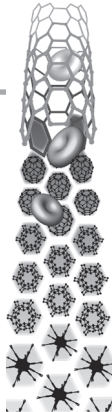


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Molecular machines drive smart drug delivery

“With nanoparticles already functioning at the subcellular level, the possibility of incorporating different molecular machineries onto their surfaces opens the door to a wide variety of intracellular possibilities.”

KEYWORDS: azobenzenes ■ drug delivery ■ mesoporous ■ molecular machines ■ nanopellers ■ nanoparticles ■ nanovalves ■ rotaxanes

In his historic lecture to the American Physical Society in 1959, ‘There is plenty of room at the bottom’, Richard Feynman, one of the greatest scientists of the 20th Century, shared a futuristic vision with his audience using molecular machines in medicine. He contemplated, “What would be the utility of such (molecular-scale) machines? ... Although it is a very wild idea, it would be interesting in surgery if you could swallow the surgeon. You put the mechanical surgeon inside the blood vessel and it goes into the heart and ‘looks’ around. It finds out which valve is the faulty one and takes a little knife and slices it out. Other small machines might be permanently incorporated in the body to assist some inadequately functioning organ” [1]. As of today, the small machine-powered surgeon predicted by Feynman has yet to be realized; however, the idea of using molecular-level machines in nanomedicine is burgeoning just as predicted [2,3]. Thus far, scientists have made significant progress in developing molecular-machine-driven, mesoporous nanoparticle-based smart drug delivery systems (DDS) that are capable of timely, on-demand drug release. By combining the robustness of mesoporous nanoparticles and the preciseness of molecular machines, these smart DDS hold great promise for future nanotechnology-based medical therapy and treatment.

Functionalizing mesoporous nanoparticles with molecular machines

Mesoporous nanoparticles have long been recognized as appealing materials for DDS, as they have the ability to encapsulate a payload of therapeutic compounds and transport them to specific locations throughout the body. The stable, rigid frame of mesoporous nanoparticles allows for resistance to pH, degradation and

mechanical stress. The diameters of the pores can be tuned between 2 and 10 nm to allow for different drug loadings. Compared with traditional oral or injectable drugs, these mesoporous nanoparticles can enhance bioavailability, improve therapeutic efficacy and minimize side effects [4]. At present, the key challenge in developing mesoporous nanoparticle-based DDS is designing the structure and surface properties of these nanoparticles to enable the targeted, on-demand release of drug molecules.

Molecular machines (e.g., rotaxanes, pseudorotaxanes and azobenzenes) are capable of delivering efficient actuations at dramatically reduced length scales when compared with traditional microscale actuators [5–10]. Their ability to precisely and cooperatively control mechanical motions at the molecular level, in addition to their comparable sizes to the mesopores of nanoparticles, makes molecular machines prime candidates for controlled release systems in mesoporous nanoparticle-based drug delivery. Molecular machines are enabling timed and controlled release of therapeutic compounds from the mesoporous nanoparticles upon various forms of external stimuli, such as chemical reduction/oxidation, light, magnetic fields, enzymes and changes in the surrounding pH [11]. This area of research is swiftly maturing with the progress in synthesis, surface functionalization, operation and characterizations of molecular machines.

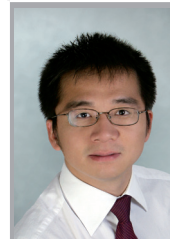
To fulfill their potential in DDS, molecular machines must retain the switching capability and full range of mechanical motion when transferred from solution to nanoparticle surfaces. Extensive research on molecular machines assembled on metallic nanoparticles reveals multiple effects of surface confinement on the switching and motions of molecular machines,

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including steric effects, excitation quenching and altered redox potentials [11]. While much can be gleaned from these results, further research on the effects of immobilization to non-metallic, porous nanoparticles should be undertaken to systematically evaluate all alterations, so that proper designs can be used to permit and enhance switching properties. Plasmonic quenching is perhaps the most significant issue facing metallic nanoparticle systems; however, most molecular-machine-powered DDS generally involve nonmetallic particles that do not support plasmon resonance, so the quenching effect will be of diminished consequence. The effects of ligands and surface conditions on redox potentials will be of greater importance, as they can alter the switching conditions for both rotaxanes and pseudorotaxanes, two widely used molecular machines in nanoparticle-based DDS. The modifications to the functionalities of molecular machines at nonmetallic mesoporous nanoparticle surfaces must be thoroughly understood and designed around before the molecular-machine-powered DDS can fulfill its potential.

Operating molecular-machine-powered DDS in abiotic conditions

Abiotic demonstration is often the first step in the development of novel biomedical technologies. With the recent progress in the synthesis of molecular machines, control of surface chemistries and surface characterization, researchers have demonstrated a series of molecular-machine-powered smart DDS under abiotic conditions. These systems have proven capable of on-demand drug delivery by employing molecular machines as nanovalves, nanoimpellers, or both, in organic solvents.

Nanovalves represent an important development along the path to smart drug delivery; they present the opportunity to transport materials without loss and release them on demand. In acting as nanovalves, molecular machines generally make use of the combination between a stalk and a capping agent [2]. Generally, the stalks are covalently attached to the pore openings, with the pores themselves being large enough to contain common drug molecules, yet small enough to be blocked by the capping agents (e.g., the macrocyclic organic molecules, such as the cyclodextrins). Under various forms of external stimuli, the capping agents experience mechanical motions, effectively opening and closing the pores and regulating the release of the encapsulated drug. Development in this research

direction has demonstrated molecular-machine-powered DDS with a series of external stimuli (e.g., optical, chemical and electrochemical) [11].

Compared with chemical and electrochemical stimuli, light has several advantages as an external and noninvasive method for actuating the nanovalves in DDS [12]. Light is abundant, clean and can be transmitted to molecules without physically contacting them, in contrast to chemical stimuli that require the addition of fresh reactants at every step of the working cycle, and result in the concomitant formation of waste products. With light, the energy input can be carefully controlled with the wavelength and intensity of the exciting photons to regulate the drug release. Brinker and coworkers have used *cis-trans* photoisomerization properties of azobenzenes to make such light-switchable nanovalves [13]. In the *cis* form, produced under ultraviolet (UV) radiation, the pendant molecules on the internal walls of the pores are shorter and leave an open channel. Upon restoration to the *trans* form using heat or green light, the molecules revert back to a pore-blocking state. In another important work, Stoddart, Zink and coworkers have devised switchable molecular valves based on pseudorotaxanes for nanoparticle drug delivery. The pores in these nanoparticles are closed by the ring-like components of the pseudorotaxanes at the pore openings; drug release is accomplished by the light-triggered removal of the ring-like component [11].

“Recently, much effort has been dedicated to moving research on molecular-machine-powered drug delivery systems from abiotic demonstrations to biological uses.”

Nanovalves have proven effective in regulating the release of drug molecules that naturally diffuse out of the mesopores; however, some drug molecules have a larger affinity with the pores that prevent their natural diffusion. In this case, forces are needed to drive the drug out of the mesopores. The nanoimpeller is a molecular device that can provide just this type of force. Zink and coworkers developed molecular nanoimpellers based on azobenzene derivatives tethered to the mesopore interiors [3]. By irradiating the system at the isosbestic point, the *cis-trans* photoisomerizations result in continual motion of the molecules, making them work like an impeller to propel the drug molecules out of the pores. By controlling the arrangement of azobenzenes, switching efficiency and switching dynamics, the drug releasing process can

be regulated. Furthermore, nanovalves can be added into the nanoimpeller system at the mesopore openings to provide an additional independent mechanism for control over drug delivery. The dual-controlled nanoparticles operate with an AND logic and allow refined control of the release dynamics [14].

One of the most common obstacles in the application of photoresponsive molecular machines in biomedical applications is the requirement of UV stimulation. UV radiation is limited to use in peripheral tissues where penetration depth and lack of transparency are not as significant. To circumvent this limitation, next-generation nanovalves and nanoimpellers must be triggered by near-infrared light that penetrates deeper into human tissue and thus increases the potential working area for the new delivery systems [15,16]. Furthermore, near-infrared radiation can avoid the cytotoxic effects of UV light due to the low-power photoirradiation under biocompatible and physiological conditions.

Moving the research from abiotic demonstrations to biological uses

Although significant progress has been made in molecular-machine-powered DDS that operate in abiotic conditions, *in vivo* applications of these systems remain the ultimate goal. The complexity of biological systems and their response to the molecular-machine-functionalized nanoparticles present great challenges to the development of molecular-machine-powered DDS for biological uses. To develop such systems, collaborated research efforts are required from multiple disciplines, including biology, medicine, chemistry, physics and engineering, to gain insights into a range of issues from molecular synthesis to system design.

Recently, much effort has been dedicated to moving research on molecular-machine-powered DDS from abiotic demonstrations to biological uses. The use of cellular stimuli, which exploit the physiological processes within a cell to affect the motion of molecular machines and ultimately the release of a drug, constitutes a significant milestone in molecular-machine-powered DDS. This process allows the DDS to operate autonomously, requiring no external stimuli. The rotaxanes, which consist of triethylene glycol chains threaded by α -cyclodextrin tori, have been attached to mesopore openings of nanoparticles for enzyme-controlled drug delivery [2,17]. The bulky stoppers, which serve to hold the tori in place, are stable under physiological conditions, but are cleaved by

the catalytic action of an enzyme, causing the dethreading of the tori and release of the guest molecules from the pores of the nanoparticles. The flexibility in choosing the stoppering unit, incorporated in the final step of the synthesis, makes it possible for the system to target any number of hydrolytic enzymes.

Another biologically inclined DDS is based on the operation of molecular nanovalves that are tightly closed at physiological pH (7.4), but capable of opening in acidifying cellular compartments. In the system, β -cyclodextrin rings (as capping agents) encircle aromatic amines (as stalks) as a result of noncovalent bonding interactions under neutral pH conditions, effectively blocking the mesopore openings. A decrease in the pH leads to protonation of the aromatic amines, followed by β -cyclodextrin cap release and drug molecule diffusion from the mesopores. The operation of this autonomous nanosystem was demonstrated successfully in human differentiated myeloid (THP-1) and squamous carcinoma (KB-31) cell lines [18].

Prospects, challenges & opportunities

In the past decade, the developments in molecular-machine-powered DDS mark substantial steps in the progression toward biological and clinical use. Flexibility in molecular design and synthesis has helped produce various molecular machines for DDS applications with a wide range of stimuli. Advanced tools in surface functionalization and characterization are enabling researchers to engineer the switching dynamics of molecular machines on nanoparticle surfaces for specific release profiles of drugs in response to biological conditions [11]. Despite these recent developments, several major obstacles need to be overcome before molecular-machine-powered DDS is fit for clinical applications. For example, biocompatibility of both the nanoparticles and molecular machines is of primary concern in these delivery systems. Work has been carried out with biocompatible stalks and caps in pseudorotaxanes, and the formation of biocompatible complexes in molecular machines holds great promise for future development in total system biocompatibility. The ultimate utility of these systems will be proven as the research moves *in vivo* and the pharmacology of the systems is thoroughly examined. It will also be important to control the dynamics of the drug delivery. While it has been characterized for most current systems, control over the release rates remain fairly novice. To effectively deliver

a wide variety of drugs, it will be necessary to have variable release rates from impulses to slow, steady release. In addition to dynamic control over a full range of delivery times, further demonstrations and refinements in logical control, which utilize multiple inputs to control delivery, will result in greater efficiency with reduced collateral damage.

The beauty of molecular nanotechnology resides in its promise to manipulate materials at the atomic level. While individual atom-by-atom manipulation does not yet exist, it is possible to work at the molecular and nanoscopic levels to create the precisely controlled molecular machines for DDS with unprecedented timed/controlled drug release, therapeutic efficiency and biocompatibility. With nanoparticles already functioning at the subcellular level, the possibility of incorporating different molecular machineries onto their surfaces opens the door

to a wide variety of intracellular possibilities (e.g., gene therapy, proteomics and individual organelle treatment). Molecular machines seem to be driving smart drug delivery toward biological and clinical viability with a full head of steam, but this research must rely on the continued efforts and harmonic collaboration of the bright minds in all related fields to achieve complete success.

Financial & competing interests disclosure

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