ULTRASOUND-PROPELLED BIOMIMETIC NANOROBOT FOR TARGETING AND ISOLATION OF PATHOGENIC BACTERIA FROM DIVERSE ENVIRONMENTAL MEDIA

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ABSTRACT. The artificial nanomachines have been demonstrated with their useful applications in the fields of bioanalytical diagnostics and environmental remediation. We designed ultrasound-propelled nanorobot, which was functionalized with cell membrane for targeting and isolation of pathogenic bacteria (e.g. *Escherichia coli*) from complex and diverse environmental media. Since the artificially prepared device was covered with cell membrane, i.e. membrane receptors were immobilized on its surface the resulted nanoscale invention was known as biomimetic nanorobot. It displayed rapid detection ability and prolonged acoustic propulsion in aqueous solution. The proposed design opens the possibilities for creation of a broad-spectrum detoxification and remediation robotic platforms.

Keywords: Biomimetic nanorobot, ultrasound-propelling, pathogenic bacteria isolation

БИОМИМЕТИЧЕН НАНОРОБОТ ЗАДВИЖВАН ЧРЕЗ УЛТРАЗВУК ЗА НАСОЧВАНЕ И ИЗОЛИРАНЕ НА ПАТОГЕННИ БАКТЕРИИ ОТ РАЗНООБРАЗНИ ПРИРОДНИ СРЕДИ

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РЕЗЮМЕ. Изкуствените наномашини са демонстрирани с техните полезни свойства в области, като биоаналитична диагностика и екологично възстановяване. Ние проектирахме нанороботот, задвижван от ултразвук, който беше функционализиран с клетъчна мембрана за насочване и изолиране на патогенни бактерии (например *Escherichia coli*) от комплексни и разнообразни природни среди. Тъй като изкуствено конструираното устройство беше покрито с клетъчна стена, т.е. мембранните рецептори бяха имобилизирани на повърхността му, полученото изобретение беше известна, като биомиметичен нанороботот. Той показа способност за бърза детекция и продължително акустично задвижване във воден разтвор. Предложеният дизай отваря възможности за създаване на широкоспектърни роботизирани платформи за детоксикация и ремедиация.

Ключови думи: Биомиметичен нанороботот, ултразвуково задвижване, изолиране на патогенни бактерии

Introduction

The development of artificial nanomachines and nanorobotics with wide range of applications has raised substantially during the last decade (Loukanov et al., 2019). Nowadays the micro- and nanorobots are already commonly used in numerous and diverse biotechnological processes (Bogue, 2010). However, a critical issues is still their way of powering and locomotion in terms of bio-application. Most of them can be actuated through various chemical methods for propulsion, which require energy from proceeding of chemical reactions in the solution (Yang et al., 2019). So in all these cases the environment must contain fuel (e.g. hydrogen peroxide, acid, etc.), which often is harmful for the living organisms (Soto & Chrostowski, 2018). Due to this reason, the efforts were focused on the research, where the triggering and control of nanorobots might occur in fuel free environment. The most references in this novel scientific field have reported about the externally powered nanodevices with magnetic force (microswimmers) or ultrasound, e.g. highly-porous nanomotors (Xu eat al., 2017). The ultrasound has been found as especially promising way to drive nanomachines in biological fluids or media, because of its deep penetration and less harmful effect to the tissues. The ultrasound-propelled nanodevices can be built from gold nanowires and then propelled by the induced ultrasound mechanical waves (Garcia-Gradilla et al., 2014). The gold nanostructures have also received considerable interest due to their attractive plasmonic, catalytic and thermal properties (Lee et al., 2018). If biomaterial as proteins, receptors or plasma membrane is immobilized on their surface, they can be considered as robust biomimetic nanorobots with multipurpose programmed applications (de Ávila et al., 2018).

The purpose of current report was to design ultrasound-propelled biomimetic nanorobot for removal of pathogenic bacteria from broth environment. Our nanorobot was fabricated from acoustic gold nanowires. Their surface was coated with monoclonal antibodies, which inherently mimic the surface properties of the cells that have been used as a source. In such a way, our resulted biomimetic nanorobots possess the advantages of both functional biomolecules of cellular membrane, as well as the dynamic movement induced by the ultrasound propulsion. The presented integration resulted also in broader and more robust application in respect to the complicated and complex tasks performance. This statement was proved by our programmed hybrid fuel-free nanorobot, which was able to simultaneous target and removal the pathogenic *E. coli* through ultrasound and adhesion, thankful to its functionalized layer of monoclonal antibodies. *Escherichia coli* O157:H7 was chosen in the conducted experiments as a model and target pathogenic bacterium, because it is known and well-characterized microorganism (Lim et al., 2010). The advantage of coupling between the biological function of antibodies and the ultrasound fuel-free navigation in our design enables a dynamic and multifunctional platform that might represent a unique tool for movement and manipulation of biological objects at cellular level. In addition, all processes in the sample occurred for relatively short time, which was measured within minutes. And last but not least, the designed nanorobot might be considered as a robust technique for potential use in different biological fields, including immune modulation, drug delivery, cellular imaging and detoxification.

Materials and methods

Reagents and materials

Commercial gold plating solution (Technic Inc., Anaheim, USA) was used for fabrication of the nanorobot. (3-Mercaptopropionic acid used for coating of the gold nanowires was purchased from Sigma-Aldrich. Methylene chloride, ethanol and isopropanol were purchased from WAKO. The PC membrane template was purchased from Whatman Nuclepore (catalog № 110407). It contained 400 nm in diameter nanopores. The nanorobot was functionalized with monoclonal antibody (anti-E. coli O157:H7) purchased from Meridian Life Science, Inc (Saco, ME). The acoustic cell consisted of a piezoelectric transducer (Ferroperm), which generated ultrasound waves. 4′, 6-diamidino-2-phenylindole (DAPI) was used as a fluorescent stain for quantitative measurement of the cells by photoluminescence spectrophotometer (Jasco analytical photoluminescence UV–VIS, model No FP-6300). Aqueous solution of 2 % uranyl acetate $UO_2(CH_3COO)_2$ was used as a reagent for the negative staining of the samples observed in the transmission electron microscope.

Nanorobot fabrication

The gold nanowires were prepared by a common electrodeposition method. For that purpose, thin film of elemental gold was sputtered on porous PC membrane template. After that the membrane was assembled in a plating cell (Teflon) with Al foil. The gold was plated and the resulted sputtered layer was removed by mechanical polishing. Thus gold wire nanorobot was created with concave shape and average size distribution between 2 – 7 μm. PC membrane residues were dissolved in methylene chloride and the nanorobots were separated from the mixed solution through centrifugation and washing with isopropanol and ethanol. The resulted precipitation was stored in ultrapure water at 4°C. The functionalization of the nanorobot with monoclonal antibody (anti-E. coli O157:H7) was done in phosphate-buffered saline (PBS) solution. The gold nanowires were first coated with 0.20 mM 3-mercaptopropionic acid (MPA) for overnight and then reacted with monoclonal antibody in a buffered solution through conjugation reaction. The fabricated biomimetic nanorobot were mixed with bacterial suspension (taken at the mid log phase) and were activated by ultrasound (frequency of 2.60 MHz and 2.0 V amplitude).

Bacterial culture

Escherichia coli O157:H7 was used for the performance of ultrasound-propelled biomimetic nanorobot. The bacterial colonies were stored –70°C and cultivated on trypticase soy agar plates (BD Bioscence, MD). A single colony was inoculated in tryptic soy broth medium (BD Bioscience, MD) and grown for 24 hours at 37°C. At the mid log phase 100 L of the microbiological suspension was transferred to a fresh broth media and incubated for 6 hours at 37°C before labeling experiment.

Transmission electron microscopy observation and analysis of the attached E. coli

The attached bacteria were removed from the suspension by centrifugation. They were washed with PBS solution to avoid the formation of artifacts and dropped on a micro-grid coated with amorphous carbon film. The buffer drop was removed with filter paper and the sample was frozen rapidly in a cryogenic storage (Loukanov et al., 2018).

Results and discussion

The biomimetic nanorobot was fabricated by using of electrochemical deposition protocol for template-assisted gold nanowires (Garcia-Gradilla et al., 2013). Its surface was modified with MPA before coating with monoclonal antibodies. It was characterized by transmission electron microscope. As shown on the micrograph (Fig. 1) the nanorobot diameter is about 400-500 nm and length up to 6000-7000 nm. Its shape is asymmetric, which is a condition for the ultrasound propulsion. The presented geometrical asymmetry enables to the gold nanowires to convert the acoustic steady streaming into axial motion with independent trajectory, as shown on Fig. 1A. Another key factor which affect the acoustic energy conversion into propulsion is the material density and its dimension. The elemental gold is relatively dense material, and therefore, the designed size is enough large to achieve autonomous propulsion in agreement with the low Reynolds number (Jarell & McBride, 2008). Thus, the acoustically propelled biomimentic nanorobot demonstrated a synergistic effect of enhancing both mass transport and directional collisions with the target bacteria. The thin coating of nanorobot with monoclonal antibody (as shown on the scheme Fig. 1B) was observed by TEM observation (Fig. 1C). The protein contained layer coated on the gold surface was visible due to the negative staining of the sample with uranyl acetate. The presence of protein contents was also proved qualitatively by ninhydrin test, which reacts with the amino groups of amino acid residues. The protein coating might remain conjugated on the nanorobot for relatively long period of time (at lease 5-6 months) if stored in refrigerator at 4°C.

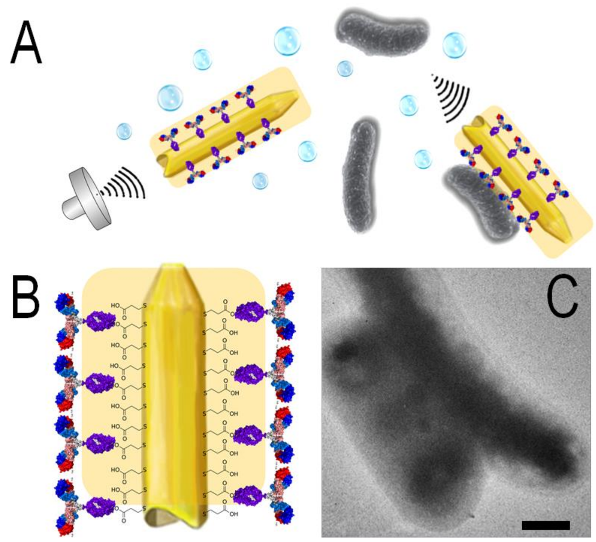

Fig. 1. Illustration of the way of operation of the ultrasound-propelled biomimetic nanorobot (A). Schematic of the nanorobot modified with MPA and coated with monoclonal antibody layer (B). TEM images of pathogenic E. coli O157:H7 attached on the nanorobot surface

As demonstrated, the presented mobile nanorobot enables both enhanced binding to pathogen in broth sample and its effective neutralization. The presence of monoclonal antibody enables selective targeting, effective adhering or binding of the pathogen to the nanorobot and its following isolation from the microbial environment. The whole process occurred in a short contact time (i.e. within 5-10 min incubation) and 2.60 MHz ultrasound (2.0 V). If the nanorobot is applied without coating with antibodies as control experiment, there was absence of the process of bacterial adhesion in the suspension. This was demonstrated by fluorescent staining (by DAPI) of the bacterial cells and measuring of the overall normalized photoluminescence intensity of the isolated cells in suspension by spectrophotometer. As shown on Fig. 2 the absence of bacteria on the bare nanorobot (in control experiment) was displayed as a negligible fluorescent intensity. In the case of antibody coated nanorobot the photoluminescence intensity was remarkable high, which is indication for successful occurred processes of biorecognition and binding.

All results demonstrated the unique characteristic of the designed ultrasound-propelled biomimetic nanorobot to achieve selective and rapid isolation of the pathogens microorganisms. However, the coating with monoclonal antibodies opens also other diverse biological function applications, which could be demonstrated with binding of existing toxins in the bacterial environment. The ability our nanorobots for absorbing and neutralization of toxins is still under investigation. The reason is that the monoclonal antibody is not able to bind the toxins molecules and accomplish their removing in the manner as the bacterial cells. Nevertheless, the possibilities for future accelerated detoxification of various produced toxins in real environment could be another important application of the designed ultrasound propelled nanorobotics.

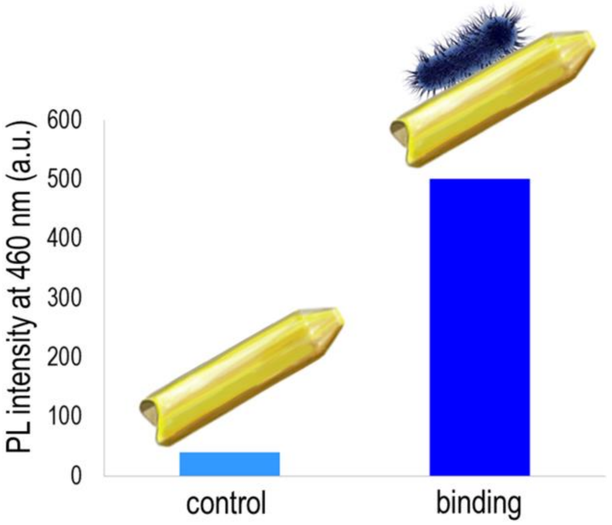

Fig. 2. Normalized photoluminescence intensity at 460 nm of DAPI-stained E. coli O157:H7 retained on a bare nanorobot (left side as control) and antibody-coated biomimetic nanorobot (right side, binding experiment)

Conclusion

Since the acoustic propulsion is used as a model of fuel-free propulsion, the reported nanorobot can be used for other diverse applications.

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