Could multiresponsive hollow shell–shell nanocontainers offer an improved strategy for drug delivery?

“In this editorial, we report a new concept using responsive nanocontainers for drug delivery, namely employing hollow nanogels with two polymer shells that have different volume phase transition temperatures.”

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In this editorial, we report a new concept using responsive nanocontainers for drug delivery, namely employing hollow nanogels with two polymer shells that have different volume phase transition temperatures. The temperature responsive swelling and collapse of the inner shell control the uptake, storage in the void and release of guest (macro)molecules, while the swelling of the outer shell controls the size of the void and the colloidal stability of the nanogels. In other words, the inner shell plays a role of a membrane of variable permeability for the molecules while the outer shell always serves as permeable membrane. These new multishell nanoparticles can be further functionalized. Their different layers may fulfill distinct requirements during transport, uptake and targeted release of pharmaceutical molecules. Therefore, two-shell hollow nanocontainers may have great potential to be used as drug delivery carriers for different applications.

Areas of interest for nanoparticle development are improved local drug release in diseased tissue, intracellular delivery of therapeutic nucleotide acids and improvement of oral bioavailability of large molecule therapeutics. Drugs for the treatment of malignant, infectious or autoimmune diseases such as chemotherapeutic drugs, antibiotics or immunosuppressors can have severe side effects due to toxicity, individual intolerances

or provocation of infectious complications because its systemic effect on the immune system. One of the major problems is the systemic release of the drugs despite most diseases being localized to specific organs or areas of the body. Drug carriers for targeted release can specifically increase drug concentrations within the diseased tissue or cells leading to improved treatment efficacy and less adverse drug effects. Therapeutic DNA or RNA that directly influences gene expression within diseased cells requires specific vectors for intracellular delivery. Nanoparticles offer a great alternative to viral or bacterial vector systems. Several antibiotics and protein drugs such as insulin or therapeutic antibodies (biologicals) have to be administered by the parenteral route. Nanoparticle systems that improve the oral bioavailability of such drugs would facilitate the treatment in an out-patient setting. Thus, the development of nanocontainers, which allow for the uptake, storage and triggered release of functional agents, is one of the key challenges in nanomedicine.

The advantage of multifunctional nanocarriers

There are various tasks that nanocarriers have to fulfill in order to improve oral bioavailability and targeted release. First, drugs need to be protected from the harsh and

Walter Richtering

Author for correspondence:
Institute of Physical Chemistry, RWTH Aachen University, Landoltweg 2, 52056 Aachen, Germany
richtering@rwth-aachen.de

Igor I Potemkin

Author for correspondence:
Physics Department, Lomonosov Moscow State University, 119991 Moscow, Russian Federation
and
DWI-Leibniz Institute for Interactive Materials e.V., 52056 Aachen, Germany
igor@polly.phys.msu.ru

Andrey A Rudov

Physics Department, Lomonosov Moscow State University, 119991 Moscow, Russian Federation
and
DWI-Leibniz Institute for Interactive Materials e.V., 52056 Aachen, Germany

Gernot Sellge

Department of Internal Medicine III, University Hospital RWTH Aachen, Pauwelsstr. 30, 52074 Aachen, Germany

Christian Trautwein

Department of Internal Medicine III, University Hospital RWTH Aachen, Pauwelsstr. 30, 52074 Aachen, Germany

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diverse environment during the passage of the GI tract (e.g., pH between 2 and 10, digestive enzymes, different salt concentrations). Second, drugs have to cross several barriers with very different behavior such as the intestinal mucosa consisting of a mucus layer and a tight cellular epithelial monolayer, the endothelium of the blood vessels to reach and exit the systemic blood circulation and cellular membranes for drugs with an intracellular mode of action. Third, nanoparticles must confer colloidal stability within the blood in order to allow sufficient transport and prevention of embolic complications in small vessels. Fourth, nanoparticles should preferentially enrich in diseased tissue or cells. Fifth, the drug cargo should be preferentially released in diseased tissue or cells.

“...microgels have the ability to adapt their size and shape to environmental requirements and enable controlled size selective mass transfer.”

Specific physicochemical conditions at organ barriers, cellular surfaces and within diseased tissue such as differences in pH, salt concentration, charge, redox potential, protease activity and different pore sizes as well as external stimuli such as light, heat and cold can be exploited as triggers for nanocarrier uptake and cargo release [1,2]. Furthermore, coating of nanoparticle surfaces with specific ligands enhances interaction with cellular membranes or extracellular matrices and therefore facilitates targeted barrier crossing and cellular uptake [3].

Nanocarriers based on stimuli-responsive polymers in different architectures as, for example, dendrimers, micelles and nanohydrogels, and employing a variety of stimuli for the release as, for example, temperature, light, pH, ultrasound or magnetic field have been developed [4–11]. The development of drug carriers that guarantee the stability of the drug and, in addition, respond to multiple stimuli may largely improve targeted drug release [3]. An example for dual-functional drug carriers is provided by Mao *et al.* [12]. They developed dual pH-responsive polymeric micelles for drug release. At weakly acidic environment at pH approximately 6.5 in tumor environment (physiological pH is 7.4) the surface charge of the micelles reversed from negative to positive facilitating binding to positively charged cellular membranes and subsequent cellular uptake. At pH <5.5 present in intracellular phagolysosomes the drug is released owing to the cleavage of imine bonds. This dual pH-responsive feature may improve the ability to precisely deliver chemotherapeutic drugs to cancer cells [12]. Shan *et al.* reported self-assembled nanoparticles for efficient oral delivery of insulin. The nanoparticles possessed a nanocomplex core composed of insulin and cell-penetrating pep-

ptide, and a dissociable hydrophilic coating of poly(N-(2-hydroxypropyl)methacrylamide) copolymer derivatives. Nanoparticles exhibited excellent permeation in mucus due to the ‘mucus-inert’ poly(N-(2-hydroxypropyl)methacrylamide) copolymer coating, as well as high epithelial absorption mediated by cell-penetrating peptide [13].

Temperature-responsive nanoparticles may be used for targeted drug release in combination with hyperthermia therapy. Radiofrequency ablation (RFA) directly induces cell death by high frequency radio waves that are applied by a needle electrode into skin or liver tumors. Mild local hyperthermia applied by microwaves, high frequency ultrasound or infusion of warmed liquids does not induce cell death by itself but renders tumor cells more responsive to radiation or chemotherapy. Temperature-responsive nanocarriers for local release of chemotherapeutic drugs may improve RFA efficiency by improving killing of tumors cells not immediately killed by RFA alone [14] and increase local drug concentration in other forms of hyperthermia therapy [2].

Multiresponsive hollow shell-shell nanocontainers

Nanogels based on responsive polymers provide characteristic features that make them characteristically different from other colloidal and polymeric systems. The combination of being soft and porous while still having a stable structure through the cross-linked network allows for the possibility to introduce orthogonal chemical functionality in different compartments within the microgel [15,16]. In contrast to rigid colloidal nanoparticles, microgels have the ability to adapt their size and shape to environmental requirements and enable controlled size selective mass transfer [17]. Thus nanogels are soft species and can achieve switchable properties that enable adaptability of form and function both in bulk solution and at interfaces [18–20].

Hollow capsules represent a very promising structure for the design of efficient drug delivery systems [4,21]. Drugs could be stored in the void of the hollow particles with the shell protecting them from external influences but also a controlled release of the drugs by external stimuli is possible [22,23]. Hollow nanogels allow combining the typical features of capsules having a void with the adaptability of soft nanogels [24]. Recently we introduced hollow nanogels with two shells of different sensitivity to external stimuli [25]. These two-shell hollow nanocontainers are shown in **Figure 1**. The images are snapshots of the nanogels obtained at different conditions in computer simulations [25]. What makes the nanogels special and why could they offer a new strategy for nanomedicine?

Figure 1. Snapshots of nanogels at different conditions obtained via computer simulations. (Upper row, left-to-right) Procedure of preparation of the hollow double-shell nanogels. (Middle row, right-to-left) Collapse ($T < 42^{\circ}\text{C}$) and swelling ($T < 32^{\circ}\text{C}$) of the inner shell of the nanogel. Immersing of the nanogel with open pores into solution of the (guest) molecules (red dots). (Bottom row, left-to-right) Penetration of the molecules into the nanogel in the solution. Locking of the guest molecules in the void via collapse of the inner shell at $T > 32^{\circ}\text{C}$. Release of the guest molecules into the pure solvent at $T < 32^{\circ}\text{C}$ via swelling of the inner shell.

First of all, the ‘membrane’ consists of two polymer networks that are chemically connected but consist of different polymers. On the one hand this allows separating different relevant functions of the membrane: the responsive swelling of the inner network allow controlling the permeability of the (inner) shell, whereas the swelling of the outer shell provides the colloidal stability. The synthesis of the two-shell nanocontainer is straightforward (shown in Figure 1) and enables a separate and controlled modification of the shell’s properties, for example, the inner shell can be modified via the copolymerization with functional molecules to introduce, for example, specific binding sites. Furthermore, the crosslink density and thus the mesh size and permeability can be controlled. The outer shell provides the colloidal stability and additional possibility to alter the permeability and can easily be modified via the adsorption of macromolecules on nanoparticles. This can occur in a

layer-by-layer fashion [26] or chemically, for example, with binding sites for specific targeting or PEGylation to achieve a stealth effect. An example for a nanogel delivery system of therapeutic siRNA delivery into ovarian carcinomas has been reported by Blackburn *et al.* [27]: they coupled a peptide mimicking the ligand ephrin-A1 to the surface of approximately 100 nm diameter core/shell nanogels harboring high concentrations of siRNA. The peptide ligand binds to its receptor expressed by the tumor cells and permits, therefore, specific targeting and subsequent intracellular delivery of siRNA.

An important aspect of the shell-shell nanocontainers is that two networks are connected. Thus, there is a ‘mechanical’ coupling of the two shells. In other words, the swelling of the outer and inner networks mutually influence each other. This allows the permeability of the inner shell, which can be influenced in two ways: directly by a stimulus that affects the swelling of the

polymer network of the inner shell; and indirectly by a stimulus that affects the swelling of the outer shell. The swelling pressure in the outer network will lead to swelling in the outward direction and consequently will stretch the inner network as well.

In our proof-of-concept study, we employed temperature-sensitive polymers. In particular, we used Poly(*N*-isopropylacrylamide) (PNIPAm), which shows a volume phase transition (VPT) in aqueous solution at about 32°C and poly(*N*-isopropylmethacrylamide) (PNIPMAm), which shows a similar temperature sensitive behavior in water as PNIPAm, but with a slightly higher VPT temperature of about 42°C. The VPT temperatures of these polymers can easily be adjusted by incorporation of functional comonomers [28], which can also be used to introduce an additional sensitivity, for example, to pH [29].

The synthesis of the hollow two-shell nanocontainers followed a straightforward procedure (Figure 1): first, silica nanoparticles were prepared as sacrificial cores. They can be synthesized within a broad range of sizes with very narrow size distributions and can easily be dissolved leading to hollow capsules. Afterward, first a chemically cross-linked shell of PNIPAm was polymerized around the silica core and second a PNIPMAm shell was polymerized around the core-shell nanoparticle. This outer PNIPMAm shell was also cross-linked chemically. Finally, the silica core was dissolved leading to temperature sensitive, hollow two-shell nanocontainers. The structure of these nanocontainers in solution was studied by small-angle neutron scattering employing contrast variation experiments and by computer simulations [25]. Both techniques revealed the unique behavior of the two-shell particles: at intermediate temperatures, the inner polymer network collapses and thus provides a barrier for molecules; the outer shell on the other hand is swollen and provides colloidal stability. The bottom row in Figure 1 corresponds to snapshots of computer simulations, which demonstrate the ability of the nanogels to uptake guest molecules (both shells are swollen), lock and store them in the void (inner and outer shells are collapsed and swollen, respectively) and release the molecules into pure solvent

upon swelling of the inner shell. It is worth mentioning that both shells consist of uncharged polymers and thus tolerate the presence of salt. Therefore, the hollow two-shell nanocontainers at intermediated temperatures are not sensitive to the ionic strength of the aqueous medium. This makes such nanogels distinctly different from, for example, nanocapsules based on layer-by-layer adsorption of polyelectrolytes due to electrostatic attraction. The physical reason for uptake and release of the neutral molecules by the hollow nanogels has entropic nature.

Future perspective

Drug nanocarriers that meet the multiple requirements of storage, transport, uptake and release will be able to more specifically reach the goal of targeted drug release. Therefore, multishell and multifunctional nanoparticles have a great potential because their different layers may fulfill distinct needs.

Two-shell hollow nanocontainers have two membranes that can potentially be further functionalized. The two layers can mediate independent functions, which also could be coupled. Our proof-of-concept polymers alter their size and permeability depending on temperature. This feature could be exploited in combination with local hyper- and/or hypothermia therapy. Further possible developments may include coupling of specific ligands as well as introducing specific responsiveness to pH or osmotic stress. Therefore, two-shell hollow nanocontainers may have great potential to be used as drug delivery carriers for different applications.

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