Temperature monitoring during light-induced release of cargo using polymer capsules modified with gold nanoparticles and nanodiamonds

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Abstract. Application of different light-sensitive drug delivery carriers is limited due to a risk of overheating of living cells. Therefore, a real-time temperature monitoring within biological objects that controls the photothermal release of different cargos from light-sensitive carriers is highly demanded. In this work, we develop a multifunctional platform comprised of polymer microcapsules modified with nitrogen vacancies (NV) centers as nanothermometers and gold (Au) nanoparticles (NPs) as heating elements for the realization of laser-induced cargo release with a simultaneous temperature measurement inside cells. Such platform allows to prevent unwanted side effects related with the overheating of living cells and tissues.

1. Introduction
To date, micro- and nanosized drug delivery systems can be designed to realize a remote non-invasive release of bioactive compounds into cells upon different stimuli, for example, a laser-induced heating. Such photothermally responsive drug delivery platforms are able to absorb light energy and transform it into heat that further lead to the rupture of drug carriers with the consequent drug release [1]. Nonetheless, excessive heating and temperature changes strongly affect cellular functions such as cell
division [2], gene expression [3], growth factor activity [4] and metabolism [5]. Thus, it is vital to provide a real-time temperature monitoring during external heating to avoid possible side effects.

The widely used technology for temperature sensing include scanning probe microscopy [6], Raman spectroscopy [7], fluorescence-based methods using quantum dots, dyes, lanthanides or fluorescent proteins [8]. However, their applications are limited due to the low sensitivity and the influence of surrounding medium on optical properties of light-responsive carriers. However, it can be overcome by applying nanoscale thermometry based on a quantum mechanical spin associated with nitrogen vacancy (NV) centers in nanodiamonds using optically detected magnetic resonance (ODMR) [9], [10].

In this work, we develop a multifunctional system based on polymer capsules for simultaneous temperature measurements inside living cells and simultaneous drug delivery. For this, we incorporate quantum nanothermometers (NV centers) and heating agents (Au nanoparticles – Au NPs) into a single platform - polymer capsules. It is worth noticing that Au NPs were incorporated either in capsules cavity (core) or in capsules wall. Laser irradiation stimulates heating of Au NPs embedded into carriers leading to their consequent rupture, which triggers a photoinduced release of cargo. We found out a strong correlation between the localization Au NPs within a capsule (either in the capsule’s cavity or attached onto the capsule’s wall) and the critical laser power of capsule rupture. The developed approach allows temperature monitoring to prevent unwanted overheating of cells and tissues during photoinduced drug delivery.

2. Materials and methods

2.1. Au NPs fabrication and NV centers coating

Au NPs with 50 nm diameter were synthesized by a seed-mediated growth approach [11]. Briefly, the first step was the preparation of the initial Au seeds. The second step included Au NPs growth up to 50 nm. The final step was stabilization of Au NPs growth up to 50 nm. The final step was stabilization of Au NPs by H2N-PEG-SH via ligand-exchange procedure [12]. Commercially available NV centers were additionally coated with a silica shell (NV@SiO2) via a Stöber method [13].

2.2. Au NPs fabrication and NV centers coating. Synthesis of polymeric capsules with incorporated Au NPs and NV centers into capsule’s core or wall, and their DAPI loading.

Micrometric capsules were synthesized as reported elsewhere with several modifications [14]. To load Au NPs and NV centers into the capsule core (CORE sample), 615 µL of calcium chloride (CaCl2, 1M in water) were mixed with 2.5 mL of water, 400 µl of NV centers and 400 µl of Au NPs under magnetic stirring. Then, 615 µL of Na2CO3 (1 M, in water) were added to the CaCl2 mixture under vigorous stirring at 1000 rpm for 30 sec at room temperature. Next, a polymer shell around CaCO3 particles was deposited using a Layer-by-Layer approach. Six monolayers of oppositely charged polyelectrolytes poly-(allylamine hydrochloride) (PAH) and poly-(sodium 4-styrenesulfonate) (PSS) were attached on the top of the CaCO3 cores yielding the following architecture PAH/PSS/PAH/PSS/PAH/PSS. Finally, CaCO3 cores were dissolved with a 0.2 M solution of ethylenediaminetetraacetic acid disodium salt (EDTA).

To incorporate Au NPs and NV centers into the capsule wall (WALL sample), the similar protocol described above was used. For CaCO3 core-template formation, 615 µL of calcium chloride (CaCl2, 1M water solution) were mixed with 2.5 mL of water under magnetic stirring. Then, 615 µL of Na2CO3 (1M, water solution) were added to the solution under vigorous stirring at 1000 rpm for 30 sec at room temperature. Next, after deposition of first two polyelectrolyte layers PSS/PAH, 400 µl of NV centers and 400 µl of Au NPs were added to the CaCO3 particles. The obtained architecture of polymer shell was PSS/PAH/NV centers + Au NPs/PSS/PAH/PSS/PAH. Finally, CaCO3 cores were dissolved by a 0.2 M EDTA.
The 4',6-diamidino-2-phenylindole (DAPI) was loaded into the capsules cavity via postloading method [12]. For this, 500 μL of 5 mg/mL DAPI aqueous solution were mixed with capsules and shaken for 2 h at 65 °C. Then the loaded capsules were washed twice and redispersed in 1 mL of Milli-Q water.

2.3. Cells
Murine melanoma cell line (B16-F10 cells) was obtained from the American Type Culture Collection.

2.4. Simultaneous visualization and temperature measurements of synthesized capsules
Temperature measurements inside living cells were realized using ODMR method under continuous wave (CW) microwave excitation [15]. The experimental setup was based on a home-built confocal microscope modified with microwave (MW) and time correlated single photon counting (TCSPC) modules, developed MW resonant antenna and Mercury Arc lamp. The MW resonant antenna was specifically designed for ODMR of NV centers to provide the resonance frequency at around 2.87 GHz. It is designed as a planar ring coil with a circular hole in the middle, inside of which resonant magnetic fields are concentrated [16]. The parameters of the microwave resonant antenna were numerically optimized using CST Microwave software. During ODMR measurements, the microwave driving and laser pumping occur simultaneously. For Au NPs heating and NV centers excitation, we applied quasi-CW laser (repetition rate RR=80 MHz) at 532 nm that was selectively focused on capsules with spot size 1.2 μm.

3. Results
Two different capsule configurations (CORE and WALL) modified with Au NPs and NV centers were fabricated. Morphology of the obtained capsules was measured with Scanning Transmission Electron Microscopy (STEM) (Figure 1). As determined from STEM images, an average diameter of capsules was around 5 μm.

![Figure 1. STEM images of capsules modified with NV-centers and Au NPs](image)

For in vitro experiments, B16-F10 cells were seeded into confocal cell imaging dishes at amount of 5.0 x 10⁴ cells per dish and left overnight. Next day, capsules loaded with DAPI were added to the cells at the ratio 1:10. Cells were washed twice with PBS to remove non-associated capsules. Afterwards, nanothermometry and photoinduced drug release was performed.

Firstly, the critical power and the temperature of capsule rupture was estimated. The temperature of capsule rupture was the same for the both sample types and it was equal to 128 ±1.12°C. Interestingly, there was a correlation between localization of Au NPs and the power, which corresponds to the capsule decomposition. For CORE sample the critical power was 1.59 mW (145 mW/cm²). In the case of WALL sample, the critical power corresponded to 2.55 mW (230 mW/cm²). It can be explained by the close proximity of Au NPs in the CORE sample, which caused higher heat release due to absorbance of the greater amounts of light energy. The ODMR spectra corresponding the temperature of carriers rupture...
and the temperature dependence on laser power are represented in Figure 2A, B. Intracellular photoinduced delivery of DAPI during temperature monitoring was observed with a fluorescence microscope. Because of the small laser spot, which was 1.2 μm, capsules could be opened selectively, without heating the whole cell. It is worth mentioning that the various applied powers of a pulse laser at 80 MHz (12.5 ns between two pulses) are low enough for the Au nanoparticles to cool down in between pulses, which allows avoiding potential side effects resulting from phototoxicity and overheating. After laser irradiation at critical power, capsule rupture led to the release of DAPI through the endo/lysosomal compartments of cells and staining of a cell nucleus (Figure 2C). It proves the ability of the developed system to deliver various cargos and perform the real-time temperature monitoring, which preserves cells from the excessive heating.

Figure 2. A – The ODMR spectra corresponding to critical temperature of capsule rupture. Points – experimental data. Line – Lorentzian fitting. Points that are greater than 1 are part of statistical error during measurements. The inset picture shows numerical simulation of the field localization in MW resonant antenna during ODMR measurements. B – Measured temperatures in capsule samples during laser treatment at various powers. C - Overlay (fluorescence + brightfield channel) images of cells with released DAPI from capsules after laser irradiation. Cells with DAPI-loaded capsules (CORE) were irradiated with laser (λ = 532 nm, 1.59 mW or 145 mW/cm²) and DAPI staining (in blue) of cell nuclei is observed. Scale bars correspond to 5 μm.

4. Conclusion
In this work, we demonstrated the real-time temperature monitoring with an accuracy ±1.12 °C and thermally induced non-invasive cargo release from polymer capsules in living cells. Carriers were developed by loading of NV centers and Au NPs into capsules synthesized by a layer-by-layer approach. We found out the correlation between Au NPs localization and their required power for carriers rupture. Obtained results presented in the current study can be applied for the site-specific photorelease in vivo.
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References
[1] I. Koryakina, D. S. Kuznetsova, D. A. Zuev, V. A. Milichko, A. S. Timin, and M. V. Zyuzin,
“Optically responsive delivery platforms: From the design considerations to biomedical applications,”
Nanophotonics, vol. 9, no. 1. De Gruyter, pp. 39–74, Jan. 01, 2020, doi: 10.1515/nanoph-2019-0423.
[2] J. Choi et al., “Probing and manipulating embryogenesis via nanoscale thermometry and
temperature control,” Proc. Natl. Acad. Sci. U. S. A., vol. 117, no. 26, pp. 14636–14641, Jun. 2020,
doi: 10.1073/pnas.1922730117.
[3] Y. Kamei et al., “Infrared laser-mediated gene induction in targeted single cells in vivo,”
Nat. Methods, vol. 6, no. 1, pp. 79–81, Dec. 2009, doi: 10.1038/nmeth.1278.
[4] H. Yukawa et al., “A quantum thermometric sensing and analysis system using fluorescent
nanodiamonds for the evaluation of living stem cell functions according to intracellular temperature,”
Nanoscale Adv., vol. 2, no. 5, pp. 1859–1868, May 2020, doi: 10.1039/d0na00146e.
[5] L. Shen, T. R. Xie, R. Z. Yang, Y. Chen, and J. S. Kang, “Application of a dye-based
mitochondrion-thermometry to determine the receptor downstream of prostaglandin E2 involved in the
regulation of hepatocyte metabolism,” Sci. Rep., vol. 8, no. 1, pp. 1–12, Dec. 2018, doi:
10.1038/s41598-018-31356-y.
[6] Y. Yue and X. Wang, “Nanoscale thermal probing,” Nano Rev., vol. 3, no. 1, p. 11586, Jan.
2012, doi: 10.3402/nano.v3i0.11586.
[7] G. P. Zograf et al., “All-Optical Nanoscale Heating and Thermometry with Resonant
Dielectric Nanoparticles for Controllable Drug Release in Living Cells,” Laser Photon. Rev., vol. 14,
no. 3, p. 1900082, Mar. 2020, doi: 10.1002/lpor.201900082.
[8] T. Bai and N. Gu, “Micro/Nanoscale Thermometry for Cellular Thermal Sensing,” Small,
vol. 12, no. 34, pp. 4590–4610, Sep. 2016, doi: 10.1002/smll.201600665.
[9] V. M. Acosta, E. Bauch, M. P. Ledbetter, A. Waxman, L. S. Bouchard, and D. Budker,
“Temperature dependence of the nitrogen-vacancy magnetic resonance in diamond,” Phys. Rev. Lett.,
vol. 104, no. 7, p. 070801, Feb. 2010, doi: 10.1103/PhysRevLett.104.070801.
[10] G. Kucsko et al., “Nanometre-scale thermometry in a living cell,” Nature, vol. 500, no. 7460,
pp. 54–58, Jul. 2013, doi: 10.1038/nature12373.
[11] Y. Zheng, X. Zhong, Z. Li, and Y. Xia, “Successive, Seed-Mediated Growth for the
Synthesis of Single-Crystal Gold Nanospheres with Uniform Diameters Controlled in the Range of 5–
150 nm,” Part. Part. Syst. Charact., vol. 31, no. 2, pp. 266–273, Feb. 2014, doi:
10.1002/ppsc.201300256.
[12] A. R. Muslimov et al., “Biomimetic drug delivery platforms based on mesenchymal stem
cells impregnated with light-responsive submicron sized carriers,” Biomater. Sci., vol. 8, no. 4, pp.
1137–1147, Feb. 2020, doi: 10.1039/c9bm00926d.
[13] T. Zhang et al., “Hybrid nanodiamond quantum sensors enabled by volume phase transitions of
hydrogels,” Nat. Commun., vol. 9, no. 1, pp. 1–8, Dec. 2018, doi: 10.1038/s41467-018-05673-9.
[14] Y. V. Tarakanchikova et al., “Layer-by-Layer-Assembled Capsule Size Affects the
Efficiency of Packaging and Delivery of Different Genetic Cargo,” Part. Part. Syst. Charact., vol. 38,
no. 2, p. 2000228, Feb. 2021, doi: 10.1002/ppsc.202000228.
[15] G. Petrini et al., “Is a Quantum Biosensing Revolution Approaching? Perspectives in NV-
Assisted Current and Thermal Biosensing in Living Cells,” Adv. Quantum Technol., vol. 3, no. 12, p.
2000066, Dec. 2020, doi: 10.1002/qute.202000066.