DESIGN OF SELF-PROPELLING JANUS NANOIMPELLER AS A NANOMACHINE FOR TARGETING AND DESTRUCTION OF PATHOGENIC MICROORGANISMS

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ABSTRACT. The design and ability of a self-propelled Janus nanoimpeller is presented as a nanomachine for targeting and destroying pathogen microorganisms as gram negative Escherichia coli 0157:H7 in natural aqueous media. The nanomachine was fabricated from mesoporous silica nanoparticles with an average diameter of about 90 nm as a platform for coating one of the hemisphere sides with a thin nano-gold layer. The mesoporous silica was chosen as a transparent material for photo-control and spectroscopic monitoring with appropriate pore sizes. Its core structure allowed transport and release of some organic compounds (Rhodamine 6G). On the other hand, the gold nano-layer enabled to conjugate chemically with cysteine amino acid. Thus, the nanomachine could readily target and specifically identify the pathogenic E. coli through biological recognition due to the occurrence of electrostatic interaction with the bacterial membrane proteins.

Keywords: Janus nanoimpeller, light-activation, pathogenic bacteria targeting

ПРОЕКТИРАНЕ НА САМОХОДЕН ЯНУС НАНОИМПЕЛЕР КАТО НАНОМАШИНА ЗА НАСОЧВАНЕ И УНИЩОЖАВАНЕ НА ПАТОГЕННИ МИКРООРГАНИЗМИ

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РЕЗЮМЕ. В този доклад е представен дизайна и способността на самоходен Янус наноимпелер, като наномашина за насочване и унищожаване на патогенни микроорганизми, като грам отрицателната Escherichia coli 0157:H7 в природни водни среди. Наномашината беше изработена от мезопорести силициеви наночастици със среден диаметър около 90 nm, като платформа за покриване на едната полусфера с тънък нано-слой от злато. Мезопорестия силициев диоксид беше избран, като прозрачен материал за фотоконтрол и спектроскопски мониторинг с подходящи размери на порите. Неговата ядрена структура позволява транспортиране и освобождаване на някои органични съединения (Rhodamine 6G). От друга страна, нанослоят от златно покритие дава възможност да се конюгира химически с аминокиселината цистеин. По този начин наномашината лесно може да се насочи и да иденстифицира спефифично патогенната E. Coli, чрез биологично разпознаване, поради възникващото електростатично взаимодействие с бактериалните мембранни протеини.

Ключови думи: Янус наноимпелер, *светлинно-активиране, насочване към патогенни бактерии*

Introduction

A widely known approach for preparation of self-propelling nanomachines involves asymmetrically coated catalytic nanoparticles (Yi et al., 2016). Such two-faced nanoparticles (NPs) are named Janus (in honour of the two-faced Roman god Janus) and they typically consist of two hemispheres with different elemental composition and surface chemistries on the opposite sides (Lattuada, Hatton, 2011). The Janus design allows diverse chemical and physical functionality of NPs, especially the induction of catalytic propulsion in liquid media (Yang, Loos, 2017). Thus, the nanomachines designed for self-propulsion motion on Janus principle must comprised also of asymmetric catalytic and non-catalytic faces on their surface. The decomposition of fuel molecules (for example H_2O_2 to H_2O and molecular oxygen) on the catalytic face generate a propulsive force that drives the nanomachine motion (Ke et al., 2010). The first active movement of Janus particles was invented by using asymmetric Pt-coated polystyrene (Howse et al., 2007) and Pt-coated silica microspheres (Gibbs, Zhao, 2009). It was proved that the coating of one hemisphere of mesoporous silica nanoparticle with elemental Pt is sufficient to achieve a self-propulsion force of the fabricated nanocomposite in dilute hydrogen peroxide fuel solution. As mentioned above, the reason for movement is due to the catalytic decomposition of the peroxide molecule (used as fuel) into oxygen molecules and water. In all experiments the obtained propulsion velocity increased with raising the peroxide fuel concentration. However, if magnetic nanoparticle is coated on the one side by platinum (named as magnetic Janus nanomachine) the motion can be guided by use of external magnetic field in biological media instead of the toxic peroxide. In this case, the magnetic field might influence not only the direction of the Janus nanomachine but also on its speed (Baraban et al., 2013), which allowed reverse direction of the movement too. The bimetallic Janus spheres with Pt and Au coating on the opposite sides can move in aqueous solution at speeds comparable to bimetallic nanowire motors (Wheat at al., 2010). Further miniaturisation of their spherical diameter, along with biocompatibility open new fields of biotechnological

applications. In addition, higher efficient propulsion can be achieved by opening of a stomatocyte ("nozzle") that serves as an outlet for the generated molecular oxygen. The self-propelling motion of such stomatocyte nanomotor is controlled by both bubbles propulsion and self-diffusiophoresis, which can operate even at very low fuel concentrations (Wilson et al., 2013). The ability for trajectory navigation of these nanomachines and for regulation of their speed is of high importance with respect to the biotechnological application and bacterial targeting. The guiding of Janus nanomachines motion to their target destinations without the use of externally applied fields is essential for the research progress of modern nanomotor science. Another alternative approach is to achieve an autonomous movement through following the concentration gradient of different signalling chemicals (Ebbens et al., 2012). Nevertheless, such external stimuli as temperature, light and/or electric potential can be also used for motion triggering and speed regulation. For example, the temperature control is an attractive method for NPs speed regulation (Balasubramanian et al., 2009). As an example, the thermally induced acceleration or deceleration might reflect on the primarily heat-induced changes of the NPs solution viscosity. By this approach a wide range of NPs speeds can be generated through simply tuning the applied temperature. The speed of artificial nanomachines can be modulated as well as accelerated also via local heating by laser irradiation (Liu et al., 2013). Upon the laser power the propulsion speed displayed a clear linear dependence, the process is reversible and it can be repeated continuously with high reproducibility. Another promising biotechnological application of the Janus nanomachines based on the properties explained above is targeting and drug delivery to single cells, imaging probe or molecular biosensing of the internal cell components. By this approach, a single Janus nanoparticle with incorporated multiple compartments can be exploited to detect and destroy various types of pathogenic cells. The purpose of this report is to develop Janus nanoimpeller for detection and inhibition of *Escherichia coli*. In our study mesoporous silica nanoparticles were coated on the one hemisphere side with a thin nano-gold layer in order to obtain a Janus like nanoimpeller, which was capable to target and destroy E. coli. For this purpose, template silica nanoparticles can be used as a convenient platform for coating with gold and attaching of biomolecules that might undergo large amplitude motions. The mesoporous silica NPs are easy to synthetise and such mesostructured particles are transparent materials (for photo-control and spectroscopic monitoring). They can be fabricated into nanoimpellers with useful morphological properties with respect to the designed pore sizes and structures. The cargo transport and release inside the mesoporous silica NPs can be controlled by photoinduced cis-trans isomerisation of diazo bonds (-N=N-) of various azobenzene derivatives which are tethered to the interiors of the mesopores (Angelos et al., 2007). In this report, upon the continuous excitation with blue light (475 nm) the nanoimpeller enabled the cis- and trans-isomers of diazo bonds to be in constant isomerisation reaction at the mesoporous interior. This caused a dynamic wagging of the untethered terminus and impelled the cargo molecules through the pores of mesoporous silica. In addition, it was proven that the transport control can be made to occur in a dynamic manner in the nanomachines with size less than 100 nm containing 2-3 nm diameter pores. The obtained mesoporous structure enabled also a high drug loading capacity as well as time and light irradiation-depending drug release. The pores might be sealed by a gatekeeper system which could be also used for additional functionalisation with ligands and improvement of the whole specific characteristics.

Experimental Procedures

Materials and analytical instrumentation

All chemicals used in the protocols below were of analytical-reagent grade. UV-VIS spectroscopy was performed on UV-VIS Jasco analytical spectrophotometer (model No V-570) using 1 cm quartz cuvette. The zeta potential of NPs was measured using a ZetaPALS Zeta Potential Analyzer (Brookhaven Instrument Corporation). Nanoparticle size distribution was determined using dynamic light scattering (DLS, Zetasizer Nano ZS) and transmission electron microscope (TEM). TEM micrographs of nanoimpellers and E. coli 0157:H7 bacteria were taken by JEOL JEM-3100FFC TEM at 300 kV accelerating voltage equipped with Hilbert differential contrast (HDC) phase plate. The collected labelled bacteria cells were dropped on a copper micro-grid coated with amorphous carbon film (20 nm in thickness). The liquid suspension was removed by a filter paper and E. coli cells were rapidly frozen in liquid ethane by rapid freezing device (Leica Microsystems, Germany). The frozen cells were kept in a liquid nitrogen for a while. Finally, the frozen labelled E. coli were transferred and observed in the transmission electron microscope by cryo-transfer system.

Synthesis of mesoporous silica nanoparticles (MSN)

MSN were synthesised based on a modified synthesis by using tetraethyl orthosilicate (TEOS) as a precursor reagent for condensation of silica, and of different other template additives, such as cetylmethylammonium bromide (CTAB) surfactant, polymers, micelle forming agents or dopants. In brief, the surfactants were stirred in a mixture of ultrapure MilliQ water and ethyl alcohol under basic conditions, and TEOS or other silicates were added under agitation of the reaction suspension. The silica sources concentrations and compositions, as well as the template-agents and stirring conditions determined the nanoparticle size, pore diameter and shape. When the surfactant precursor concentration was above the critical micelle concentration, CTAB is self-aggregating into micelles and the silica reagents condensate at the surface. Thus the silica structure was formed around the surface of obtained micelles. Then, the surfactants were completely removed through centrifugation to obtain biocompatible mesoporous silica nanoparticles which were further coated with gold and modified with diazobenzene derivatives and conjugated cysteine amino acid (as capping ligands). The obtained common pore diameter distribution of MSN ranges between 2 and 5 nm. The nanoimpeller pores were saturated with the organic dye Rhodamine 6G.

Deposition of gold nanolayer on MSN

The used electroless metal plating technique for Au deposition on MSN spheres includes three steps (Kobayashi et al., 2005). The first step is surface sensitisation of the silica spheres. The second step is surface activation and the final third step is gold plating. The obtained Janus nanoparticles

were washed and kept in a refrigerator in order to be used in next experiments.

Surface functionalisation of the Janus nanoimpeller with cysteine capping on the gold hemisphere and detection of E. coli

Stock solution of cysteine was prepared in CH_3COONa buffer (0.01 M sodium acetate, pH 10). The immobilisation of cysteine amino acid on the surface of gold coated hemisphere was done by mixing the biomolecule solution with NPs in a 1: 1 ratio and leaving the solution to react for 24 hours at ambient temperature. During the reaction there was a visible colour change of the mixed solution. After that the suspension was subjected to ultracentrifugation in order to separate the nanoparticles with the unreacted cysteine in the suspension. The culture of E. coli 0157:H7 was prepared as follows: 50 μl of the anabiotic bacterial suspension was inoculated into 5 ml M9 medium at 37°C for 4h to reach the exponential growth phase.

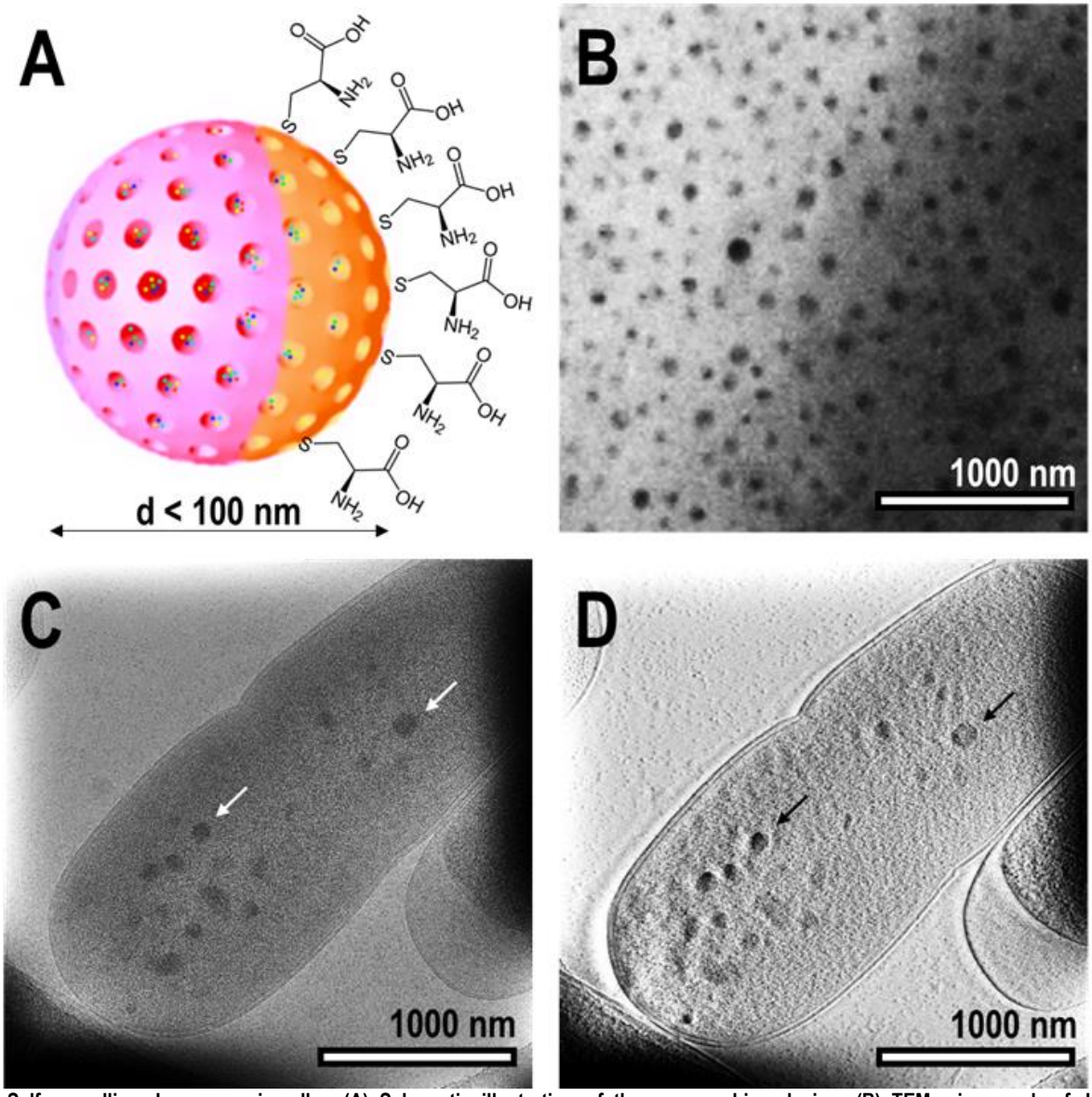

Fig. 1. Self propelling Janus nanoimpeller. (A) Schematic illustration of the nanomachine design. (B) TEM micrograph of Janus nanoimpellers on graphite carbon film with 20 nm thickness. (C) TEM micrograph of cryo-embedded E. coli labelled with Janus nanoimpellers (as shown with the arrows). (D) Same object observed with phase-plate Hilbert-differential contrast TEM in order to obtain higher contrast image; scale bar for B, C and D = 1000 nm (1 m)

Results and discussion

Characterisation of the design of Janus nanoimpeller as a functionalised nanomachine for self-propulsion and drug delivery system

The design of fabricated two-faced Janus nanoimpeller is shown on Fig. 1A. This self-propelling nanomachine was functionalised with amino acid cysteine on the gold hemisphere for targeting pathogenic microorganisms. The obtained bonds between the nanoimpeller surface coated with a gold nanolayer and the cysteine ligands shell were covalent through the thiol groups from the amino acid residue. The photo transparent mesoporous core of silica can be loaded with various payload materials (in our experiment with Rhodamine

6G) and capped with the so-called gatekeeper (azobenzene derivatives). The fabricated nanoimpeller particles possess spherical geometric shapes (Fig. 1B) with an average diameter of about 90 ± 8 nm as confirmed with TEM observation and the measurement with dynamic light scattering. The pores inside the nanoparticle core have much smaller diameter with average distribution range between 2 and 5 nm, which enabled to be saturated as a drug delivery system with various organics (in the current experiment as mentioned above with the photosensitizer Rhodamine 6G). The surface plasmon resonance (SPR) peak maximum of the gold coated hemisphere was red-shifted due to the conjugation with cysteine amino acid residues. Under excitation with blue laser diode (InGaN) at 475 nm (for about 10 min) and 9 mW energy dose the azobenzene motion was excited. The organic dye Rhodamine 6G was released in the analysed solution and its amount was measured as a plot function of absorbance maximum and concentration. As a control experiment the nanoimpeller particles were irradiated with equal power but at wavelength of 650 nm at which there was not any absorption of the azobenzene group. In the control experiment there was no releasing of organic dye. These data proved unambiguously that the Janus nanoimpeller responds only to the wavelength that drives the photo-induced cis-trans isomerisation and motion of the azobenzene group, which was tethered to the interiors of the nanomachine mesoperes. The releasing of dye molecules inside the nanomachine mesopores upon continuous excitation was in irradiation-depending manner, which demonstrates an "impeller" mechanism of operation of the designed Janus nanoimpeller. The reason is because the excitation of the azobenzene on the nanoparticle surface caused the Rhodamine 6G dyes molecules to wag back and forth and thus effectively imparted the motion of trapped organic compounds in the drug delivery system. This physicochemical process allows them to traverse the pore interior until they escape in the bacterial suspension solution. These data proved the drug-releasing ability of the nanomachine to be controlled by the laser irradiation dose and wavelength.

Bio-detection ability of the nanomachine for targeting and destruction of gram negative pathogenic microorganisms

To demonstrate the bio-detection ability of the Janus nanoimpeller as a functionalised nanomachine it was determined whether the capping amino acid cysteine ligands on the gold hemisphere surface facilitate targeting and anchoring of the nanomachine onto bacterial cell membrane surface. When the functionalised Janus impeller is added in a bacterial suspension of E. coli 0157:H7 shifting of the plasmon absorption peak to longer wavelength region (so called red-shifting effect) can be measured. The reason for the occurred SPR optical effect is that the nanomachine can readily target and specifically identify E. coli through biological recognition due to the electrostatic interaction between amino acid charges and bacterial membrane proteins. It was also found that the degree of resulted aggregation onto the cell membrane is completely dependent on the nanoparticle concentration. Analytical cryo- TEM analysis was performed in order to prove the hypothesis of biological recognition occurrence and anchoring of functionalised Janus nanoimpeller on the gram negative E. coli bacteria (as shown on Fig. 1C). As shown on the TEM micrographs the nanomachines have come closer to one another on the cell membrane, which is a clear indication of the occurred electrostatic forces of bio-recognition interactions. To obtain higher contrast imaging of the objects the same, ice-embedded cells were observed and analysed also with 300 kV phase-plate Hilbert-differential contrast TEM (Fig. 1D). On this highly-contrasted image the discrete ultrastructure of bacteria without any preliminary chemical preparation could be observed. The microscopic analysis demonstrated that the nanomachines are attached to the cell membrane but they have not entered inside the cell cytoplasm. Under photo-controlled conditions with laser irradiation the nanomachines can release their payload and generate reactive oxygen species, which have a lethal effect on the pathogenic bacteria.

Conclusion

We have designed and fabricated a functionalised Janus nanoimpeller as a self-propelling nanomachine for targeting and destruction of gram negative pathogenic microorganisms. For that purpose, the optically transparent mesoporous silica nanoparticles were loaded with the photosensitizer Rhodamine 6G and their hemispheres were coated with a gold nano-layer. The release of the organic dye can be controlled by photoinduced isomerisation of the azobenzene molecules, which were tethered to the interiors of the mesoporous silica. The process can be controlled by excitation with blue light. The proposed design opens a new field for development of multifunctional nanomachines for inhibition of pathogenic microorganisms. The reported Janus impeller might find numerous applications in the field of biosensors technology, mineral biotechnology and environmental protection.

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References

Angelos, S., E. Choi, F. Vögtle, L. De Cola, J. Zink. I.2007. Photo-driven expulsion of molecules from mesostructured silica nanoparticles. – *J. Phys. Chem*. C 111, 6589–6592.

Balasubramanian, S., D. Kagan, C. M. Hu, S. Campuzano, M. J. Lobo-Castanon, N. Lim, D. Y. Kang, M. Zimmerman, L. Zhang, J. Wang. 2011. Micromachine enables capture and isolation of cancer cells in complex media. – *Angew. Chem. Int. Ed*., *50*, 4161–4164.

Baraban, L., D. Makarov, O. G. Schmidt, G. Cuniberti, P. Leiderer, A. Erbe. 2013. Control over Janus micromotors by the strength of a magnetic field. – *Nanoscale,* 5, 1332–1336.

Ebbens, S. J., G. A. Buxton, A. Alexeev, A. Sadeghi, J. R. Howse. 2012. Synthetic running and tumbling: an autonomos navigation strategy for catalytic nanoswimmers. – *Soft Matter,* 8, 3077–3082.

Gibbs, J. G., Y. P. Zhao. 2009. Autonomously motile catalytic nanomotors by bubble propulsion. – *Appl. Phys. Lett*., *94*, 163104–163107.

Howse, J. R., R. A. L. Jones, A. J. Ryan, T. Gough, R. Vafabakhsh, R. Golestanian. 2007. Self-motile colloidal particles: from directed propulsion to random walk. – *Phys. Rev. Lett., 99*, 048102.

Ke, H., Y. Shengrong, R. L. Carroll, K. Showalter. 2010. Motion analysis of self-propelled Pt-silica particles in hydrogen peroxide solutions. – *J. Phys. Chem*. A 114, 5462–5467.

Kobayashi, Y., Y. Tadaki, D. Nagao, M. Konno. 2005. Deposition of gold nanoparticles on silica spheres by electroless metal plating technique. – *J. Colloid and Interface Sci., 283*, 601–604.

Lattuada, M., T. A. Hatton. 2011. Synthesis, properties and application of Janus nanoparticles. – *Nano Today,* 6, 286–308.

Liu, Z., J. Li, J. Wang, G. Huang, R. Liu, Y. Mei, 2013. Small-scale heat detection using catalytic microengines irradiated by laser. – *Nanoscale,* 5, 1345–1352.

Wheat, P. M., N. A. Marine, J. L. Moran, J. D. Posner. 2010. Rapid fabrication of bimetallic spherical motors. – *Langmuir 26*, 13052–13055.

Wilson, D. A., B. van Nijs, A. van Blaaderen, R. J. M. Nolte, J. C. M. van Hest. 2013. Fuel concentration dependent movement of supramolecular catalytic nanomotors. – *Nanoscale,* 5, 1315–1318.

Yang, Q., K. Loos. 2017. Janus nanoparticles inside polymeric materials: interfacial arrangement toward functional hybrid materials. – *Polym. Chem*., 8, 641–654.

Yi, Y., L. Sanchez, Y. Gao, Y. Yu. 2016. Janus particles for biological imaging and sensing. – *Analyst, 141*, 3526–3539.