To achieve better control, we used guidance of laser
2. Materials and methods

2.1. Cells

In vitro model with myeloid K-562 cell line was utilized to evaluate bubble generation and cell damage at single cell level. We also have used cryopreserved human cells, tumor cells (patient-derived acute B lymphoblast leukemia) and normal stem cells for estimating clinical applicability of LANTCET for suspensions of cells. We have used well-defined specific MABs as primary MAB for targeting: CD15 and Glycophorin-A for K562 cells and CD19 for acute B-lymphoblast leukemia cells. Selection of MAB for experiments was realized by using flow cytometry. MABs CD 15 and Glycophorin A were selected for K562 cells based on their superior level of expression on surface of those cells. Other cell surface markers (CD14, CD33, CD13, CD11b) revealed lower level of expression for K562 and therefore were not used for our experiments. CD 19 was selected in the same way for acute B lymphoblast leukemia cells. We will refer further K562 and acute B-lymphoblast leukemia cells as tumor cells. For control we have used: a single-cell model, untreated K562 cells and a suspension model, normal stem cells.

Three different protocols for accumulation of NPs in the cells were investigated: (1) specific targeting, i.e. when a receptor-specific MAB1 was used as primary antibody and NPs were conjugated with the secondary MAB2 and incubated with cells targeted with the primary MAB (so-called ‘sandwich’ scheme—NP-MAB2-MAB1-cell)—that may provide coupling of more NP to one receptor in comparison with conventional scheme—NP-MAB1-cell), and (2) non-specific targeting, i.e. when either NPs were conjugated to a non-specific MAB and no primary MAB was used, and (3) no MAB was employed at all (bare NPs were incubated with cells).

2.2. Specific targeting protocol

Cells at concentration of 800,000/ml in Phosphate Buffer Solution (PBS) with 1% Fetal bovine serum (FBS) were preincubated with two primary MABs CD15 and Glycophorin-A (20µl/ml of each) (K562 cells in single-cell model) and CD19 (acute B lymphoblast leukemia cells and normal stem cells in suspension model) for 30 min with shaking in the dark at 4 °C. After incubation, the cells were centrifuged at 300g for 4 min and washed twice. Then the cells were incubated again for 30 min hour with $2 \times 10^{10}$/ml of 30 nm gold NPs conjugated to secondary MAB, Goat anti-Mouse IgG (these bioconjugates is a product of British Biocell International (Cardiff, UK) that was purchased from Ted Pella Inc (Redding, CA). Then cells were separated from free uncoupled NPs by centrifugation at 300g for 4 min. The bottom pellet of cells was resuspended in PBS–FBS solution and immediately used in laser experiments. Same cells were used for preparation of cell samples with modified protocols.

2.3. Non-specific targeting protocol

The same procedure as described above was applied, however, without primary MAB, only with secondary MAB-NP complex. As a simple control, bare gold NPs without any MAB were incubated with cells under the same conditions.

To determine cell viability, trypan blue dye was used according to a routine protocol, on an aliquot of cells from the stock suspension before the laser treatment and on every treated sample after the laser treatment. We have prepared four K562 cell samples using three described protocols for the single-cell model. Two cell samples (leukemic and normal stem cells) were prepared for the suspension model using the specific targeting protocol. Additional control sample of nanoparticles in solution without cells was also prepared.

2.4. Laser treatment procedure and cell damage measurements

LANTCET was experimentally studied in the two models, single cell and suspension of cells. Both of them employ a protocol graphically depicted in Fig. 1. In the first model, single cells were irradiated one by one and their possible damage was immediately detected. After the incubation with gold NPs, the samples of K562 cells were immediately placed in
the sample chamber (S-24737, Molecular Probes, OR) mounted on a microscopic slide to produce a monolayer of cells. Individual cells (total of 150) were irradiated within 7 min with 10-ns long single focused laser pulse at 532 nm. The green light at 532-nm is strongly absorbed by NPs, but not by the cells. All samples were exposed to the same laser irradiation conditions, i.e. a specific optical fluence. We used photothermal (PT) microscope previously developed by us for visualization of vapor bubbles around laser-irradiated NP [30]. PT microscopy may be used not only for detection of microbubbles around clusters of nanoparticles, (3) the bubbles destroy only cells targeted with nanoparticles and non-targeted cells (left) remain intact.

Cells were irradiated one by one with single focused laser pulse at 532 nm (10-ns duration) and PT response from each cell was recorded with a photodetector measuring changes in the power of the probe laser at 633-nm (temporal profile of probe laser signal) [31,32]. Two quantitative parameters [31] were measured: (1) probability of cell damage at a specific laser fluence as $DP = M/N$, where $N$ is the total amount of irradiated cells, $M$ is the number of cells that yield bubble-specific PT response, and the damage threshold fluence, i.e. the fluence corresponding to $DP = 0.5$. Then $DP$ was calculated for each cell sample.

In the second model, feasibility of LANTCET for purging was evaluated using suspension of cells. Suspensions of test and control cells were injected into a rectangular glass chambers and then were irradiated with single laser pulses. In this experiment, a laser beam with diameter of 1 mm was scanned along the cuvette with dimensions of $0.4 \times 0.1 \times 10.0 \text{ mm}$. This procedure took about one minute for each sample. After the irradiation, the laser-induced cell damage was assessed within 5 min in the same cuvette using the standard trypan blue dye, optical microscope and digital camera for visualization and counting the level of positively stained cells. The damage probability, $DP$, was then calculated.

2.5. Electron microscopy of leukemic cells

Binding of IgG-conjugated gold nanoparticles to primary MAB attached to CD15 and Glycophorin-A receptors on the surface of K562 cells causes internalization of the complex. Thus, we next tested whether phagocytosis took the NPs into the cells using transmission electron microscopy [21]. The targeted cells were fixed in situ in a mixture of 2.5% glutaraldehyde and 1.5% formaldehyde in 0.05 M cacodylate buffer pH 7.2 to which 0.03% trinitrophenol and 0.03% CaCl2. After washing in 0.1 M cacodylate buffer cells were pelleted and processed further as a pellet. They were post-fixed in 1% OsO4 in 0.1 M cacodylate buffer, en bloc stained with 2% aqueous uranyl acetate, dehydrated in ethanol and embedded in Poly/Bed 812 (Polysciences, Warrington, PA). Ultrathin sections were cut on Reichert-Leica Ultracut-S ultramicrotome, stained with lead citrate and examined.
3. Results

3.1. Evaluation of gold NP uptake by cells with electron microscopy

Electron microscopy (EM) images of K562 cells (Fig. 2) revealed that NPs fill the entire volume of the cell and do not concentrate only on its outer membrane, where target receptors are located. Clusters of closely packed 5–20 particles were found in cells that were specifically targeted using the primary MAB and bioconjugates of NPs with the secondary MAB (Fig. 2b). The total average number of NPs per cell estimated by extrapolating the number of NPs counted in 60-nm thin slices corresponding to EM images was about $31650 \pm 4810$ in specifically targeted cells. In contrast, much smaller number of NPs was found in non-specifically targeted cells: about $1500 \pm 350$ NPs per cell, and only in about $200 \pm 90$ NPs per cell in control cells incubated with bare nanoparticles. No NP clusters were observed in non-specifically targeted and control samples (Fig. 2a). High NP contrast (ratio of NP level for specific targeting to that of non-specific targeting was 21. This ratio further increased to 158 for specific targeting relative to the control of bare NPs. The selectivity achieved by specific targeting of tumor cells promises a high degree of LANTCET safety: only cells with clusters of NPs may be destroyed leaving other cells not damaged by the laser pulse.

3.2. Laser elimination of tumor cells–single cell model

Using the single-cell model we studied PT response from individual K562 cells to pulsed laser irradiation (532 nm, 10 ns). PT response signals from (1) free space between the cells (background), (2) from individual cells targeted with NPs, and (3) from suspension of NPs without cells were obtained. The microbubble generation and the cell damage were monitored simultaneously with the probe laser pulse by analyzing profiles and amplitudes of PT responses as depicted in Fig. 3. Among all studied samples, only the cells specifically targeted with NP showed prominent bubble-specific PT response (Fig. 3c). These cells also frequently exhibited apparent visual signs of damage, such as fragmentation after irradiation with a single laser pulse. We detected micro-bubbles with duration from 1 to 3.5 \(\mu s\) (Fig. 3c) only in specifically targeted cells, while in other test samples such long micro-bubbles were never observed even at optical fluences 10 times exceeding the threshold of bubble generation. So such long bubbles were detected only in those cells that manifested clusters of NPs in their EM images.

Explanation for our observation is given in the recently developed model of a vapor bubble generated around superheated nanoparticle [29]. The temperature of laser-heated NP is proportional to the ratio \(\tau_{HD}/\tau_L\) of heat diffusion time and the laser pulse duration, where the heat diffusion time from a spherical NP into aqueous medium can be found as the ratio of NP diameter squared and the heat diffusion coefficient \((\chi = 0.0013 cm^2/s)\) in water \(\tau_{HD} = d^2/24\chi\) [21]. Heat diffusion time equals 0.3 ns for a single gold NP with diameter of 30 nm and this time is much less than laser pulse length (10 ns). Such rapid heat diffusion distributes the laser pulse energy over a larger volume, so that only a 0.033 fraction of laser energy is used to heat the NP. Even more important for generation of vapor bubbles around nanoparticles

![Fig. 2. Electron microscopy images of K562 cells. A control cell without Nanoparticles (left) and a cell specifically targeted with primary MABs (CD 15 and glycophorin A) and 30 nm diameter spherical gold nanoparticles conjugated to secondary MAB (right). Small black dots are single nanoparticles, larger black spots are clusters of nanoparticles.](image-url)
is that the surface tension and the dynamic viscosity of water prevent formation of expanding bubbles around nanoparticles superheated substantially above the critical point temperature of water (647 K).

The situation changes for NP clusters with effective diameter of more than 300 nm consisting of about 10-20 NPs, so that the initial surface tension is 10 times lower and the full thermal energy of a laser pulse is utilized to heat the cluster of gold NPs. Therefore, the threshold of pulsed laser interaction with clusters of NPs is significantly lower than that for a single nanoparticle. Superheating of the NP clusters generates a much bigger vapor microbubble capable of damaging even large cells. We found that NP clusters were destroyed by single laser pulses with optical fluence of 5 J/cm², which could superheat NP to a temperature significantly above the boiling temperature of gold (2873 K). Laser pulses that followed the first microbubble-generating pulse produced no bubbles, and no bubble-specific PT response was detected for the second pulse (Fig. 3a). It is important to note the difference in PT response amplitude and lifetime, the parameters dependent on the bubble diameter, between the control suspension of NPs (Fig. 3b) and the cells specifically targeted with gold NPs (Fig. 3c). Small amplitude and a short 0.3 μs duration of the PT response signal detected in the suspension of gold NPs (Fig. 3b) indicates that only a very short-lived nanobubble was generated even with optical fluence of 35 J/cm². In contrast, cells specifically targeted with NPs (test sample) produced a much stronger PT response (Fig. 3c), which indicates that the bubble was almost one order of magnitude larger and, therefore, lasted longer. Such increase in the bubble lifetime and size may be explained only by formation of clusters of NPs in tumor cells.

Cell damage probability DP for test and control samples was analyzed at several different levels of laser fluence: 5, 35 and 90 J/cm² (Fig. 4). We have found that both, the DP level and the laser fluence threshold for bubble generation depend on the presence of NP clusters in cells: the DP increases and the damage threshold decreases for target cells that may have clusters of NPs compared with control samples having for which no clusters were found during EM examination.

For example, the damage probability, DP, for specifically targeted cells reached its absolute maximum of 100% at a fluence of 5 J/cm², while for non-specifically targeted cells it was only 0.07 at the same fluence and for the control sample of cells incubated with bare NPs, the DP level was only 0.09 at much higher fluence.
higher fluence of 35 J/cm². We have estimated the damage threshold for the specifically targeted K562 cells to be about 1–2 J/cm², which is 30–50 times lower than the threshold fluence level for destruction of the same cells without particles (control). Thus, the specific targeting protocol provided a significant decrease of the laser damage threshold compared to intact untargeted cells and more than 10 times decrease compared with control K562 cells incubated with NPs not conjugated to a MAB. The length of PT response signal in targeted cells statistically varied from 0.2 to 3.5 ms. Maximal micro-bubble diameter reached 20 μm, as calculated using previously developed model of cell damage based on the experimentally measured lifetime of a microbubble [31].

3.3. Laser elimination of tumor cells–cell suspension model

In addition to laser irradiation of individual cells and their real-time monitoring with the PT microscope, we also irradiated suspensions of the human leukemic and normal stem cells in the cuvettes, simulating the first approximation to the bone marrow purging procedure. In this case many cells (200–400) were irradiated simultaneously with a broad laser beam. Damaging effects of a single laser pulse was monitored by microscopic examination of trypan blue uptake by cells after the laser treatment. Cells with visual signs of destruction as well as positively-stained were considered damaged. Laser fluence in the range of 0.5–2 J/cm² was used in this experiment. We have found that at the level of 1.7 J/cm² 100% of specifically targeted tumor cells were damaged (as shown in Fig. 5b) and 16% of normal stem cells were damaged (Fig. 4), while 84% of normal cells survived laser pulses (Fig. 5a). Bubble generation threshold for tumor cells was found to be in the range 0.1–0.3 J/cm². This is 100–300 times lower than the bubble generation threshold of optical fluence (30–70 J/cm²) determined earlier by us [31] for intact untreated cells: lymphocytes, K562 and lymphoblasts.

4. Discussion

4.1. Cell damage

Correlation of the cell damage and observation of microbubbles with the optical fluence threshold of the bubble-related photothermal response signal (such as shown in Fig. 3b and c) demonstrates that the PT response is caused by the bubbles in cells and simultaneously indicates that the cell is damaged. This correlation was confirmed with visual observation of cell disintegration (Fig. 5b) and also with positive staining of cells with trypan blue. We consider laser-induced microbubbles as the main damaging factor in LANTCET. The microbubble generation may occur simultaneously in several locations inside the cell volume. The size of laser-induced microbubbles with lifetime of 0.5–10 μs may be comparable with

Fig. 4. Cell damage probabilities, DP, experimentally obtained for different pump laser pulse (532 nm, 10 ns) fluence levels for specifically targeted common B acute lymphoblasts (lymphoblasts) and normal stem cells, for specifically targeted K562 cells (test), for non-specifically targeted K562 cells (control # 1), for K562 cells targeted with bare NP (control # 2) and for untargeted K562 cells (control # 3).

Fig. 5. Optical microscopic images of normal stem cells (a) and common B acute lymphoblasts (b) in the cuvette after irradiation of cell suspension with a single broad laser pulse with optical fluence of 1.7 J/cm²; cells were prepared using specific targeting protocol.
the cell dimensions. Such large microbubbles mechanically damage the cell membrane causing necrosis and lysis. However, smaller bubbles may also induce apoptosis without rupturing the membrane. Even smaller bubbles (nanobubbles) that emerge around NPs located at the distance from cell membrane do not damage the cells due to their limited diameter of less than a micrometer.

Although we did not study the influence of NP concentration during incubation process on the cell damage threshold, this study is the subject of our future publication. For the feasibility study reported here, we have chosen rather high concentration of nanoparticles to achieve the lowest possible fluence threshold for cell damage. On the other hand, the concentration of nanoparticles we used has been shown nontoxic for injection in animals [25,26].

The most surprising and important finding of this study is that formation of NP clusters in laser-irradiated target cells caused microbubbles around them and the cell destruction, though the same laser pulses were safe not only for intact cells, but also for cells containing single nanoparticles. We think that the nanoparticulate clusters first formed at the membrane surface of a target leukemia cell via aggregation of secondary MAB around the primary MAB, and then these clusters are internalized into the cell by phagocytosis. In order to minimize endocytosis of single nanoparticles in target cells, the cells pretreated with the primary MAB are incubated with NPs conjugated with the secondary MAB at a low temperature of 4 °C. Then uncoupled NPs are washed out. At the final targeting stage the temperature in the incubation chamber is elevated to a normal temperature of 37 °C, so that the process of endocytosis can occur. As a result the clusters of NPs may appear inside cells within 20–40 min.

4.2. Monoclonal antibodies

The safety of LANTCET depends upon specificity of expression of certain antigens by tumor cells. When tumor-specific MAB can be found, NP can be selectively delivered only into tumor cells and no bubbles would emerge in normal cells without NP. In this point LANTCET is similar to other therapeutic and cell-processing methods that use MAB to recognize and kill target cells. In our studies we have used primary MABs that showed high expression level for K562 cells and high specificity of expression (more than 2 orders of magnitude) to human leukemic cells. The relative expression of CD15 in normal and leukemic cells is comparable. We have chosen CD 15 and Glycophorin A as the MABs that yielded highest expression level for K562 cells and then applied ‘sandwich’ targeting scheme to study selectivity of NP delivery to tumor cells. To distinguish normal and leukemic cell types a specific immunophenotype should be determined (with flow cytometry for example) for each cell sample. This will provide several MABs that are specific for leukemic cells. We have applied this approach for determining optimal MAB for human leukemic cells in described above experiments in suspension model. In clinical application, the specific immunophenotype should be determined for each population of tumor cells to distinguish normal and leukemic cell types and determine the most specific genes and related membrane receptors for targeting. An optimal bioconjugation and targeting conditions could enhance clusterization of NPs on the surface and inside tumor cells thus providing necessary efficacy and safety of LANTCET as a purging method for treating autologous bone marrow transplantation grafts and peripheral blood stem cells transplants.

4.3. Purging techniques

There are several purging methods that use metal (magnetic) particles and tumor-cell-specific MAB—magnetic sorting [33–35], drugs [36,37], MAB with complement [2], MAB with immunotoxins [35], photodynamic effects [9,35], adenoviral vector-based methods [38]. Neither of them provides up to the date sufficient efficacy in elimination of residual cells. Unsolved for those methods problems include the loss of normal cells, risk of xenobiotics by infection agents, costs, need for additional safety and validation procedures, delays of engrafment after transplantation [1]. The other general problem for any equipment that is used for ex vivo manipulations with transplants is the increasing standards and requirements for such equipment. LANTCET excludes toxicity problems because it is chemical-free method and gold conjugates were shown to be non-toxic [25,26]. Also decreasing laser fluence can minimize
potential laser damage and negative influence on the graft. Our future plans include experiments to determine whether low fluence laser irradiation of cells with small concentration of single NPs can cause apoptosis and delayed cell death. We expect that laser damage thresholds of target tumor cells can be further reduced at least by several times after replacement of spherical gold NP with elongated nanorods or nanoshells possessing exceptionally strong optical absorption in the near-infrared spectral range. Simultaneously, laser irradiation in the near-infrared will be much safer for all types of normal cells. We plan also to study in detail the influence of NP properties (size, shape, composition, concentration) on efficacy and safety of LANTCET. Studied cultured and human cells and MAB-NP complexes cannot represent all real leukemic cells though they were sufficient for demonstration of basic features and potential of LANTCET. Similar experiments will be performed by us in future with patient-derived leukemic cells and the efficacy and safety of LANTCET will be evaluated in clinic. There is also the possibility for limited in vivo purging application of LANTCET. Combination of specific cell targeting protocols with the methods of spectrally selective pulsed laser irradiation guided by photothermal microscopy may help LANTCET to match high standards and requirements for graft processing.

5. Conclusions

New method of Laser Activated Nano-Thermolysis Cell Elimination Technique was experimentally demonstrated in vitro using model K562 and human leukemic cells treated with light-absorbing spherical gold nanoparticles and irradiated with laser pulses of selected wavelength and optical fluence. The efficacy of suggested targeting protocols was directly verified by electron microscopy. The protocol developed for specific targeting resulted in the total damage of K562 and human leukemic cells with single laser pulse through generation of intracellular microbubbles around clusters of gold nanoparticles. The same cells without NP clusters were not damaged. Provided success of further development, LANTCET may allow better selectivity, safety and control for laser elimination of residual tumor cells from bone marrow and blood grafts for autologous stem cell transplantation.

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