Constructing Tunable Nanopores and Their Application in Drug Delivery

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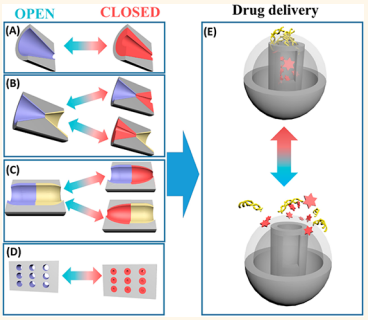
ABSTRACT Inspired by biological cell membranes, various "smart" and efficient gating nanoporous devices have been proposed to imitate and to understand life processes. Nanodevices under development with enhanced gating efficiency could play pivotal roles in biosensing and drug delivery. In this Perspective, we highlight an important development by Willner and colleagues that is detailed in this issue of *ACS Nano*. They designed a new "smart" nanodevice with both "sense" and "release" functionalities for drug delivery based on a nanoporous material, mesoporous silica nanoparticles. We outline recent progress in designing intelligently gated nanoporous devices in material science and nanotechnology. We also summarize new strategies designed for drug delivery based on mesoporous materials. With continuing efforts, we expect more powerful nanodevices to be developed and used in clinical and other real-word applications.



The biological cell employs ion channels located in the cell membrane to communicate chemically and electronically with the extracellular world or with subcellular compartments. Ion channels implement their physiological functions during life processes *via* controlling the flow of ions across cell membranes, which include ionic selectivity, ionic rectification, and ionic gating.[1] Nanogating devices that have the ability to simulate the functions of biological ion channels would be valuable for applications in materials science and drug delivery.

Specifically, solid-state nanopores and nanochannels equipped with biological or synthetic molecules are emerging as an intensely studied field, and these systems provide highly efficient means of controlling ionic or molecular transport in response to pH,[2,3] light,[4] and temperature.[5] It should be noted that "nanopore" is defined simply as a nanoscale pore having a diameter larger than its depth, whereas "nanochannel" means the pore depth is much larger than the nanoscale diameter.[6]

Interestingly, tunable nanopores can also be used to cap and to release drugs. Nanomaterials that can encapsulate free drug to overcome limitations such as poor solubility and stability, unwanted toxicity, and/or the inability to cross cell membranes, afford fascinating therapeutic capabilities. In this context, mesoporous silica nanoparticles (MSNs) have attracted much attention in therapeutics due to their high long-term stability, high surface area, ease of synthesis, availability in porous forms, and easy surface modification.[7] Because common causes of treatment failure include low drug concentration at the tumor or nonspecific targeting and releasing, it is critical to develop approaches to increase uptake efficacy of MSNs and to enhance selectivity for drug delivery. In this Perspective, we highlight recent progress made in "smart" nanodevices and drug-delivery systems based on MSNs, and focus on the following two keys aspects: (1) constructing "smart" nanopores and (2) tuning the nanopores to release drugs efficiently and selectively.

"Stimuli-Responsive" Single Nanopore. In recent years, solid-state nanopores have emerged in order to overcome the limitations of nanopore channels that are made from proteins, which depend on lipid membranes. For example, Jiang and colleagues demonstrated a strategy to gate a synthetic nanopore reversibly by switching DNA motors (Figure 1A).[2] The DNA molecule motor, which is sensitive to pH, was immobilized inside the nanopore. In a low-pH solution, the motor DNA folds into a densely packed rigid quadruplex i-motif structure

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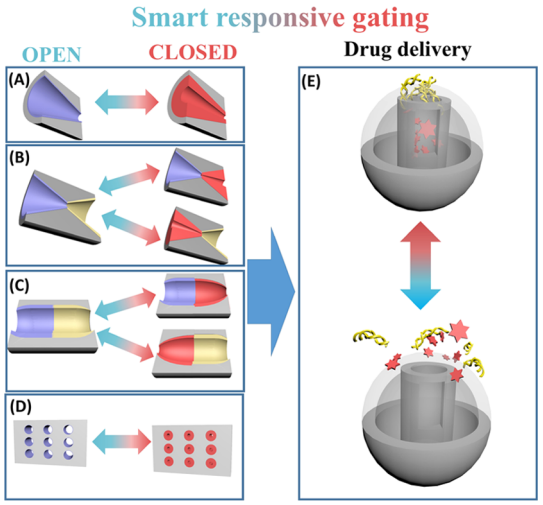

Figure 1. Schematic representations of biomimetic single nanopore (A); asymmetric responsive single nanochannel system (B); bioinspired functional single ion pump (C); multiple nanopores (D). (E), Drug-delivery system based on mesoporous silica nanoparticles.

that partially decreases the effective diameter of the nanopore, resulting in a high conductance state (on-state). At high pH, the motor DNA relaxes to a loosely packed single-stranded and more negatively charged structure that enhances the total ion conductivity inside the nanopore, leading to a low conductance state (off-state).

In this Perspective, we highlight recent progress made in "smart" nanodevices and drug delivery systems based on mesoporous silica nanoparticles.

In another study, Brinker and colleagues prepared a photoresponsive nanoporous membrane based on azobenzene ligand-modified monosized pores.[4] In this system, alternating UV and visible light exposure controls the photoisomerization state of the azobenzene ligands. Meanwhile, the conformation of azobenzene ligands tunes the effective pore size, and thus the change of oxidative current can be measured.

Guo *et al.* show that a temperature-responsive ionic current rectifier can be realized by attaching poly(*N*-isopropylacrylamide) [PNIPAM] into single, gold-coated conical nanopores, which can be switched between a rectifying state below 34 °C and a nonrectifying state above 38 °C.[5] When the temperature is below 34 °C, the dehydration of the attached PNIPAM brushes strengthen the rectifying capability, and the nanopore is in the rectifying state. When the temperature is above 38 °C, the PNIPAM brushes have sufficiently collapsed, and the nanopore switches to the nonrectifying state.

To endow artificial nanodevices with more functions and make them "smart" like a biological ion channel, Jiang and colleagues developed an asymmetric responsive single nanochannel system, which provides both pH- and temperature-tunable properties (Figure 1B).[8] In this dual-responsive single nanochannel, there is a negative correlation between the ionic current rectification ratio and the temperature with varying pH. The temperature-responsive capabilities can be reduced by increasing the pH, while the pH-responsive capabilities are also reduced by increasing the temperature.

More recently, with the purpose of achieving high-level intelligent ion transport features in biological ion pumps, Jiang and colleagues reported a cooperative pH response double-gate nanochannel (Figure 1C).[3] This artificial, single-ion pump has three essential features that are like those of biological ion pumps: an alternating gate ion pump, reversible transformation of the ion pump into an ion channel, and a fail-safe ion pump. These "smart" characteristics are achieved by continuous switching of the symmetric/asymmetric pH stimuli.

"Stimuli-Responsive" Multiple Nanopores. Although substantial progress has been achieved in gating synthetic single nanopores, efforts to construct efficient multiple nanopores continue. For example, in order to expand the work environment of nanodevices, Jiang and colleagues developed a temperature-responsive nanodevice based on solid-state nanopores embedded in anodic aluminum oxide (AAO) membranes, which can work in room-temperature ionic liquids.[9] They tiled the solid-state nanopores with poly(benzyl methacrylate) (PBzMA) molecular brushes, and demonstrated that the transport of the organic charge carriers through the nanopores can be controlled by the conformational change of the PBzMA brushes.

As a distinctive nanoporous material that has two defined surfaces, mesoporous silica nanoparticles are good candidates for "smart" drug delivery.

Enhancing the gating efficiency and signal-to-noise ratios is still required because the DNA gatekeepers are permeable to small ions, which

TABLE 1. Summary of the Mesoporous Silica Nanoparticle-Based Drug Delivery Systems Discussed in This Perspective[a]

emphasized functionality	stimulus	trigger	interaction	ref
Reversible	Photo	UV light	UV light-thymine	14
Controlled release	Redox	glutathione	glutathione — S—S	20
RT monitoring	Redox	glutathione	glutathione — S—S	23
Biocompatibility	Ionic	pH	pH-multiamine chains	16
selective targeting	Antibody	Antibody	Antibody—antigen	17
MDR reversion	Peptide	cRGDyK peptide	Peptide—integrin	18
selective targeting	Surface receptor of cancer cells	Folate	Folate—folate receptor	19
Both "sense" and release	Apatamer	ATP	ATP—ATP amptamer	21, 22

[a] DTT = dithiothreitol; S—S = disulfide bond; RT = real time.

would significantly reduce the resolution of the resistive-pulse sensing. For this purpose, gating devices based on multiple channels and aptamer-assisted super-sandwich structures are emerging.

Aptamers isolated from the systematic evolution of ligands by exponential enrichment (SELEX) are single-stranded oligonucleotides.[10] They have higher affinity to their targets such as proteins, metal ions, and other molecules than to their complementary DNA, which make them promising tools for a variety of important applications. Recently, Jiang *et al.* developed an efficient, smart gating system based on ATP molecules—ATP aptamer and its complementary DNA—which exhibited extremely high ON—OFF ratios (up to ~106) and nearly perfect electric seals (~GΩ) in its closed state (Figure 1D).[11] The open-to-closed process is achieved by self-assembling super-sandwich structures consisting of an ATP aptamer and its complementary DNA into solid-state nanochannels, while the closed-to-open process is realized by the disassembly of ATP—ATP aptamer binding. Additionally, Liu *et al.* developed hybrid nanoporous biosensors with the sensitivity and selectivity to detect DNA and ATP simultaneously through self-assembly and disassembly of supramolecular DNA nanostructures in nanopores.[12] A detection limit of 10 fM for target DNA and 1 nM for ATP can be realized using this strategy.

Tuning the Nanopores To Release Drugs Efficiently and Selectively. As a distinctive nanoporous material that has two defined surfaces, an internal one and an external one, MSNs are good candidates for "smart" drug delivery. "Smart" drug delivery represents the ability to control both the rate and the location in the body of the drug release. The development and application of controlled-release systems increase the overall efficacy of drugs *via* maintaining the drug level within the optimum therapeutic range and under the toxicity threshold. To date, various stimuli have been applied to release drugs based on MSNs (Table 1).

Photoresponsive drug-delivery systems, which use light of suitable wavelengths to trigger drug release, are of interest due to their noninvasive nature and the high spatiotemporal resolution of light. For example, Fujiwara and colleagues developed a reversible photocontrolled release system by attaching coumarin derivatives onto the pore entrances of MSNs.[13] However, the hydrophilicity and biocompatibility of the above system is not sufficient for drug delivery applications. Zhao and colleagues designed a reversible, photoresponsive molecular-gated system based on the surface modification of mesoporous silica nanoparticles with thymine.[14] In this system, the lock of the pore is realized by the dimerization of thymine monomers induced *via* irradiation of thymine. The opening of the pore is achieved by the photocleavage of the cyclobutane dimer *via* 240 nm UV light irradiation.

In another study, Feng and colleagues developed a redox-responsive drug delivery system based on poly(*N*-acryloxysuccinimide)-grafted mesoporous silica.[15] The guest can be locked by cystamine, which can crosslink the polymer chain around the pore opening, and can be released by the disulfide-reducing agent dithiothreitol, which can cleave the disulfide linker of the polymeric network.

In this issue of *ACS Nano*, Willner and colleagues present a new ATP aptamer capping and release mechanism for drug delivery based on mesoporous SiO_2 nanoparticles, which has the functionalities to both "sense" and "release."

Additionally, a pH-responsive drug-delivery system *via* grafting propyldiethylenetriamine groups (multiamine chains) on MSNs was prepared by Deng and colleagues.[16] Multiamine chains come close to each other through hydrogen bonding interactions at high pH, and tend to stay as far away from each other as possible at low pH. In the proposed delivery system, when pH was about 7.5, MSNs released drugs rapidly and completely;, whereas when pH was about 4.0, only 13 wt %

of the drug was slowly released from MSNs.

Nevertheless, in most of the above-mentioned methods, a large amount of the administered drug is directed to those body sites that are sensitive to the toxic effects of the drug. The lack of selectivity of drug delivery often causes severe side effects and decreases the concentration of the drug in the tumor site, which affects the treatment efficacy of the cancer. This highlights the need for strategies to target MSNs to cancers. Up to now, various targeting biomolecules, such as antibodies, peptides, aptamers, or others (folate) have been used for selective drug delivery and therapeutics.

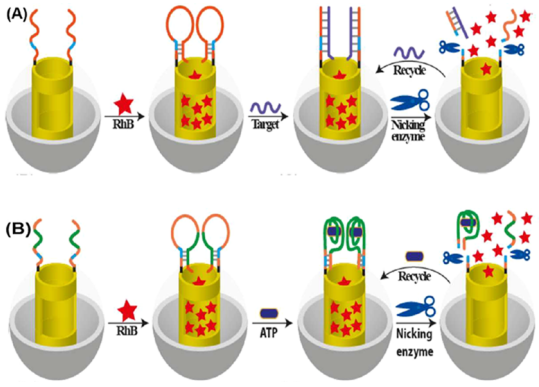

Figure 2. "Smart" DNA-gated mesoporous SiO_2 nanoparticles possess both "sense" and "release" functionalities. (A) Unlocking the DNA pore-capping units is achieved by using an analyte–DNA biomarker as an activator for opening the hairpins, while regenerating the DNA–biomarker with the Exo III. (B) Unlocking the DNA pore-capping units is achieved by the formation of an ATP–aptamer complex, while regenerating the APT-biomarker with the Exo III. Reprinted from ref 22. Copyright 2011 American Chemical Society.

Antibodies with remarkably high binding affinities and selectivities for targets of interest have been used to increase the specificity of the therapeutic agent and, thus, minimize side effects while maximizing desired effects. Amorós and colleagues described a controllable delivery system based on antibody–antigen specific interactions.[17] They functionalized the pore outlets of MSNs with a certain hapten that was able to interact with an antibody to block the guest release. In the presence of the antigen to which the antibody is selective, the pores opened and the enwrapped guest was released as a result of a highly effective displacement reaction.

Peptides, another low cost, low immunogenicity, smaller-sized variety of ligands, have recently attracted more attention. Their small sizes could couple nicely with the optimized physicochemical properties of nanoparticles, and also make them easy to conjugate with MSNs. Lo and colleagues reported tri-functionalized MSNs for traceable imaging of particle targeting, photodynamic therapy (PDT), and targeted delivery to cancer *via* cRGDyK peptides.[18] cRGDyK peptides bind selectively to avb3 integrin, which is overexpressed in tumor metastatic and endothelial cells. In this method, with receptor-mediated endocytosis, cRGDyK peptide was tiled on the outmost surface of MSNs and efficiently focused MSN delivery, minimizing MSN uptake by healthy cells, which may trigger deleterious side effects.

Specific cellular targeting can be achieved by employing ligands that have the capability to bind selectively to the cell surface and trigger receptor-mediated endocytosis. Recently, Lindén and colleagues developed selective MSNs that were modified by poly(ethylene imine) and conjugated with folic acid to target cancer cells specifically.[19] They demonstrated that the total number of such particles internalized by the cancer cells was about an order of magnitude higher than the number internalized by normal cells. In comparison with normal cells, cancer cells overexpress the folate receptor, which makes folic acid emerge as an interesting targeting ligand for selective delivery. In addition, Yang and colleagues immobilized collagen (Col) on the exterior surface of MSNs by disulfide bonds to block the guest, and introduced lactobionic acid (LA) with a galactose group to achieve receptor-mediated endocytosis.[20] The cell-expressed glutathione can cleave disulfide bonds, releasing the guest from MSNs. They demonstrate that the LA-Col-linker-MSNs displays efficiently cell-specific intracellular drug delivery and cellular uptake properties. For example, the endocytosis efficiency of the LA-Col-linker-MSNs is around 3 or 2 times higher than that of naked MSNs after 2 and 4 h, respectively. In addition, the number of internalized LA-Col-linker-MSNs by HepaG2 cells is 2 and 2.2 times higher than that of endothelial cells after incubation for 2 and 4 h, respectively.

It is worth noting that aptamers can be used for therapeutic purposes in much the same way as antibodies. More importantly, they can be easily modified, are more stable to biodegradation, and can meet the stringent requirements in bioassays better than antibodies. Wang and colleagues designed a stimuli-responsive delivery system based on high affinity and specificity between aptamer and target.[21] In this system, gold nanoparticles modified with ATP aptamers block the pores of MSNs. The guest can be released from MSNs *via* a competitive displacement reaction that results in the separation of gold nanoparticles from MSNs.

Developing "smart" drug delivery systems with multifunctionalities will be beneficial not only for therapeutics but also for diagnosis and clinical work. In this issue of *ACS Nano*, Willner and colleagues present a new ATP aptamer capping and release mechanism for drug

delivery based on mesoporous SiO2 nanoparticles, which has the functionalities to both "sense" and "release" (Figure 1E and Figure 2).[22] They locked the anticancer drug camptothecin (CPT) in MSNs by tailored ATP aptamer caps, and unlocked the pores by ATP-induced rearrangements of ATP aptamer caps that can be digested by Exo III, or a nicking enzyme. After digestion, the ATP regenerates, which makes this amplified sensing process release the CPT continuously. They demonstrate that the number of cell deaths of MDA-231 breast cancer cells is about 2.5 times higher than that of MCF-10a normal breast cells. Moreover, the more ATP that is synthesized in the cancer cells, the higher the CPT-induced death of the cancerous cells.

Another strategy established by Lee and colleagues enables real-time monitoring of drug release to investigate the accumulation of the drugs.[23] They developed redox-responsive fluorescent MSNs to track drug release from the pores of MSNs in the present of glutathione by measuring the change in the fluorescence resonance energy transfer (FRET) signals. Their results have demonstrated that with incremental increases of the glutathione, the quantity of released drugs increased and, thus, enhanced the change in the FRET signals. This real-time monitoring system is promising for direct investigation of drug release kinetics.

CONCLUSION AND FUTURE DIRECTIONS

We have highlighted some exciting biomimetic ion channels and drug delivery systems based on nanoporous materials. "Smart" nanodevice systems show great potential for efficient gate-tuning in response to pH, temperature, light, and some specific biomolecules. In particular, MSNs, which have both internal and external surfaces, show great potential for drug delivery due to their biocompatibility, large load capacity, and ability to engage in stimulated release. Although these results are encouraging, new breakthroughs are still needed. For example, endowing nanopores with more sensitive and efficient gating abilities will benefit real-world applications, such as biosensing. Constructing "smart" MSNs with multifunctionalities is required. Although selectivities have been improved, side effects remain, which highlights the need for strategies to avoid delivering drugs to normal cells or tissues. Another important issue to be addressed is how to apply those promising MSNs to advance from preclinical research into clinical development.

Conflict of Interest: The authors declare no competing financial interest.

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