Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Pharmacological aspects of release from microcapsules — from polymeric multilayers to lipid membranes

Pieter Wuytens[1,2,3], Bogdan Parakhonskiy[4,5], Alexey Yashchenok[5,6], Mathias Winterhalter[7] and Andre Skirtach[2,3,6]

This review is devoted to pharmacological applications of principles of release from capsules to overcome the membrane barrier. Many of these principles were developed in the context of polymeric multilayer capsule membrane modulation, but they are also pertinent to liposomes, polymersomes, capsosomes, particles, emulsion-based carriers and other carriers. We look at these methods from the physical, chemical or biological driving mechanisms point of view. In addition to applicability for carriers in drug delivery, these release methods are significant for another area directly related to pharmacology — modulation of the permeability of the membranes and thus promoting the action of drugs. Emerging technologies, including ionic current monitoring through a lipid membrane on a nanopore, are also highlighted.

Addresses
[1] Photonics Research Group, Department of Information Technology, Ghent University — imec, B-9000 Ghent, Belgium
[2] Department of Molecular Biotechnology, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium
[3] Center for Nano- and Biophotonics (NB-Photonics), Ghent University, Belgium
[4] A.V. Shubnikov Institute of Crystallography RAS, 119333 Moscow, Russia
[5] Institute of Nanostructures and Biosystems, Saratov State University, 410012 Saratov, Russia
[6] Max-Planck-Institute of Colloids and Interfaces, 14476 Potsdam, Germany
[7] Biophysics, School of Engineering and Sciences, Jacobs University of Bremen, 28759 Bremen, Germany

Corresponding author: Skirtach, Andre (Andre.Skirtach@UGent.be)

Current Opinion in Pharmacology 2014, **18**:129–140

This review comes from a themed issue on **New technologies**

Edited by **Gleb B Sukhorukov**

For a complete overview see the Issue and the Editorial

Available online 14th October 2014

http://dx.doi.org/10.1016/j.coph.2014.09.016

Introduction

Successful delivery of pharmaceuticals [1], medicine and action of drugs — hinge upon two interconnected subfields: First, delivery of drugs by carriers [2] and second, assuring the transport to the target [3]. We provide a generalized view of release mechanisms which, on one hand, are acting on delivery carriers, while on the other hand can be used for inducing permeability of the membranes for drug targeting. Polyelectrolyte multilayer capsules not only represent and an excellent model system, but they are directly relevant to biomedical [4] and drug delivery applications [5•,6•,7]. Flexibility of LbL assembly building [8,9] permits for incorporation of various entities, which permit triggering of release, both of organic and inorganic nature into the polymeric shell of capsules.

This review analyzes mechanisms of release from polyelectrolyte multilayer capsules identifying their advantages and disadvantages. Some examples in which application of release methods led to significant developments are highlighted. At the end, we draw a parallel between applicability of the release methods described in this review with their suitability for affecting such alternative carriers as liposomes, polymersomes, particles, emulsion based carriers, films. Applicability of remote release methods is also considered for studying fundamental properties of biomolecules (i.e. application of nanopores to study lipid properties) from which these carriers are made.

Polyelectrolyte multilayer capsules

Only very brief description of capsules is provided here.

Mechanisms of formation and polymer interaction

Although such new methods of polymer assembly as hydrogen bonding [10,11•,12] and non-electrostatic assembly mechanisms [13•] were considered, electrostatics still remains a popular option.

Particles as templates on which capsules are assembled and polymers for assembly

For preparation of polyelectrolyte multilayer capsules different particles (namely silica, melamine formaldehyde (MF), polystyrene (PS), gold nanoparticles, poly(-lactic acid) (PLA) particles, calcium carbonate, calcium phosphate), serving the role of removable templates, are used [14]. Advantages of carbonate based templates [15] include inexpensiveness and porosity. Resent research revealed a way to produce calcium carbonate particles with a defined size from hundreds of nanometer [14] to micrometers [16]. The presence of pores in carbonate particles is used for direct encapsulation of molecules by

adsorption [16–18]. Development of microcapsules of different shapes may be important for bio-applications [19], anisotropicity [20–24] plays a significant role: elliptical particles [25] with controllable loading [26]. Microcapsules have been originally designed on micrometer sized templates, but nanometer scale carriers have been also pursued. Aggregation of capsules is a potential hurdle.

Very different polymers [27] and molecules including those containing charged groups, a variety of proteins, small molecules (under 1 kDa) and dyes, nanoparticles, various nanotubes [28,29], and even nanoplates [30] have been used for formation of polymeric layers and their subsequent functionalization.

Methods of release

Three main types of stimuli: those based on manipulating physical principles, affecting chemical composition or influencing biological reactions [31•] can be used for release [27]. Figure 1 depicts all these stimuli, which in fact provide complementary measures not only for simply inducing release, but also for achieving desired release profiles [32–34].

Release by chemical methods

Locality of the action is essential. All of chemical stimuli carry non-local character: the effects are transferred through a solution and act on the whole network of polymers, thus affecting the state of polymers of the whole capsules as a whole entity.

pH

Among other stimuli, pH [35] of the solution is used to control the interaction between the charges on the oppositely charged polyelectrolytes. Manipulation of the charges takes place through the so-called weak polyelectrolytes, that is those whose charge or degree of protonation changes depending on pH. Bringing in or removing protons around the charged groups results in the build-up of the excess of either positive or negative charges (such a process can be conducted, e.g. by changing pH [36,37] of the surrounding solution [38]). That leads to repulsion of the prevailing charged groups and tends to increase the spacing between polymers. This elegant approach is applied either for encapsulation (by closing the pores of capsules filled-in with encapsulated materials) or for release (by permitting encapsulated materials to leave the interior of capsules at a desired site) [39].

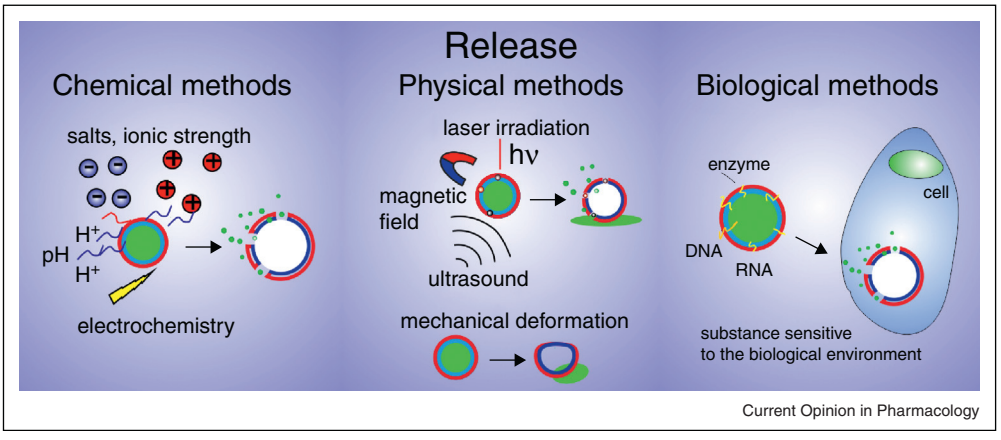
Applications of a pH-responsive release system are attractive *in vivo*. Here, inherent pH values of different organs [1]: stomach (pH varies from 1 to 3), duodenum (pH 5–8), colon (pH ~ 8), blood (pH ~ 7.8), oral cavity (pH 6–7) or subcompartments of cells act as a trigger for releasing the cargo. The permeability of polyelectrolyte capsules containing tannic acid and different polyelectrolytes (most notably PNIPAM (poly(*N*-isopropylacrylamide))) can be adjusted by pH [40].

Pros and cons: although reversible permeability change is possible, application of these capsules in biological applications, wherein pH cannot be changed, is questionable. Weak mechanical properties might be another concern for such capsules.

Salts, ionic strength of the solution and sensitivity to reduction

Salt has been generally used for preparation of microcapsules. A small amount of salt, added to solutions with polymers, somewhat screens the charges on polymers transforming the polymers from the spread to a somewhat smudged state. The effect of salt on polyelectrolyte multilayer capsules takes place on the whole surface of

Figure 1



Schematic illustration of various stimuli used for release from polyelectrolyte multilayer microcapsules. The same stimuli, which are in fact also used for encapsulation, are broadly applied for release.

microcapsules. The same principle described above in regard with the state of polymers applies here in case if salt is added to a solution containing microcapsules. Salt ions would screen the charges, also increasing the osmotic pressure, thus providing means for loosening the interaction of the polymers forming the polyelectrolyte multilayers shells. That in turn would lead to disintegration of microcapsules and release of encapsulated materials. Permeability regulation based on reduction is an attractive chemical stimuli based method with potential to be also applied to other carriers. Microcapsule expansion and the permeability change has been demonstrated for poly(ferrocenylsilane) containing microcapsules with redox-active ferrocine in the polymeric chain [41].

Interestingly, decrease of the interaction of polymers upon addition of a high (3 M) of salt can be used for fusion [42] of capsules (or releasing the contents of two capsules into a jointly formed capsule). The fusion can be also achieved by salt or pH [43]. The thickness change of polyelectrolyte multilayers due to extreme pH differences [44] supports the influence of salt on the structure of multilayers.

Pros and cons: advantages of ionic strength-based methods include possibilities to affect a large number of capsules, versatility of affecting the layers, while disadvantages concern biological applications, where salt concentration needs to be kept constant.

Solvents

Solvents along with salts are some of the most frequently used methods to affect the permeability of polyelectrolyte multilayer capsules [45]. Solvent-exchange methods have been frequently used for manipulating, forcing and encapsulating molecules [46].

Pros and cons: water has been traditionally used as an universal solvent for assembling capsules and films; although organic solvents have been often used, particularly for incorporation of water-insoluble molecules. It can be noted that solvent-exchange may affect the activity of encapsulated materials.

Electrochemical methods of release

Research in the area of electrochemical release methods is directed toward understanding of the effects of electrochemistry on polyelectrolytes and their interactions, and toward applying them to control loading and release. Polypyrrole, which possesses interesting electrical properties, has been reported as a good agent for reducing the permeability of microcapsules [47]. A film of polypyrrole was deposited on an electrode, which acted to induce the fields increasing the influx of counterions/solvent molecules increasing the osmotic pressure in the film [48]. The expanded film promotes release of molecules, while its reversibility provides means for encapsulation. Electrochemical potential change has been also recently used for releasing plasmid DNA from coated surfaces [49].

Pros and cons: the biggest advantages of such a method include high potential for sensing applications, a possibility of using electrical field pulses to induce the release, while disadvantages concern a limited volume of applicability, that is in vicinity of an electrode.

Biological methods for release

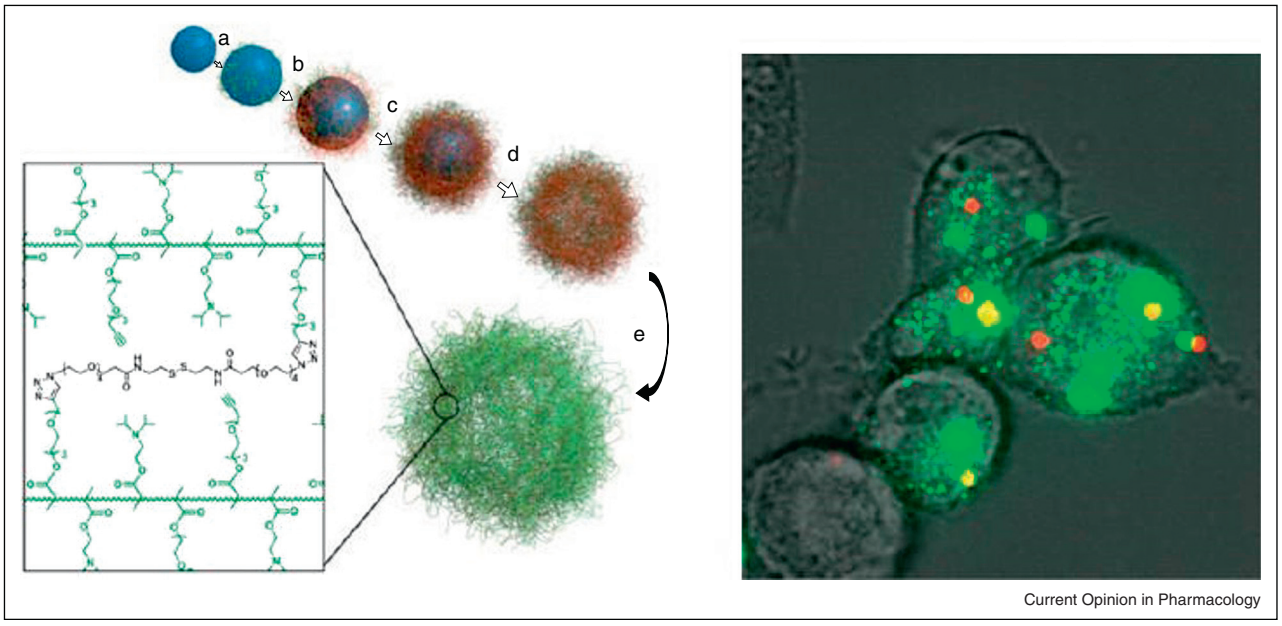
Affecting microcapsules by proteins, peptides or other biologically relevant molecules is attractive in regard with actions of such molecules when capsules are placed into cells or *in vivo*. Enzymatic degradation is another route for inducing release by biological stimuli. Substantial research activity was devoted to encapsulation of enzymes, for example enzyme [50] incorporation into polyelectrolyte multilayers [51]. Alginate particles combined with polyelectrolyte coatings have been also shown as an effective container for enzymes [52]. pARG (polyarginine) and DEXS (dextrane sulfate) were used as polyelectrolyte multilayers [53] and intracellular release by degradation has been shown. Controllability of release is essential: by increasing the number of layers in the polyelectrolyte coating [54] or controllable cross-linking [55] may be used for slowing down the release.

'Click'-chemistry [56] induced release as well as disulfide-bond disintegration [57] represent yet another class of biological stimuli, which can be effectively applied for release from microcapsules. Disulfide bond disintegration serves as an interesting example of inducing release from microcapsules. It is interesting to note that the release took place upon changing pH [35], but importantly pH was not adjusted externally. The response here is triggered upon pH change when microcapsules enter a subcompartment of cells (or tissue) possessing different pH values. A combination of redox-responsive capsules as well as the degree of cross-linking has been reported to lead to controlled degradation [55], Figure 2.

Glucose sensitive microcapsules are yet another method of using biological stimuli to generate release. In this case release is facilitated by incorporating phenylboronic acid, which exists in a charged and uncharged form. The latter is hydrophobic, while the former is hydrophobic one. The principle of inducing release from such type of capsules is based on inducing increased solubility of by shifting the equilibrium toward charged phenylborate upon forming a complex with glucose. Assembly of polyelectrolyte multilayers was accomplished by using PSS and 3-Acrylamidophenylboronic acid [58]. Glucose initiated release from such type of capsules was seen to be quite fast.

Suitability of polyelectrolyte multilayer capsules responsive to biological stimuli to biomedical applications, work

Figure 2



Redox-responsive bisazide cross-linked microcapsules (left-hand side), and their intracellular uptake (right-hand side) for subsequent degradation. Adapted with permission from [55].

with cells and *in vivo* are their biggest advantages, the disadvantages include difficulty of locally modulation of properties of polyelectrolytes.

Pros and cons: applicability of this class of stimuli in biology is its important asset, however, it is difficult to attain the same degree of control as that induced by physical methods.

Release by physical methods

Laser-light effects, nanoparticles and their assembly

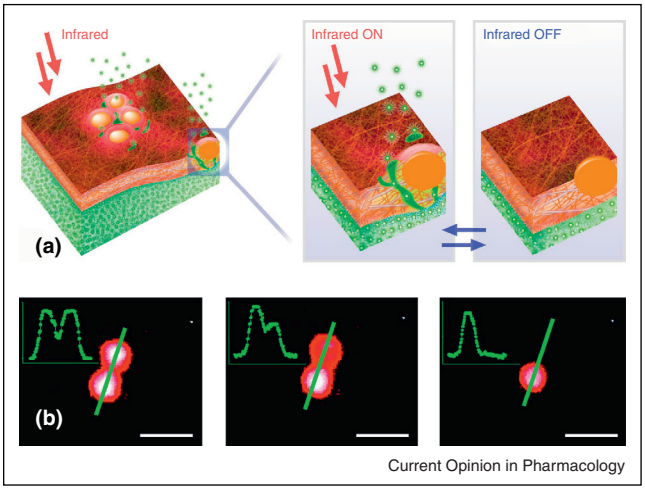
As outlined before temperature is an important parameter to control the stability and permeability of polyelectrolytes. Subsequently infrared laser pulses in the absorption window of nanoparticles convert laser energy into a localized temperature rise [59]. This laser-nanoparticle interaction has been used to create specific release-suitable interaction [60–63], which takes place also due to temperature. The temperature increase, measured around microcapsules [62], in this case can take place either through the interaction of laser with absorbing centers: nanoparticles or absorbing dyes [60], which are located in the shell of capsules.

Exposure of [PAH/PAzo(poly[[(carboxy-4-hydroxyphenylazo)benzenesulfoamido]-ethanediyl)l](PAzo)$_3$/PAH/poly(vinylsulfonate) (PVS)] microcapsules to UV (ultraviolet) light resulted in pore closure of capsules [64]. This process was interpreted as rearrangement of PAzo polymers in the polymeric network; similar processes were also observed for microgels [65]. Photocleavable polyurea can be another candidate for UV based release [66]. For non-biological applications the use of UV-light [67] represents an interesting alternative.

Distribution of nanoparticles (and intensity of incident laser light) regulates the temperature rise around absorbing centers. Polymers can be used for controlling the distribution of nanoparticles on capsules, both aggregated and non-aggregated distributions can be obtained [68]. In an alternative case, which is applicable to non-localized release of encapsulated materials with explosion of a capsule a direct reduction of nanoparticles at the surface of microcapsules is possible [69]. Laser exposure of such capsules literally leads to explosion of capsules [60]. Nanoparticle reduction under temperature controlled environment can be also used for controlling the distribution of nanoparticles. Such methods of nanoparticle growth control can be also used to control the size of reduced nanoparticles. Although this method is facile and relatively fast, true control over the nanoparticle distribution is rather complicated. In addition to these materials, release can be achieved with carbon nanotubes [29], graphene [70], and organic molecules [60,64].

Polymeric microcapsules and release from them are broadly studied [71]; as a result, new approaches have been identified: release using photodynamic therapy (PDT) [72], time-specific release [73], and remotely controllable bioreactors [74].

Figure 3



Top row: schematics of the reversible 'on'-and-'off' switching of the polymeric membrane of capsules. Bottom row: transiently opening and closing, and opening again the permeability of a top-right microcapsule. Reproduced with permission [34].

A lucrative application of release has been shown for reversible switching of the polyelectrolyte membrane of microcapsules, Figure 3. Release was shown taken place when the capsule is exposed to laser, but was arrested and temporarily stopped when the laser was shut-off. An interesting practical application is seen for controlling the dose of the delivered biomolecules or medicine.

Ultrasound

Ultrasound has been broadly used for synthesis of materials. Recently ultrasound has been used for release of encapsulated materials [68,75,76]. Ultrasound at relatively high powers (100–500 W, in the kilohertz frequency range) was sufficient to destroy microcapsules. Ultrasound operating at the most attractive, medically allowed range of power was unfortunately not sufficient to induce release from capsules.

The efficiency of ultrasound can be enhanced by increasing the density of materials introduced into the shell of polymeric microcapsules. For example, ZnO nanoparticles were used for opening of microcapsules [77], while enhancement of release was observed upon incorporation of metal nanoparticles in polymeric shells. In this case the effectiveness of this process can be controlled by tuning the shell thickness and its roughness.

Similarly to possibility of using temperature and laser for encapsulation and release, ultrasound has been used for encapsulation of drugs, for example, rifampicin.

Magnetic fields

Magnetic field has been traditionally used for targeting. The idea is to act by magnetic field on magnetic nanoparticles adsorbed into the shell of polyelectrolyte multilayer capsules. Further extension of application is foreseen by using magnetic fields for releasing from microcapsules.

Lvov *et al.* showed that the permeability change of microcapsules can be induced by exposing microcapsules functionalized with magnetic nanoparticles immobilized onto the polyelectrolyte shell. In that work introduction of ferromagnetic cobalt (Co/Au) nanoparticles into polyelectrolyte multilayer shell and application of magnetic field led to increase of permeability increase of microcapsules. During the experiments, an alternating magnetic field was applied (1200 Oe) in a solution containing PSS/PAH capsules functionalized with magnetic, cobalt containing nanoparticles, and the influx of fluorescently labeled molecules was observed. Not only cobalt, but also iron nanoparticles were used for such a release [78]. One of the disadvantages of the above approach is that the application of magnetic field was carried out for substantially long time. Investigation of heating properties [79] of small superparamagnetic nanocrystals provided important information about magnetic field–nanoparticles interaction.

Mechanical deformation

Mechanical deformation belongs to some of the most basic and most obvious release methods. Its links with release have been dating back to early work on carbon paper. The simple idea of pressing on microcapsules and by this inducing the release appears to be indeed an attractive option.

Early work on investigation of mechanical properties included application of colloidal probe AFM (atomic force microscopy) for pressing on microcapsules [80]. Mechanical deformation of microcapsules filled with fluorescent polymer was investigated. That was carried out by combining of AFM (atomic force microscopy) with fluorescent microscopy [81].

Gold nanoparticles were shown also to increase of the stiffness of microcapsules, and that paved the way to enhancing the mechanical properties of soft exponentially grown polyelectrolyte multilayer films [82]. On the other hand, mechanical effect can also be achieved by stretching [83]. Peculiarly, stretching can be also applied for films for determining their mechanical properties [84].

Directionality-control, time-control and wavelength-control of release

Directionality of release is essential for application in which pricewise targeting is required. For example, for capsule-in-capsule intermixing applications it is desirable to target a specific compartment or a part of a compartment of a more complicated multicompartment structure. In regard with nanoparticles, the feasibility of

direction-specific release was shown on example of giant microcapsules functionalized with gold nanoparticles [85]. Depending on which spatial area was illuminated, release was originated in a certain direction. Another example of direction-specific release was achieved by mechanical force. For direction specific release a sharp AFM tip [86], instead of a flat (and large compared to microcapsule) colloidal probe bead [87•], was used. The encapsulated molecules were released in a specific direction precisely controlled by the site of sharp AFM press.

Time specific release allows for providing specific doses of release at a desired period of time. Partial release through laser-nanoparticle interaction is certainly one of the options of controlling the dose and controlling time of the release [34]. Non-destructiveness and controllability [33] are one of the most important characteristics of such a release. Although such an approach was demonstrated on the individual capsule level, its extension to a large number of capsules is possible. One potential obstacle for providing uniformity of doses and perfect control of release properties is precise control of amount and distribution of nanoparticles from capsule to capsule. Organic molecules have better potential for uniformity of the distribution, and release was demonstrated for microcapsules functionalized with organic molecules. Perhaps even more attractive option for inducing release suitable for *in vivo* application is that based on enzymatic degradation. The idea is based on incorporation of mechanisms, like disulfide degradation [88] or biomolecules [53] which can undergo degradation upon action of enzymes inherently present in body/tissue. Here a simple approach of controlling the number of layers in the shell of polyelectrolyte multilayer capsule permits for slowing-down or accelerating the release [54].

Ability to control release from capsules by wavelength of laser light brings another mechanism of external and remote control. In one implementation nanorods, absorbing both in the visible and near-infrared parts of spectrum were used for inducing release [89]. Another interesting approach was suggested by Thomas *et al.*, who used photocleavable esters to provide means of using organic molecules [90].

Selected applications of release from polymeric capsules and other carriers

Immune system response, release inside cancer cells and neurons

Application of microcapsules to induce immune system response is a growing research area. Investigation of the surface presentation of small peptides by the major histocompatibility complex (MHC) class I molecules is of particular importance to immunology. Mechanically stable (thermally shrunk four-bilayer PSS/PDADMAC) polyelectrolyte multilayer capsules were introduced by electroporation assisted method into cells. Laser exposure of microcapsules allowed for induction of time and space specific release, so that the so-called surface-presentation of peptides was investigated.

Cell viability, which is important for such intracellular [91•] delivery and release, was investigated on an example of MDA-MB-435S cancer cells [92]. Substantial temperature rise inside cells was shown to lead to cancer cell death, while non-destructive delivery was proposed for delivery of drugs. Release inside neurons [93] opens up a way to investigate a number of processes in the area of neurology. *In vivo* applications of microcapsules have been also conducted, for example, in vaccine [94•] delivery for pharmaceutical [95] applications. Cell viability after delivery of coated nanoparticles [96] is another confirmation of suitability of polyelectrolytes.

Multiple stimuli release and multicompartment structures–relevance for theranostics

One application area — theranostics [97] — is an emerging application area which necessitates development of multicompartment delivery carriers as well as multiple stimuli for inducing release from them. Microcapsules functionalized with both magnetic and gold nanoparticles were magnetically manipulated and opened with laser [98]. Such a concept can be also used for inducing release either by laser or magnetic field. Microparticles functionalized with carbon nanotubes and gold nanoparticles can be used as sensors [99].

Multicompartmental capsules [100] is relevant for theranostics. Different approaches have been pursued for multicompartmentalization including shell-in-shell structures and other subcompartments spread over the surface of capsules. In this class of applications some subcompartments are responsible for sensing and diagnostics, while other are responsible for therapy by releasing drugs needed for treatment. Microcapsules containing enzymes have attracted particular attention [101]. Microcapsules possessing both a substrate and a corresponding enzyme in the same structure are yet another important step toward designing advanced drug delivery carriers [102]. Microcapsule fusion may first of all be of interest for studying the state of molecules inside polyelectrolyte multilayers (e.g. their mobility, which is so different in exponentially grown thick as opposed to normal or thin films), but it is also related to intermixing of subcompartments relevant for theranostics. Reactions in multicompartmental microcapsules were monitored under a microscope. A summary of developments in the area of enzymes was recently presented. In another study, a coupled chain reaction in multiple concentric $CaCO_3$ particles/capsule was investigated [103]. Research in the area of enzyme-catalyzed reactions revealed very promising approaches for treatment of diseases; there preserving catalytic activity of enzyme is one of the most significant factors [104]. Inward interweaving assembly of

capsules [105] represents an interesting alternative for build-up of multicompartment capsules.

Sensing is first and foremost relevant for cell biology. Sensing pH in cells [106] or oxygen [107] is relevant for sensoring part of theranostics [97]. This can be implemented by designing a semipermeable capsule which would permit ions to go through its shell [108], but would protect and preserve inside the larger molecules (proteins), which are used as indicators or sensors. Recent advances include triple [109] sensing implementation.

Mechano-biology benefits from mechanical means of investigate. However, study of mechanical pressure induced release enabled determining forces which cells exert upon uptake, that is microcapsules were used as sensors for studying cell mechanics [110]. Deformation was accurately measured by AFM: these measurements were correlated to uptake of microcapsules by cells. The stiffness and release from microcapsules undergone thermal treatments (at fifty, sixty and seventy degrees) were characterized through an *ex situ* method using AFM (by a technique referred to as colloidal probe). The correlation between the forces measured by AFM and release induced upon uptake revealed that cells utilize forces on the order of 0.2 μN.

Planar films are relevant for many *in vivo* delivery platforms. Microcapsules on planar polymeric films represents an interesting opportunity to add drug delivery functionality to the films [111]. So-called exponentially grown thick multilayer films may themselves carry molecules, but incorporation of microcapsules opens additional opportunities for modulating the release, which can be additionally accomplished from microcapsules embedded into the films [111]. It can be noted that functionalization of such films with nanoparticles [112] enhances their mechanical properties [82] and avails laser induced release. In the area of films those capable of free-standing [113], reconfigurable [114], and self-regulating [115] capabilities are particularly important.

Release from other types of carriers

Liposomes are probably the most studied type of drug delivery carriers, partially owing to the fact that molecules which comprise their walls, lipids, are also abandoned in cells and constitute its surface. In regard with release, the above mentioned methods were used for release from liposomes [116,117]. Both laser induced release and magnetic field induced release from liposomes whose surface was functionalized with nanoparticles were demonstrated.

Polymersomes represent an interesting carrier paving a bridge between liposome and polymeric capsules. Recent activity of in the area of polymersomes was directed at synthesizing new polymers and designing novel types of polymersome carriers, while on the developmental side multicompartmentalization [118] and induction of interaction between subcompartments is one of the emerging trends.

Capsosomes [119], which combine polymer microcapsules and liposomes, were recently introduced by the analogy between polymersomes and polyelectrolyte multilayer capsules. This class of carriers is somewhat closer linked with actual microcapsules, but provides an opportunity of inclusion of relatively small molecules.

Particulate delivery carriers are very closely related to microcapsules because particles from which capsules are made could be directly used as carriers [120] and inducing release from particles is essential. Layer-by-layer assembly offers very attractive means of particle modification and controllability of release [121,122]. An interesting approach for obtaining particles follows from obtaining polymer-filled template [123] or for surface initiated polymerization on silica [124]. Specific targeting, for example endothelial [125], is regarded an important aspect of delivery.

Emulsion template capsules [126] is another area wherein application of describe above release mechanisms find reflection. Emulsions offer interesting means of encapsulation and release of non-water soluble molecules and have been widely used in industry. Microfluidics has been used in this area for assisting in assembly.

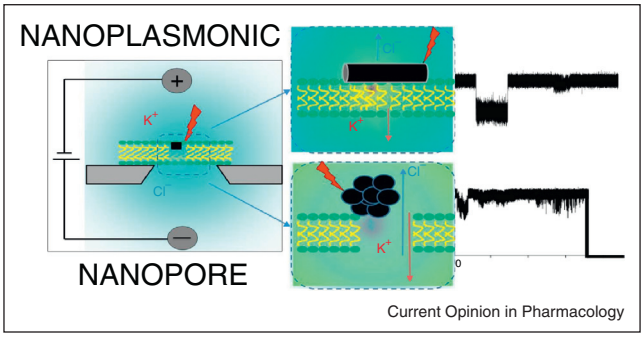
New types of polymeric capsules are carriers functionalized or made from novel materials, like silk and spider silk [127,128] provide attractive means for construction of improved carriers and release from them. Development of different materials, particularly in regard with strengthening capsules and providing additional means of controlling the permeability and release are some of the directions in this area.

Red blood cells are probably the most attractive carriers because of their inherent potential as long circulating delivery platform. Functionalization of red blood cells by gold nanoparticles [129] was recently demonstrated, because development of red blood cell-like microcapsules [130] is another complementary research direction relevant for therapy [131]. It is interesting to note that in the same way as laser was used for release of biomolecules from microcapsules, it was also applied for releasing of large (over 10 kDa) and small (under 1 kDa) molecules from vicinity of red blood cells upon laser illumination.

Facilitating traffic through lipid membranes

The permeability of a lipid membrane for internal function regulation of cells as well as for small molecules, peptides relevant for therapy is gaining a particular attention [3]. Insights into this process with unmatched

Figure 4



Schematic of laser-nanoparticle induced permeability study through lipid bilayer using ionic current monitoring at a nanopore.
Reproduced with permission [132•].

precision can be studied by monitoring ionic current through a lipid membrane. If necessary any of the porins and channel proteins can be also immobilized on the membrane.

External modulation of a membrane represents thus a desired means of facilitating the delivery. It was recently shown that aggregates of gold nanoparticles can be used for irreversible ionic current change, while ionic current through nanorod functionalized membrane can oscillate upon laser-nanorod interaction [132•].

The schematics of the experiment is shown in Figure 4: a giant liposome was spread across a pore thus sealing the pore and blocking the ionic current, which would have been generated due to the ions present in the solution. Several types of nanoparticles have been used in that study, while their aggregation affected the interaction with the membrane and the laser. Laser was used here in a similar way as that for release from polymeric microcapsules and liposomes. Laser-nanoparticles interaction may be of non-thermal nature [133], lead to nanoparticle transport across the membrane [134] or even lead to bubble formation [135,136]. Bubble formation around laser nanoparticles has been recently explored [135], wherein the effect of medium, its composition, and aggregation state of nanoparticles have been investigated. Lipid membrane modulation opens attractive opportunities to monitor ionic current with a very high precision; so far opening and closing of the lipid membrane was observed for gold nanorods, but breakage of the membrane was reported for gold nanoparticles. The difference was attributed to aggregation in the latter case and that was also confirmed for gold nanocages which aggregated the most. Pharmacologically relevant application of such membrane modulation relates to delivery of molecules, which was recently demonstrated for different types of molecules [137].

Conclusions

We have described insights of various release methods for polyelectrolyte multilayer microcapsules and highlighted pharmacological relevance, which is associated with drug delivery by carriers or modulating the membrane facilitating the drugs reaching their target. Applicability of a broad range of physical, chemical and biological methods makes microcapsules an unique and attractive carrier not only for studying fundamental properties of polymers, but also in regard with their practical applications. Some of applications, such as intracellular delivery, vaccine delivery, enzyme-catalyzed reactions are described here, multicompartmentalization and sensing applicable to theranostics. Described here release based methods are also looked at from the applicability to other carriers standpoint of view. Liposomes, polymersomes, capsosomes, emulsion based carriers, particles can all be affected by the methods of release described here. We have also highlighted applicability of release based methods, in particular laser-nanoparticle interaction, to permeability study of lipid membranes. This emerging technology based on ionic current monitoring through a nanopore is capable of providing information about the state of lipids with unprecedented precision.

Conflict of interest statement

Nothing declared.

Acknowledgements

We acknowledge support of FWO (Fonds Wetenschappelijk Onderzoek, Vlaanderen, Belgium, grant numbers FWO13/ASP/287 and 3G016613N), BOF (Bijzonder Onderzoek Fonds, Universiteit Gent, grant numbers 01B08912W and 01MRH110), DFG (Germany, grant number Wi 2278/18-1) and Government of the Russian Federation (grant number 14.Z50.31.0004 to support scientific research projects implemented under the supervision of leading scientists at Russian institutions and Russian institutions of higher education). B P. acknowledges support of the EU Marie Curie program. We would like also thank EU FP-7-People-IRSES project 'DINaMIT'.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hollinger MA: *Introduction to Pharmacology*. London & New York: Taylor & Francis; 2003, .

2. Timko BP, Dvir T, Kohane DS: **Remotely triggerable drug delivery systems**. *Adv Mater* 2010, **22**:4925-4943.

3. Stavenger RA, Winterhalter M: **TRANSLOCATION project how to get good drugs into bad bugs**. *Sci Transl Med* 2014, **6**:3.

4. Hammond PT: **Building biomedical materials layer-by-layer**. *Mater Today* 2012, **15**:196-206.

5. • Antipina MN, Sukhorukov GB: **Remote control over guidance and release properties of composite polyelectrolyte based capsules**. *Adv Drug Deliv Rev* 2011, **63**:716-729.
This paper described different applications, in which released from capsules play an important role.

6. • Yan Y, Such GK, Johnston APR, Lomas H, Caruso F: **Toward therapeutic delivery with layer-by-layer engineered particles**. *ACS Nano* 2011, **5**:4252-4257.

In this work, the authors described microcapsules as delivery carriers of biomolecules and medicine.

7. Ariga K, McShane M, Lvov YM, Ji Q, Hill JP: **Layer-by-layer assembly for drug delivery and related applications**. *Expert Opin Drug Deliv* 2011, **8**:633-644.

8. Yan Y, Bjonmalm M, Caruso F: **Assembly of layer-by-layer particles and their interactions with biological systems**. *Chem Mater* 2014, **26**:452-460.

9. Ariga K, Yamauchi Y, Rydzek G, Ji QM, Yonamine Y, Wu KCW, Hill JP: **Layer-by-layer nanoarchitectonics: invention, innovation, and evolution**. *Chem Lett* 2014, **43**:36-68.

10. Kharlampieva E, Kozlovskaya V, Tyutina J, Sukhishvili SA: **Hydrogen-bonded multilayers of thermoresponsive polymers**. *Macromolecules* 2005, **38**:10523-10531.

11. • Stockton WB, Rubner MF: **Molecular-level processing of conjugated polymers. 4. Layer-by-layer manipulation of polyaniline via hydrogen-bonding interactions**. *Macromolecules* 1997, **30**:2717-2725.
This is one of the trend-setting works in regard with hydrogen bonding interaction relevant for polyelectrolytes.

12. Driver K, Baco S, Khutoryanskiy VV: **Hollow capsules formed in a single stage via interfacial hydrogen-bonded complexation of methylcellulose with poly(acrylic acid) and tannic acid**. *Eur Polym J* 2013, **49**:4249-4256.

13. • Quinn JF, Johnston APR, Such GK, Zelikin AN, Caruso F: **Next generation, sequentially assembled ultrathin films: beyond electrostatics**. *Chem Soc Rev* 2007, **36**:707-718.
Here, a reader will find other (than electrostatic) strategies of building capsules.

14. Parakhonskiy BV, Yashchenok AM, Konrad M, Skirtach AG: **Colloidal micro- and nano-particles as templates for polyelectrolyte multilayer capsules**. *Adv Colloid Interface Sci* 2014, **207**:253-264.

15. Volodkin D: **$CaCO_3$ templated micro-beads and -capsules for bioapplications**. *Adv Colloid Interface Sci* 2014, **207**:306-324.

16. Volodkin DV, Schmidt S, Fernandes P, Larionova NI, Sukhorukov GB, Duschl C, Moehwald H, von Klitzing R: **One-step formulation of protein microparticles with tailored properties: hard templating at soft conditions**. *Adv Funct Mater* 2012, **22**:1914-1922.

17. Kiryukhin MV, Man SM, Gorelik SR, Subramanian GS, Low HY, Sukhorukov GB: **Fabrication and mechanical properties of microchambers made of polyelectrolyte multilayers**. *Soft Matter* 2011, **7**:6550-6556.

18. Wang XL, Zeng Y, Zheng YZ, Chen JF, Tao X, Wang LX, Teng Y: **Rose Bengal-grafted biodegradable microcapsules: singlet-oxygen generation and cancer-cell incapacitation**. *Chem Eur J* 2011, **17**:11223-11229.

19. Shimoni O, Yan Y, Wang YJ, Caruso F: **Shape-dependent cellular processing of polyelectrolyte capsules**. *ACS Nano* 2013, **7**:522-530.

20. Chen J, Kozlovskaya V, Goins A, Campos-Gomez J, Saeed M, Kharlampieva E: **Biocompatible shaped particles from dried multilayer polymer capsules**. *Biomacromolecules* 2013, **14**:3830-3841.

21. Shchepelina O, Lisunova MO, Drachuk I, Tsukruk VV: **Morphology and properties of microcapsules with different core releases**. *Chem Mater* 2012, **24**:1245-1254.

22. Shchepelina O, Kozlovskaya V, Kharlampieva E, Mao W, Alexeev A, Tsukruk VV: **Anisotropic micro- and nano-capsules**. *Macromol Rapid Commun* 2010, **31**:2041-2046.

23. Kohler D, Madaboosi N, Delcea M, Schmidt S, De Geest BG, Volodkin DV, Moehwald H, Skirtach AG: **Patchiness of embedded particles and film stiffness control through concentration of gold nanoparticles**. *Adv Mater* 2012, **24**:1095-1100.

24. Kozlovskaya V, Alexander JF, Wang Y, Kuncewicz T, Liu XW, Godin B, Khariampieva E: **Internalization of red blood cell-mimicking hydrogel capsules with pH-triggered shape responses**. *ACS Nano* 2014, **8**:5725-5737.

25. Yashchenok A, Parakhonskiy B, Donatan S, Kohler D, Skirtach A, Moehwald H: **Polyelectrolyte multilayer microcapsules templated on spherical, elliptical and square calcium carbonate particles**. *J Mater Chem B* 2013, **1**:1223-1228.

26. Parakhonskiy BV, Yashchenok AM, Donatan S, Volodkin DV, Tessarolo F, Antolini R, Moehwald H, Skirtach AG: **Macromolecular loading into spherical, elliptical, star-like, and cubic calcium carbonate carriers**. *ChemPhysChem* 2014, **15**:2817-2822.

27. Skirtach AG, Yashchenok AM, Moehwald H: **Encapsulation, release and applications of LbL polyelectrolyte multilayer capsules**. *Chem Commun* 2011, **47**:12736-12746.

28. Lvov Y, Aerov A, Fakhrullin R: **Clay nanotube encapsulation for functional biocomposites**. *Adv Colloid Interface Sci* 2014, **207**:189-198.

29. Yashchenok AM, Bratashov DN, Gorin DA, Lomova MV, Pavlov AM, Sapelkin AV, Shim BS, Khomutov GB, Kotov NA, Sukhorukov GB *et al.*: **Carbon nanotubes on polymeric microcapsules: free-standing structures and point-wise laser openings**. *Adv Funct Mater* 2010, **20**:3136-3142.

30. Kozlovskaya V, Wang Y, Higgins W, Chen J, Chen Y, Kharlampieva E: **pH-triggered shape response of cubical ultrathin hydrogel capsules**. *Soft Matter* 2012, **8**:9828-9839.

31. • Delcea M, Moehwald H, Skirtach AG: **Stimuli-responsive LbL capsules and nanoshells for drug delivery**. *Adv Drug Deliv Rev* 2011, **63**:730-747.
In this review, different stimuli for affecting polyelectrolytes are discussed.

32. Ye C, Drachuk I, Calabrese R, Dai H, Kaplan DL, Tsukruk VV: **Permeability and micromechanical properties of silk ionomer microcapsules**. *Langmuir* 2012, **28**:12235-12244.

33. Xu WN, Choi I, Plamper FA, Synatschke CV, Muller AHE, Tsukruk VV: **Nondestructive light-initiated tuning of layer-by-layer microcapsule permeability**. *ACS Nano* 2013, **7**:598-613.

34. Skirtach AG, Karageorgiev P, Bedard MF, Sukhorukov GB, Möhwald H: **Reversibly permeable nanomembranes of polymeric microcapsules**. *J Am Chem Soc* 2008, **130**:11572-11573.

35. Shutava T, Prouty M, Kommireddy D, Lvov Y: **pH responsive decomposable layer-by-layer nanofilms and capsules on the basis of tannic acid**. *Macromolecules* 2005, **38**:2850-2858.

36. Dergunov SA, Durbin J, Pattanaik S, Pinkhassik E: **pH-mediated catch and release of charged molecules with porous hollow nanocapsules**. *J Am Chem Soc* 2014, **136**:2212-2215.

37. Du CL, Zhao J, Fei JB, Cui Y, Li JB: **Assembled microcapsules by doxorubicin and polysaccharide as high effective anticancer drug carriers**. *Adv Healthcare Mater* 2013, **2**:1246-1251.

38. Lavalle P, Voegel J-C, Vautier D, Senger B, Schaaf P, Ball V: **Dynamic aspects of films prepared by a sequential deposition of species: perspectives for smart and responsive materials**. *Adv Mater* 2011, **23**:1191-1221.

39. Liang K, Such GK, Johnston APR, Zhu ZY, Ejima H, Richardson JJ, Cui JW, Caruso F: **Endocytic pH-triggered degradation of nanoengineered multilayer capsules**. *Adv Mater* 2014, **26**:1901-1905.

40. Doane TL, Burda C: **The unique role of nanoparticles in nanomedicine: imaging, drug delivery and therapy**. *Chem Soc Rev* 2012, **41**:2885-2911.

41. Ma YJ, Dong WF, Hempenius MA, Mohwald H, Vancso GJ: **Redox-controlled molecular permeability of composite-wall microcapsules**. *Nat Mater* 2006, **5**:724-729.

42. Zhang RJ, Kohler K, Kreft O, Skirtach A, Mohwald H, Sukhorukov G: **Salt-induced fusion of microcapsules of polyelectrolytes**. *Soft Matter* 2010, **6**:4742-4747.

43. Pechenkin MA, Moehwald H, Volodkin DV: **pH- and salt-mediated response of layer-by-layer assembled PSS/PAH microcapsules: fusion and polymer exchange**. *Soft Matter* 2012, **8**:8659-8665.

44. Irigoyen J, Han L, Llarena I, Mao ZW, Gao CY, Moya SE: **Responsive polyelectrolyte multilayers assembled at high**

ionic strength with an unusual collapse at low ionic strength. *Macromol Rapid Commun* 2012, **33**:1964-1969.

45. Kim BS, Lebedeva OV, Koynov K, Gong HF, Glasser G, Lieberwith I, Vinogradova OI: **Effect of organic solvent on the permeability and stiffness of polyelectrolyte multilayer microcapsules**. *Macromulecules* 2005, **38**:5214-5222.

46. Lvov YM, Pattekari P, Zhang X, Torchilin V: **Converting poorly soluble materials into stable aqueous nanocolloids**. *Langmuir* 2011, **27**:1212-1217.

47. Andreeva DV, Gorin DA, Shchukin DG, Sukhorukov GB: **Magnetic microcapsules with low permeable polypyrrole skin layer**. *Macromol Rapid Commun* 2006, **27**:931-936.

48. Shchukin DG, Kohler K, Mohwald H: **Microcontainers with electrochemically reversible permeability**. *J Am Chem Soc* 2006, **128**:4560-4561.

49. Aytar BS, Prausnitz MR, Lynn DM: **Rapid release of plasmid DNA from surfaces coated with polyelectrolyte multilayers promoted by the application of electrochemical potentials**. *ACS Appl Mater Interfaces* 2012, **4**:2726-2734.

50. Ghan R, Shutava T, Patel A, John VT, Lvov Y: **Enzyme-catalyzed polymerization of phenols within polyelectrolyte microcapsules**. *Macromolecules* 2004, **37**:4519-4524.

51. Caruso F, Trau D, Mohwald H, Renneberg R: **Enzyme encapsulation in layer-by-layer engineered polymer multilayer capsules**. *Langmuir* 2000, **16**:1485-1488.

52. Srivastava R, Brown JQ, Zhu HG, McShane MJ: **Stable encapsulation of active enzyme by application of multilayer nanofilm coatings to alginate microspheres**. *Macromol Biosci* 2005, **5**:717-727.

53. De Geest BG, Vandenbroucke RE, Guenther AM, Sukhorukov GB, Hennink WE, Sanders NN, Demeester J, De Smedt SC: **Intracellularly degradable polyelectrolyte microcapsules**. *Adv Mater* 2006, **18**:1005-1009.

54. Marchenko I, Yashchenok A, Borodina T, Bukreeva T, Konrad M, Mohwald H, Skirtach A: **Controlled enzyme-catalyzed degradation of polymeric capsules templated on $CaCO_3$: influence of the number of LbL layers, conditions of degradation, and disassembly of multicompartments**. *J Control Release* 2012, **162**:599-605.

55. Liang K, Such GK, Zhu Z, Dodds SJ, Johnston APR, Cui J, Ejima H, Caruso F: **Engineering cellular degradation of multilayered capsules through controlled cross-linking**. *ACS Nano* 2012, **6**:10186-10194.

56. Ochs CJ, Such GK, Yan Y, van Koeverden MP, Caruso F: **Biodegradable click capsules with engineered drug-loaded multilayers**. *ACS Nano* 2010, **4**:1653-1663.

57. Zelikin AN, Li Q, Caruso F: **Disulfide-stabilized poly(methacrylic acid) capsules: formation, cross-linking, and degradation behavior**. *Chem Mater* 2008, **20**:2655-2661.

58. De Geest BG, Jonas AM, Demeester J, De Smedt SC: **Glucose-responsive polyelectrolyte capsules**. *Langmuir* 2006, **22**:5070-5074.

59. Zhou J, Pishko MV, Lutkenhaus JL: **Thermoresponsive layer-by-layer assemblies for nanoparticle-based drug delivery**. *Langmuir* 2014, **30**:5903-5910.

60. Skirtach AG, Antipov AA, Shchukin DG, Sukhorukov GB: **Remote activation of capsules containing Ag nanoparticles and IR dye by laser light**. *Langmuir* 2004, **20**:6988-6992.

61. Radt B, Smith TA, Caruso F: **Optically addressable nanostructured capsules**. *Adv Mater* 2004, **16**:2184-2189.

62. Skirtach AG, Dejugnat C, Braun D, Susha AS, Rogach AL, Parak WJ, Mohwald H, Sukhorukov GB: **The role of metal nanoparticles in remote release of encapsulated materials**. *Nano Lett* 2005, **5**:1371-1377.

63. Angelatos AS, Radt B, Caruso F: **Light-responsive polyelectrolyte/gold nanoparticle microcapsules**. *J Phys Chem B* 2005, **109**:3071-3076.

64. Bedard MF, Sadasivan S, Sukhorukov GB, Skirtach A: **Assembling polyelectrolytes and porphyrins into hollow capsules with laser-responsive oxidative properties**. *J Mater Chem* 2009, **19**:2226-2233.

65. Zakrevskyy Y, Richter M, Zakrevska S, Lomadze N, von Klitzing R, Santer S: **Light-controlled reversible manipulation of microgel particle size using azobenzene-containing surfactant**. *Adv Funct Mater* 2012, **22**:5000-5009.

66. Dispinar T, Colard CAL, Du Prez FE: **Polyurea microcapsules with a photocleavable shell: UV-triggered release**. *Polym Chem* 2013, **4**:763-772.

67. Yi QY, Sukhorukov GB: **UV light stimulated encapsulation and release by polyelectrolyte microcapsules**. *Adv Colloid Interface Sci* 2014, **207**:280-289.

68. Skirtach AG, Dejugnat C, Braun D, Susha AS, Rogach AL, Sukhorukov GB: **Nanoparticles distribution control by polymers: aggregates versus nonaggregates**. *J Phys Chem C* 2007, **111**:555-564.

69. Parakhonskiy BV, Bedard MF, Bukreeva TV, Sukhorukov GB, Mohwald H, Skirtach AG: **Nanoparticles on polyelectrolytes at low concentration: controlling concentration and size**. *J Phys Chem C* 2010, **114**:1996-2002.

70. Kurapati R, Raichur AM: **Graphene oxide based multilayer capsules with unique permeability properties: facile encapsulation of multiple drugs**. *Chem Commun* 2012, **48**:6013-6015.

71. Zhu Z, Senses E, Akcora P, Sukhishvili SA: **Programmable light-controlled shape changes in layered polymer nanocomposites**. *ACS Nano* 2012, **6**:3152-3162.

72. Le Droumaguet B, Nicolas J, Brambilla D, Mura S, Maksimenko A, De Kimpe L, Salvati E, Zona C, Airoldi C, Canovi M *et al.*: **Versatile and efficient targeting using a single nanoparticulate platform: application to cancer and Alzheimer's disease**. *ACS Nano* 2012, **6**:5866-5879.

73. Maity S, Downen LN, Bochinski JR, Clarke LI: **Embedded metal nanoparticles as localized heat sources: an alternative processing approach for complex polymeric materials**. *Polymer* 2011, **52**:1674-1685.

74. Kreft O, Skirtach AG, Sukhorukov GB, Möhwald H: **Remote control of bioreactions in multicompartment capsules**. *Adv Mater* 2007, **19**:3142-3145.

75. Shchukin DG, Gorin DA, Mohwald H: **Ultrasonically induced opening of polyelectrolyte microcontainers**. *Langmuir* 2006, **22**:7400-7404.

76. Skirtach AG, De Geest BG, Mamedov A, Antipov AA, Kotov NA, Sukhorukov GB: **Ultrasound stimulated release and catalysis using polyelectrolyte multilayer capsules**. *J Mater Chem* 2007, **17**:1050-1054.

77. Cuomo F, Lopez F, Ceglie A, Maiuro L, Miguel MG, Lindman B: **pH-responsive liposome-templated polyelectrolyte nanocapsules**. *Soft Matter* 2012, **8**:4415-4420.

78. Hu SH, Tsai CH, Liao CF, Liu DM, Chen SY: **Controlled rupture of magnetic polyelectrolyte microcapsules for drug delivery**. *Langmuir* 2008, **24**:11811-11818.

79. Dong JY, Zink JI: **Taking the temperature of the interiors of magnetically heated nanoparticles**. *ACS Nano* 2014, **8**:5199-5207.

80. Dubreuil F, Elsner N, Fery A: **Elastic properties of polyelectrolyte capsules studied by atomic-force microscopy and RICM**. *Eur Phys J E* 2003, **12**:215-221.

81. Fernandes PAL, Delcea M, Skirtach AG, Möhwald H, Fery A: **Quantification of release from microcapsules upon mechanical deformation with AFM**. *Soft Matter* 2010, **6**:1879-1883.

82. Schmidt S, Madaboosi N, Uhlig K, Koehler D, Skirtach A, Duschl C, Moehwald H, Volodkin DV: **Control of cell adhesion by mechanical reinforcement of soft polyelectrolyte films with nanoparticles**. *Langmuir* 2012, **28**:7249-7257.

83. Vogt C, Mertz D, Benmlih K, Hemmerle J, Voegel JC, Schaaf P, Lavalle P: **Layer-by-layer enzymatic platform for stretched-induced reactive release**. *ACS Macro Lett* 2012, **1**:797-801.

84. Nolte AJ, Rubner MF, Cohen RE: **Determining the young's modulus of polyelectrolyte multilayer films via stress-induced mechanical buckling instabilities**. *Macromolecules* 2005, **38**:5367-5370.

85. Bedard MF, De Geest BG, Moehwald H, Sukhorukov GB, Skirtach AG: **Direction specific release from giant microgel-templated polyelectrolyte microcontainers**. *Soft Matter* 2009, **5**:3927-3931.

86. Schmidt S, Fernandes PAL, De Geest BG, Delcea M, Skirtach AG, Moehwald H, Fery A: **Release properties of pressurized microgel templated capsules**. *Adv Funct Mater* 2011, **21**:1411-1418.

87. • Fernandes PAL, Delcea M, Skirtach AG, Moehwald H, Fery A: **Quantification of release from microcapsules upon mechanical deformation with AFM**. *Soft Matter* 2010, **6**:1879-1883.

The answers on the question: how strong polymeric microcapsules are, can be found in this paper.

88. Zelikin AN, Quinn JF, Caruso F: **Disulfide cross-linked polymer capsules: en route to biodeconstructible systems**. *Biomacromolecules* 2006, **7**:27-30.

89. Skirtach AG, Karageorgiev P, De Geest BG, Pazos-Perez N, Braun D, Sukhorukov GB: **Nanorods as wavelength-selective absorption centers in the visible and near-infrared regions of the electromagnetic spectrum**. *Adv Mater* 2008, **20**:506-510.

90. Gumbley P, Koylu D, Pawle RH, Umezuruike B, Spedden E, Staii C, Thomas SW: **Wavelength-selective disruption and triggered release with photo labile polyelectrolyte multilayers**. *Chem Mater* 2014, **26**:1450-1456.

91. • Skirtach AG, Munoz Javier A, Kreft O, Kohler K, Piera Alberola A, Mohwald H, Parak WJ, Sukhorukov GB: **Laser-induced release of encapsulated materials inside living cells**. *Angew Chem Int Ed* 2006, **45**:4612-4617.

This paper established a possibility of non-destructive release of the contents of microcapsules inside living cells. Thus, cellular processes can be studied and affected from outside, remotely.

92. Javier AM, del Pino P, Bedard MF, Ho D, Skirtach AG, Sukhorukov GB, Plank C, Parak WJ: **Photoactivated release of cargo from the cavity of polyelectrolyte capsules to the cytosol of cells**. *Langmuir* 2008, **24**:12517-12520.

93. Pavlov AM, Sapelkin AV, Huang XY, P'ng KMY, Bushby AJ, Sukhorukov GB, Skirtach AG: **Neuron cells uptake of polymeric microcapsules and subsequent intracellular release**. *Macromol Biosci* 2011, **11**:848-854.

94. • De Geest BG, Willart MA, Hammad H, Lambrecht BN, Pollard C, Bogaert P, De Filette M, Saelens X, Vervaet C, Remon JP *et al.*: **Polymeric multilayer capsule-mediated vaccination induces protective immunity against cancer and viral infection**. *ACS Nano* 2012, **6**:2136-2149.

Vaccine delivery relevant for pharmacology is highlighted in this paper.

95. Pastorino L, Erokhina S, Erokhin V: **Smart nanoengineered polymeric capsules as ideal pharmaceutical carriers**. *Curr Org Chem* 2013, **17**:58-64.

96. Ochs M, Carregal-Romero S, Rejman J, Braeckmans K, De Smedt SC, Parak WJ: **Light-addressable capsules as caged compound matrix for controlled triggering of cytosolic reactions**. *Angew Chem Int Ed* 2013, **52**:695-699.

97. Xiong R, Soenen SJ, Braeckmans K, Skirtach AG: **Towards theranostic multicompartment microcapsules: in-situ diagnostics and laser-induced treatment**. *Theranostics* 2013, **3**:141-151.

98. Gorin DA, Portnov SA, Inozemtseva OA, Luklinska Z, Yashchenok AM, Pavlov AM, Skirtach AG, Moehwald H, Sukhorukov GB: **Magnetic/gold nanoparticle functionalized biocompatible microcapsules with sensitivity to laser irradiation**. *Phys Chem Chem Phys* 2008, **10**:6899-6905.

99. Yashchenok A, Masic A, Gorin D, Shim BS, Kotov NA, Fratzl P, Moehwald H, Skirtach A: **Nanoengineered colloidal probes for Raman-based detection of biomolecules inside living cells**. *Small* 2013, **9**:351-356.

100. Delcea M, Yashchenok A, Videnova K, Kreft O, Möhwald H, Skirtach AG: **Multicompartmental micro- and nanocapsules: hierarchy and applications in biosciences**. *Macromol Biosci* 2010, **10**:465-474.

101. Chandrawati R, Caruso F: **Biomimetic liposome- and polymersome-based multicompartmentalized assemblies**. *Langmuir* 2012, **28**:13798-13807.

102. Yashchenok AM, Delcea M, Videnova K, Jares-Erijman EA, Jovin TM, Konrad M, Moehwald H, Skirtach AG: **Enzyme reaction in the pores of $CaCO_3$ particles upon ultrasound disruption of attached substrate-filled liposomes**. *Angew Chem Int Ed* 2010, **49**:8116-8120.

103. Baeumler H, Georgieva R: **Coupled enzyme reactions in multicompartment microparticles**. *Biomacromolecules* 2010, **11**:1480-1487.

104. Karamitros CS, Yashchenok AM, Moehwald H, Skirtach AG, Konrad M: **Preserving catalytic activity and enhancing biochemical stability of the therapeutic enzyme asparaginase by biocompatible multi layered polyelectrolyte microcapsules**. *Biomacromolecules* 2013, **14**:4398-4406.

105. Pan HM, Beyer S, Zhu Q, Trau D: **Inwards interweaving of polymeric layers within hydrogels: assembly of spherical multi-shells with discrete porosity differences**. *Adv Funct Mater* 2013, **23**:5108-5115.

106. Rivera-Gil P, Nazarenus M, Ashraf S, Parak WJ: **pH-sensitive capsules as intracellular optical reporters for monitoring lysosomal pH changes upon stimulation**. *Small* 2012, **8**:943-948.

107. Kazakova LI, Shabarchina LI, Anastasova S, Pavlov AM, Vadgama P, Skirtach AG, Sukhorukov GB: **Chemosensors and biosensors based on polyelectrolyte microcapsules containing fluorescent dyes and enzymes**. *Anal Bioanal Chem* 2013, **405**:1559-1568.

108. Sato K, Shiba T, Anzai JI: **Ion permeability of free-suspended layer-by-layer (LbL) films prepared using an alginate scaffold**. *Polymers* 2013, **5**:696-705.

109. Song XX, Li HB, Tong WJ, Gao CY: **Fabrication of triple-labeled polyelectrolyte microcapsules for localized ratiometric pH sensing**. *J Colloid Interface Sci* 2014, **416**:252-257.

110. Delcea M, Schmidt S, Palankar R, Fernandes PAL, Fery A, Moehwald H, Skirtach AG: **Mechanobiology correlation between mechanical stability of microcapsules studied by AFM and impact of cell-induced stresses**. *Small* 2010, **6**:2858-2862.

111. Volodkin DV, Delcea M, Möhwald H, Skirtach AG: **Remote near-IR light activation of a hyaluronic acid/poly(l-lysine) multilayered film and film-entrapped microcapsules**. *ACS Appl Mater Interfaces* 2009, **1**:1705-1710.

112. Skirtach AG, Volodkin DV, Moehwald H: **Bio-interfaces-interaction of PLL/HA thick films with nanoparticles and microcapsules**. *ChemPhysChem* 2010, **11**:822-829.

113. Caridade SG, Monge C, Gilde F, Boudou T, Mano JF, Picart C: **Free-standing polyelectrolyte membranes made of chitosan and alginate**. *Biomacromolecules* 2013, **14**:1653-1660.

114. Geryak R, Tsukruk VV: **Reconfigurable and actuating structures from soft materials**. *Soft Matter* 2014, **10**:1246-1263.

115. Skorb EV, Andreeva DV: **Surface nanoarchitecture for bio-applications: self-regulating intelligent interfaces**. *Adv Funct Mater* 2013, **23**:4483-4506.

116. Wu GH, Milkhailovsky A, Khant HA, Fu C, Chiu W, Zasadzinski JA: **Remotely triggered liposome release by near-infrared light absorption via hollow gold nanoshells**. *J Am Chem Soc* 2008, **130**:8175-8177.

117. Troutman TS, Leung SJ, Romanowski M: **Light-induced content release from plasmon-resonant liposomes**. *Adv Mater* 2009, **21**:2334-2338.

118. Peters R, Marguet M, Marais S, Fraaije MW, van Hest JCM, Lecommandoux S: **Cascade reactions in multicompartmentalized polymersomes**. *Angew Chem Int Ed* 2014, **53**:146-150.

119. Chandrawati R, Odermatt PD, Chong S-F, Price AD, Staedler B, Caruso F: **Triggered cargo release by encapsulated enzymatic catalysis in capsosomes**. *Nano Lett* 2011, **11**:4958-4963.

120. Musyanovych A, Landfester K: **Polymer micro- and nanocapsules as biological carriers with multifunctional properties**. *Macromol Biosci* 2014, **14**:458-477.

121. Morton SW, Poon ZY, Hammond PT: **The architecture and biological performance of drug-loaded LbL nanoparticles**. *Biomaterials* 2013, **34**:5328-5335.

122. Reibetanz U, Schoenberg M, Rathmann S, Strehlow V, Goese M, Lessig J: **Inhibition of human neutrophil elastase by alpha(1)-antitrypsin functionalized colloidal microcarriers**. *ACS Nano* 2012, **6**:6325-6336.

123. Volodkin D: **Colloids of pure proteins by hard templating**. *Colloid Polym Sci* 2014, **292**:1249-1259.

124. Mullner M, Cui JW, Noi KF, Gunawan ST, Caruso F: **Surface-initiated polymerization within mesoporous silica spheres for the modular design of charge-neutral polymer particles**. *Langmuir* 2014, **30**:6286-6293.

125. Hood ED, Chorny M, Greineder CF, Alferiev IS, Levy RJ, Muzykantov VR: **Endothelial targeting of nanocarriers loaded with antioxidant enzymes for protection against vascular oxidative stress and inflammation**. *Biomaterials* 2014, **35**:3708-3715.

126. Datta SS, Abbaspourrad A, Amstad E, Fan J, Kim SH, Romanowsky M, Shum HC, Sun BJ, Utada AS, Windbergs M *et al.*: **25th anniversary article: double emulsion templated solid microcapsules: mechanics and controlled release**. *Adv Mater* 2014, **26**:2205-2218.

127. Shchepelina O, Drachuk I, Gupta MK, Lin J, Tsukruk VV: **Silk-on-silk layer-by-layer microcapsules**. *Adv Mater* 2011, **23**:4655-4660.

128. Blum C, Nichtl A, Scheibel T: **Spider silk capsules as protective reaction containers for enzymes**. *Adv Funct Mater* 2014, **24**:763-768.

129. Delcea M, Sternberg N, Yashchenok AM, Georgieva R, Baeumler H, Moehwald H, Skirtach AG: **Nanoplasmonics for dual-molecule release through nanopores in the membrane of red blood cells**. *ACS Nano* 2012, **6**:4169-4180.

130. She SP, Li QQ, Shan BW, Tong WJ, Gao CY: **Fabrication of red-blood-cell-like polyelectrolyte microcapsules and their deformation and recovery behavior through a microcapillary**. *Adv Mater* 2013, **25**:5814.

131. Vergaro V, Scarlino F, Bellomo C, Rinaldi R, Vergara D, Maffia M, Baldassarre F, Giannelli G, Zhang X, Lvov YM *et al.*: **Drug-loaded polyelectrolyte microcapsules for sustained targeting of cancer cells**. *Adv Drug Deliv Rev* 2011, **63**:847-863.

132 • Palankar R, Pinchasik B-E, Khlebtsov BN, Kolesnikova TA, Moehwald H, Winterhalter M, Skirtach AG: **Nanoplasmonically-induced defects in lipid membrane monitored by ion current: transient nanopores versus membrane rupture**. *Nano Lett* 2014, **14**:4273-4279.
In this paper release methods developed for capsules and liposomes are applied to the area of nanopore. Monitoring ionic current through the nanopore covered with a lipid membrane allows for precise monitoring of the permeability change upon an external modulation. Universality and applicability of this approach to different membranes is remarked here.

133. Krpetic Z, Nativo P, See V, Prior IA, Brust M, Volk M: **Inflicting controlled nonthermal damage to subcellular structures by laser-activated gold nanoparticles**. *Nano Lett* 2010, **10**:4549-4554.

134. Urban AS, Pfeiffer T, Fedoruk M, Lutich AA, Feldmann J: **Single-step injection of gold nanoparticles through phospholipid membranes**. *ACS Nano* 2011, **5**:3585-3590.

135. Huhn D, Govorov A, Gil PR, Parak WJ: **Photostimulated Au nanoheaters in polymer and biological media: characterization of mechanical destruction and boiling**. *Adv Funct Mater* 2012, **22**:294-303.

136. Lukianova-Hleb EY, Mutonga MBG, Lapotko DO: **Cell-specific multifunctional processing of heterogeneous cell systems in a single laser pulse treatment**. *ACS Nano* 2012, **6**:10973-10981.

137. Xiong RH, Raemdonck K, Peynshaert K, Lentacker I, De Cock I, Demeester J, De Smedt SC, Skirtach AG, Braeckmans K: **Comparison of gold nanoparticle mediated photoporation: vapor nanobubbles outperform direct heating for delivering macromolecules in live cells**. *ACS Nano* 2014, **8**:6288-6296.