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Polymeric microcapsules with light responsive properties for encapsulation and release

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Abstract

This review is dedicated to recent developments on the topic of light sensitive polymer-based microcapsules. The microcapsules discussed are constructed using the layer-by-layer self-assembly method, which consists in absorbing oppositely charged polyelectrolytes onto charged sacrificial particles. Microcapsules display a broad spectrum of qualities over other existing microdelivery systems such as high stability, longevity, versatile construction and a variety of methods to encapsulate and release substances. Release and encapsulation of materials by light is a particularly interesting topic. Microcapsules can be made sensitive to light by incorporation of light-sensitive polymers, functional dyes and metal nanoparticles. Optically active substances can be inserted into the shell during their assembly as a polymer complex or following the shell preparation. Ultraviolet-addressable microcapsules were shown to allow for remote encapsulation and release of materials. Visible- and infrared-addressable microcapsules offer a large array of release strategies for capsules, from destructive to highly sensitive reversible approaches. Besides the introduction and conclusions, this review contains in four sections reviewing the effects of light 1) on polymer-based microcapsules, 2) microcapsules containing metal nanoparticles and 3) functional dyes, as well as a fourth section that revisits the implications of light addressable polymeric microcapsules as a microdelivery system for biological applications. © 2009 Elsevier B.V. All rights reserved.

Keywords: Microcapsules; polyelectrolytes; layer-by-layer; drug delivery; metal nanoparticles; encapsulation; UV; IR; porphyrin; azobenzene

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1. Introduction

Selective permeability is an essential attribute of membranes. The ability of certain ions and molecules to pass across membranes of living cells is the result of biological compartmentalization that separates certain cellular components into sub-environments appropriate to carry on their activities. Microencapsulation technologies inspire themselves from natural occurrences to create artificial micron- and nano-sized containers with the ability to encapsulate or release substances with the help of triggers. Over the last 50 years, there were reports on various hollow shells with the capability to encapsulate and release materials. However, most of these present a fragile construction and come with many limitations in terms of the template materials that could be used and the shell constituents.

In the 1990s Decher and the group led by Möhwald (MPI-KG, Germany) developed and perfected the electrostatic self-assembly (ESA) method \(^1\) (first proposed by Iler in 1966 \(^2\)) by which thin films can be repetitively deposited onto a surface to produce multilayered films. Unlike multilayer films obtained by the well established Langmuir-Blodgett deposition technique for instance, ESA was completely indifferent to the type of substrate used so long as it is charged. The driving force for ESA resides in creating a surface charge excess, or overcompensation, at the interface of a material of interest. This technique is now commonly referred to as layer-by-layer (LbL) assembly. Originally designed on planar surfaces, LbL films were soon used to coat colloidal particles which are later dissolved to produce hollow polymeric shells (Fig. 1.1) \(^3, 4\). LbL polymeric microcapsules possess significant advantages over other types of microshells (e.g. micelles, liposomes, inorganic hollow shells) notably in their mechanical behavior and the possibility to control shell composition, thickness, stiffness, roughness, density, hydration, diameter and permeability to various degrees \(^5, 6\). Emulsion-based microcapsules and encapsulation of substances within them was demonstrated as early as the 1960s, with the publication of a method to stabilize droplets of organic solvents in aqueous media using polyelectrolytes \(^8\).

![Fig. 1.1. Schematic of the LbL assembly of polymeric microcapsules. The procedure begins by coating a charged colloid with a polyelectrolyte of opposite charge (1). An oppositely charged molecule (2) or polyelectrolyte (3) can then be used to coat the first layer. These steps may be repeated taking care to alternate the charge of the coating material at each addition. The subsequent decomposition of the template (4) yields a hollow polymeric shell.](image)

This review is meant to give to the reader an overview of the recent development on the optical approaches for the release and encapsulation of substances using polymer-based microcapsules. Microcapsules constructed of polyelectrolytes only, or impregnated with dyes or metal nanoparticles were shown to exhibit different types of behavior when irradiated with light from the near-UV to near-IR region of the electromagnetic spectrum. For microcapsules built of polyelectrolytes only, the effects of UV have been investigated. In section 2, it will be shown that UV irradiation has a strong effect on the chemical composition of capsules, yet can also be used to encapsulate substances. Small molecules such as dyes are typically used for visualization purposes. In section 3, we will discuss how metal nanoparticles such as TiO\(_2\), silver and gold can be used both, to destructively and non-destructively change the permeability and mechanical attributes of microcapsules when exposed to light. In section 4, the effects of visible and IR irradiation on the
stability and morphology of microcapsules containing fluorescent and functional dyes will be presented. Finally, section 5 gives the reader a brief summary of the recent biological studies using these optically addressable microshells. The review is an attempt to summarize the work done in the past five years as well as the current developments on the topic of light addressable polymeric microcontainers. Recently emerging challenges and newly found solutions in using these capsules for in vivo studies suggest that the topic of optically addressable microshells is on the rise.

1.1. Preparation of polyelectrolyte microcapsules and proximal factors for encapsulation and release

The preparation procedures of LbL polymeric microcapsules have been thoroughly described in the literature and are summarized in Fig. 1.1. Briefly, capsules are constructed in solution using one of two self-assembly procedures: 1) sequential additions of polymer solution, followed by centrifugation to separate the coated colloids from the supernatant, and 2) using a microfiltration setup that allows the removal of supernatant without the extra steps of centrifugation. Microcapsules can be constructed on solid particles, liquid droplets and more recently microbubbles. The latter two templates are more fragile and challenging to manipulate and are occasionally used to stabilize multiphase systems. Solid sacrificial templates can be of organic nature (latexes, gel beads) or inorganic micro- and nanometer sized particles (silica, CaCO₃).

The shell constituents usually require the use of at least one polyelectrolyte and can be anything with sufficient charge density, from synthetic polyelectrolytes to sugars and DNA. The creation of layers composed of small or low charge density molecules by electrostatics can be difficult since all the charges on low molecular weight materials can easily find a countercharge, neutralizing the surface charge as opposed to reversing it. Amongst recently published novelties in the production of single component microcapsules, emphasis can be put on the use of chemical cross-linking and click chemistry which enabled to fix small molecules or same charge materials into multilayer complexes, or to strengthen the LbL film. Diblock copolymers were also used to produce single component microcontainers. Recently, Wang et al. constructed thick-walled microcapsules using a mesoporous substrate treated with a single layer of polyelectrolyte and chemically cross-linking the polymers before dissolving the template.

Polyelectrolyte microcapsules have a number of interesting properties and carry a great potential for diverse applications. For one, capsules are essentially semi-permeable membranes with selective permeability to certain molecules. Controlling the permeability of the thin shell of microcontainers is perhaps the most challenging and interesting topic in the area of micro-encapsulation. Encapsulation of a substance that initially permeates the LbL microshells can be accomplished by increasing the wall density, either chemically (i.e. forming cross-links in the shell) or by physical means. The latter involves changing the permeability of microcapsules using one of various strategies including light, solvent exchange, high temperature and pH. Another way to encapsulate a substance that initially does not permeate the shell is by co-precipitation with CaCO₃ and use of the loaded CaCO₃ as a sacrificial template for the capsules. Emulsion-emulsion synthesized microgels constitute alternative templates for microcapsule construction. The most important consequence of being able to decrease the permeability of microcapsules is, therefore, the possibility to encapsulate substances in the cavity of the microcontainer.

Triggers that can be used to release encapsulated substances from the microcapsule cavity can be grouped in two categories: 1) proximal triggers that require a physical interaction with the capsule solution or the capsules themselves and 2) external triggers, which require no interaction or alteration of the capsule or capsule solution properties. Proximal triggers that were found to affect LbL capsules include the application of force on the capsule to damage it. Physical interaction was used to study the mechanical behavior of microcapsules and determine the parameters that improve their resistance to deformation. Other proximal triggers to induce release of microcapsules require softening the electrostatic interactions changing solution parameters such as pH and ionic strength, or in the case of capsules built from biological polyions (e.g. DNA, polypeptides, polysaccharides), the addition (or presence) of appropriate enzymes. However, for many applications such as biomedical or surface coating technologies, it is desirable to address the microcontainer using external means that require no direct interaction with the capsule medium.

1.2. Magnetism, ultrasound and microwaves as external triggers for release

External triggers with the ability to affect microstructures come in the form of magnetic force as well as in three flavors of electromagnetic waves; ultrasonic, microwaves and light. The development of microcontainers that are highly sensitive to any of these triggers has important implications, especially in the fields of health and surface sciences, where sometimes microdelivery systems cannot be addressed with proximal triggers. The magnetic force is used primarily to localize and visualize materials that contain magnetic or paramagnetic constituents. Lu et al. were able to use an alternating magnetic field to change the permeability
of microcapsules impregnated with Co-Au core shell nanoparticles. The magnetic field in this case, induced the nanoparticles to rotate damaging the shell, irreversibly increasing the wall permeability. A clear example of release of encapsulated material using magnetism as a remote trigger was demonstrated very recently by Hu et al. Magnetic microcapsules with an anti-cancer drug could allow the medical practitioner to position and localize the microcontainers around a target area of sick tissue, minimizing the dose necessary and the corresponding side effects of the treatment.

Ultrasound radiation with frequencies in the order of kilo and mega Hertz (20 kHZ- 10 MHz) are extensively used for the synthesis of nanomaterials as well as for imaging and diagnostic purposes in medicine. Ultrasound can lead to the cavitation and collapse of microbubbles from dissolved gases, a highly energetic process with the capability of breaking molecular bonds. Taking advantage of this effect, ultrasound was recently introduced as an alternative approach to release encapsulated materials from polymeric microcapsules. Microwaves on the other hand, possess shorter waves than ultrasound spanning electromagnetic frequencies between 0.3 GHz and 300 GHz. Microwaves have more subtle interactions with matter than ultrasound and are not energetic enough to ionize matter. It was recently shown that microwaves can be used to control the aggregation of proteins and demonstrated that low intensity microwaves (2-8 GHz) can lead to the destruction of LbL microcontainers. The destructive effect of microwave exposure was especially strong in shells that contained silver nanoparticles. This nanoparticle-microwave interaction could be another promising approach to remotely affect the properties of capsules. Light radiation with wavelength in the approximate range 200-1200 nm is the most studied type of remote trigger used with polymeric capsules. The remaining of this text will be dedicated to the various aspects of optically addressable polymeric microcapsules. One of the prominent applications of microcapsules is intracellular delivery and release. In this regard, the optical (or more specifically near-IR ~ 800 nm) spectral range is one of the most biologically friendly regions of the electromagnetic spectrum.

1.3. Light addressable strategies to affect microcapsule permeability

One of the priorities of recent research on polymeric microcontainers has been to control the shells’ permeability using mild external triggers such as light. The first studies to remotely release encapsulated materials from microcapsules involved the use of pulsed (modulated) laser sources or microcontainers doped with very large amounts of metal nanoparticles, both resulting in the destruction of the microcontainer. Concerns with regards to the explosive destruction of microcontainers in various applications and the potential toxicity of metal nanoparticles in cells led to focus the research on significantly reducing the radiation energy requirements for capsule activation and minimizing the concentration of light sensitizing agents in microshells.

2. Effect of Ultraviolet Light on Polymer-Based Microcontainers

The main constituents of LbL microcapsules are polymers consisting primarily of atoms of oxygen, carbon, nitrogen and hydrogen held together by covalent bonds with energies of the order of 100 kcal·mol⁻¹. Far-UV irradiation (λ < 200 nm) is energetic enough to ionize covalent bonds of such polymers (and most molecules around them) and therefore polymeric microcapsules are assumed to absorb irradiation at far-UV wavelengths. At sufficient intensity, the saturated bonds of the polymers are cleaved by laser ablation, the thermodynamic process by which bonds within molecules are broken upon absorption of photons. However, the underlying mechanisms of polymer ablation under far-UV irradiation are complex and not suited for this review. Because of its highly unspecific, destructive nature towards organic materials, far-UV is not a useful source of irradiation to control the encapsulation and release of relatively fragile structures such as polyelectrolyte microcapsules. At longer wavelengths (> 200 nm), specific absorption may occur in polyelectrolytes but in this case correlates with other materials or chromophores present in the capsules, such as nanoparticles or unsaturated side chains attached to the polymers. Many polyelectrolytes discussed in the literature, which were used in the construction of LbL capsules absorb somewhere in the near-UV region (≈200-400 nm). This section summarizes those investigations on the effects of near-UV irradiation of microcapsules built from polyelectrolytes only.

2.1. Destruction of polymer-based microcapsules by UV

Experiments using a pulsed laser (Nd:YAG, B.M. Industries, YAG 502 DPS 7910DP) operating at 355 nm and fluence up to 1 J/cm² to irradiate microcapsules built from polymers such as poly(allylamine hydrochloride) (PAH), (PDADMAC), poly(vinylsulfonate) (PVS) and aromatic containing poly(styrene sulfonate) (PSS) were conducted (unpublished results). None of these polymers absorb significantly at 355 nm. Irradiation of microcapsule solutions was found to leave the capsules undamaged when using relatively low laser fluence (<400 mJ/cm²) after an exposure time of up to one hour (35 ps per pulse, repetition rate of 10 Hz). Between energy densities of 40-100 mJ/cm² capsules remained unaffected
or became deformed after a few pulses. This is illustrated in Fig. 2.1 before (A) and after (B) exposure to the UV laser beam for (PAH/PSS)$_n$ microcapsules at a fluence of 70 mJ/cm$^2$. At fluence greater than 100 mJ/cm$^2$, the microcontainers became severely damaged. This is shown in Fig. 2.1 D where capsules (C) were irradiated with 10 pulses at 200 mJ/cm$^2$ resulting in some shells appearing as if cut to pieces. Because the investigated capsules lacked strong absorption, this effect can be attributed to (1) pressure of light 78–80 acting on relatively weak capsules 84, 45, (2) weak absorption and strong field and (3) plasma generation (which depends on factors such as; molar absorptivity of the irradiated material, penetration depth of the laser beam and irradiation wavelength used 75). While capsules damaged by this approach could also be effectively opened to release encapsulated substances, UV ablation is a very energetic process which could equally damage the encapsulated substance. To avoid these undesirable effects, much milder approaches, such as longer wavelengths, continuous wave (CW) lasers or lamps are preferable for microcapsule applications involving remotely addressable encapsulation and release.

Fig. 2.1. Ablation effects on (PAH/PSS)$_n$ microcapsules in water after exposure to pulsed UV irradiation at 355 nm. Top: three capsules before (A) and after exposure to 20 laser pulses at a fluence of 70 mJ/cm$^2$ tend to deform or break. Bottom: five capsules before (C) and after (D) exposure to 10 laser pulses at a fluence of 200 mJ/cm$^2$ are often completely destroyed.

The effects of CW near-UV irradiation on microcapsules were also investigated 25, 81. Katagiri et al. demonstrated that the irradiated LbL capsules containing near UV absorbing chromophores (i.e. PSS/PDADMAC, DNA/PAH, PSS/PAH) are degraded to various oligomers, ions, radicals and oxides 81. From their observations it was concluded that the decomposition products originated from the destruction of capsule materials that absorb significantly in the near-UV such as the aromatic styrene groups of the polymer PSS, which possesses an absorption maximum at ~225 nm. The capsules remained seemingly unaffected by UV when constructed from only non-aromatic polymers 87. A second notable effect of capsule irradiation with CW near-UV was that the shells had a tendency to shrink during illumination 83, which is relevant because shrinkage could mean wall densification and the possibility to encapsulated materials. In such capsules, however, the diameter decreased similarly for shells with an even and uneven number of layers putting aside UV-induced heating as a causative parameter for shrinkage. Changes in capsule diameter due to temperature belong to an important entropy related effect of polyelectrolyte microcapsules that is a direct consequence of the charge balance across the shell 82.

Heat-shrinkage of microcapsules as a function of LbL structure was thoroughly investigated by Köhler and coworkers who noted that shells constructed with an even number of layers tended to shrink upon heating, while built from an uneven number of layers the shells become larger 82. The opposing behaviors upon heating the capsules are attributed to a balance between two forces; (1) hydrophobic surface forces and (2) electrostatic repulsive forces due to like charges 83, 84. For example, when the net charge across the capsule wall is close to neutrality the hydrophobic main chains of the polymers within the shell force this one to minimize its surface area in contact with water, resulting in the capsule shrinking. It should be noted that this effect can be different, see completely reversed depending on the solution properties (e.g. pH, buffer) 85. Since no thermal related effects were found for shrinking capsules irradiated with UV, it can be assumed that UV effects are predominantly of chemical origin rather than physico-chemical.

A far more versatile approach to sensitize microshells to UV irradiation, however, involves impregnating polymer capsule constructs with metal oxide photocatalytic nanoparticles (e.g. TiO$_2$) to form polymer/nanoparticle composite films. This will be discussed in more detail in section 3.1.

### 2.2. Microcapsules containing azobenzene moieties

An interesting group of molecules that responds to both near-UV and visible light are azobenzenes. An azobenzene molecule contains two phenyl groups joined by a azo (N=N) bond 86. Azobenzene groups respond to UV absorption by undergoing reversible internal rearrangements termed cis-trans (Z-E) isomerizations (Fig. 2.2). Thermal isomerization typically occurs from the least stable cis to the most stable trans moiety (Z→E), while optical isomerization can occur either way. At the
molecular level, these cis-trans isomerizations come with significant changes in terms of molecular geometry and polarity, but they are rather weak when incorporated in macroassemblies. The largest of absorbance region of azobenzene molecules is situated in the near-UV (Fig. 2.2, II→II* but can extend to the visible range depending on the phenyl substitution, solvent and other factors 87. A second less intense absorption region (Fig. 2.2, n→II*) is typically located at longer wavelengths. Fig. 2.2 illustrates that the intensity of the two peaks varies oppositely depending on the degree of cis-trans isomerization. Cis-trans isomerization is often reversible and it was shown very recently that lipid vesicles containing azo moieties could be made to bud into multiple vesicles upon UV light exposure and fuse with one another when exposed to green light 88. Diazoresins (DZR) were shown to form covalent bonds with poly(acrylic acid) or PSS in LbL films upon UV irradiation 13, 89-92. Similarly, composite shells of diazoerin (DZR) and PSS could be polymerized by exposure to ultraviolet light 93, 94. The resulting cross-linked DZR/PSS microcapsules displayed superior mechanical stability over capsules that were not exposed to light 94. Photosimerization in LbL films on planar support and microcapsules was shown using main chain polyazo molecules as a mean to destroy polymer aggregates by optically forcing cycles of trans-cis-trans isomerization within the films 95.

The incorporation of the azobenzene molecule Brilliant Yellow (BY) in LbL films of the type BY/PAH showed slow isomerization kinetics in comparison to pure azo films 96. Additionally, isomerization in such films was irreversible whereas photodegradation was detected at low levels. The potential use of azobenzene molecules to control the permeability of microcapsules was briefly investigated 97. Reversible isomerization of azobenzene groups in LbL films was shown to occur in various films 96,100 including some containing the polyanion poly{1-4[4-(3-carboxy-4-hydroxyphenyl-azo)benzene-sulfonamido]-1,2-ethanediyl] (PAZO) 101-103. PAZO is a polyelectrolyte with carbonated azobenzene side chains and a II→II* absorption centered at 366 nm. This polyelectrolyte was used in the design of capsules that shrink upon UV exposure in the study of the optical properties of gold nanoparticles attached to the shell 104.

LbL films constructed with PAZO were used to develop the first light induced encapsulation strategy for polymer microshells 25. Azobenzene-substituted PAH with substitution ratio from 1 to 10%, were used to assemble LbL microcapsules. Cis-trans isomerization was detected in those polymers but failed to occur when these were present in a LbL system of the type (Azo-PAH/PSS)ₙ or (Azo-PAH/PVS)ₙ (unpublished results). Alternatively, microcapsules built from the polyelectrolytes PAH, PAZO and PVS to obtain the construction (PAH/PAZO)ₙ/PVS were reported to shrink and allow the encapsulation of macromolecules after irradiation under near-UV light (300-400 nm); however, the permeability change was found to be irreversible 25. PAH and PVS possess no significant absorption for this irradiation. Heating the capsules up to 90°C for prolonged time did not lead to shrinking and local rearrangements due to annealing effects were excluded.

![Fig. 2.2. Cis-trans isomerization of azobenzene molecules upon irradiation. Top and middle panels: Exposure to light in the 300-400 nm window (pink) typically leads to an E → Z conformational change whereas heat or exposure to light at wavelengths above 400 nm (blue) favors the Z → E change. A change in conformation in the E → Z direction is accompanied by a decrease in the main II→II* absorption peak and a small increase in the n→II* region. The spectroscopic changes are reversible. Bottom panel: Confocal microscope image of (PAH/PAZO)ₙ/PVS capsules, showing that the shell is permeable to fluorescent dextran, before UV irradiation (A) and washing the supernatant left empty capsules (B). Encapsulation of the fluorescent dextran (C) occurred after exposure of the capsules to UV light in the presence of fluorescent dextran. Figure reprinted from [25]. Copyright Wiley-VCH Verlag GmbH & Co. KGaA.](image-url)
3. Metal and Metal Oxide Nanoparticles in Microcontainers

Metal and metal oxide nanoparticles lend themselves to a variety of applications from the formation of inorganic thin films with well defined pore size \(^{105}\) and the catalytic synthesis of other nanoparticles \(^{106, 107}\), to the formation of polymer/nanoparticles composite films \(^{108}\). Nanoparticles of silver and gold absorb light in the visible spectrum and release this energy as heat in their surroundings \(^{109-112}\). This is due to surface plasmon resonance absorption of the nanoparticles, whose absorption cross-section is drastically more intense (> 10\(^6\)) than for a typical dye. In the case of silver and gold, these oscillations termed plasmons have frequencies of approximately 425 nm and 525 nm, respectively. The heat produced by such nanoparticles can be harvested to release encapsulated substances from microcapsules destructively (1) or non-destructively (2) as illustrated in Fig. 3.1. A review on nanoparticle-modified polymeric capsules with much emphasis on the biological applications of such shells was recently published \(^{35}\).

![Fig 3.1. Schematic of the two possible release scenarios of encapsulated material by the laser nanoparticle interaction. (1) Upon illumination, the nanoparticles produce a large amount of heat that breaks the capsule wall open. (2) During illumination, the nanoparticles produce a small quantity of heat sufficient to exceed the glass transition of the polymer complex of the capsule, decreasing the shell’s permeability until illumination is stopped. The increased permeability allows for encapsulated material to be released from the capsules without the shell being damaged.](image)

3.1. Titanium oxide nanoparticles

Titanium dioxide particles and related composites are well known for their catalytic activity and oxidative potential \(^{105, 113}\). They have low production costs and a strong absorbance in the UV, making such nanoparticles a smart choice of compound to sensitize thin films to UV light \(^{114, 115}\). Several reports have been already published on the incorporation of TiO\(_2\) nanoparticles into microcapsules, via in-situ synthesis of the nanoparticles within films \(^{116-119}\), doping \(^{106, 120}\) as a component layer in LbL films \(^{121}\) and as an encapsulated substance \(^{122}\). Katagiri et al. \(^{123}\) showed that organic-inorganic microcontainers impregnated with TiO\(_2\) rupture upon exposure to UV light at relatively low intensity (5-20 mW cm\(^{-2}\)) \(^{118}\). The organic part of such hybrid capsules contributes to considerably reduce the permeability of the shell to small hydrophilic materials. Such capsules have a great potential as a container for self-healing coatings \(^{124}\) where light activated release could be triggered by any irradiation source. This system would also be particularly interesting as a drug delivery candidate if; (1) inorganic templates (e.g. CaCO\(_3\), SiO\(_2\)) instead of melamine formaldehyde (MF) particles, which are known to affect the microcapsules mechanics and leave toxic melamine oligomers in the shell structure after core decomposition \(^{125, 126}\), (2) visible or near-IR addressable particles were incorporated in the structure \(^{127, 128}\). An overview of various templates for microcapsules and various encapsulation and release mechanisms was recently published \(^{129}\).

3.2. Silver nanoparticles

The use of noble metal nanoparticles to optically induce changes in the shell of microcapsules was first demonstrated by Skirtach and coworkers who doped silver nanoparticles into PAH/PSS microshells and studied their response to light irradiation \(^{73}\). Absorption of light in the surface plasmon spectral region of the nanoparticles results in heat production that can affect the capsule wall (Fig 3.1). The main absorption region of silver nanoparticles spans the 380-430 nm wavelengths and the position of its absorption maximum depends largely on the size and shape of the particles \(^{130, 131}\). The use of wavelengths in the main absorption region of silver nanoparticles is not ideal for therapeutic treatments such as drug or gene delivery since many biological molecules absorb light outside the so-called “biologically friendly window”, i.e. 700-1000 nm. The residual absorption of silver nanoparticles with a diameter of 20 nm in the near-IR was shown to be sufficient to produce heat and break microcapsules \(^{73}\) but insufficient for microcapsules containing 4 nm silver nanoparticles \(^{132}\). The irradiation time necessary to break microcapsules also depends on the size of the nanoparticles. Using a 532 nm CW laser, Radzuk et al. \(^{131}\) showed that capsules impregnated with 10 nm silver nanoparticles took on average 22 seconds to open whereas it took 10 seconds of laser exposure using 18 nm nanoparticles \(^{133}\). Interestingly, the combination of the two nanoparticle sizes in capsules shells decreased the laser exposure requirements to 5 seconds or less \(^{133}\). Antimicrobial and self-healing coatings are great candidates for light sensitization with silver nanoparticles \(^{134}\).
3.3. Gold nanoparticles

Like silver nanoparticles, gold nanoparticles absorb light predominantly in the visible range, at around 520 nm. It is also known, however, that changing the surface properties, shape, size, synthetic approach and the distance between neighboring gold particles can affect the absorption spectra of gold nanoparticles. In general, these effects come with a red-shift in the main plasmon absorption region of the nanoparticles or in the appearance of a second absorption region at longer wavelengths. The possibility of tuning the main absorption region of gold nanoparticles significantly increases the versatility of the laser/nanoparticle interaction and consequently makes it possible to get the most out of the gold nanoparticles as a visible light and near-IR sensitizer for microcontainers.

The effects of modulated near-IR pulses (1064 nm) were investigated using an average intensity of 30-700 mJ cm\(^{-2}\) per pulse to simultaneously damage multiple polymeric microcapsules containing one or more layer of densely packed gold nanoparticles. While it was demonstrated that many capsules could be damaged by continuous irradiation, this remote release approach has rather large energy requirements despite the presence of nanoparticles. More recently, a modulated IR source was used to open multi-compartment, “shell-in-shell” capsules. Figure 3.2 illustrates a shell-in-shell container designed by coating CaCO\(_3\) particles with polyelectrolytes and gold nanoparticles (inner capsule), followed by precipitating a CaCO\(_3\) shell around the former and coating the whole with polyelectrolytes (outer capsule). After the dissolution of the inorganic CaCO\(_3\) matrices, the inner, light sensitive polymeric container is encapsulated within a larger polymer capsule.

![Diagram of laser opening a shell-in-shell type microcontainer.](image)

Using an 840 nm near-IR pulsed laser, it was possible to open the inner container and release its content into the larger container. At the low radiant power used (10 \(\mu\)J/pulse), the outer shell, which contained no light sensitive particles, could not be opened in control experiments. De Geest et al. made light responsive hybrid capsules that could be destroyed by laser irradiation using CaCO\(_3\) as a sacrificial template. Microcapsules constructed using mesoporous CaCO\(_3\) as a template are known to possess rather thick walls and are consequently assumed to be mechanically more stable than shells built from “smooth” templates (MF, PS, SiO\(_2\)), which typically possess thin shells. Wu et al. used gold nanoshells to remotely release the content from liposomes using pulsed IR light. The incorporation of nanoparticles in the capsule wall was recently shown to have a beneficial impact on the mechanical stability of microcapsules, but this effect tends to be outweighed by the effects of wall thickness which have a greater influence on the shell’s relative stability.

Concerns with regards to the destructive use of pulsed lasers to remotely open polymer microcapsules were raised in section 2.1. Unlike UV laser ablation, a photochemical process in which energetic photons disrupt molecular bonds, the effects of IR ablation are due to thermal processes. Pola and coworkers have conducted several investigations on the decomposition of various bulk polymers by IR laser ablation. However, laser irradiation in liquids also involves molecular collisions of the irradiated material with the solvent which can deactivate the excited molecule. The shell of multilayered polymeric capsules containing metallic nanoparticles is partially hydrated and contains molecular interactions absent in bulk polymer making IR ablation events even more complex and less likely to occur. With regards to the presence of gold nanoparticles in the capsule wall, Volkov et al. recently investigated the theoretical energy transfer of colloidal gold in water when irradiated with laser pulses. Their results show that at high energy density, irradiating gold nanoparticles in water can result in exceeding the critical temperature of water (T\(_c\) = 647 K) with the explosive formation of vapor bubbles. This effect is accompanied by significant pressure and temperature increment that exponentially decays with distance from the nanoparticle surface. The effects of IR ablation on living tissues have been thoroughly reviewed by Vogel and Venugopalan.

These effects also support the observations made by the groups of Caruso and Möhwald by which release of material by pulsed IR irradiation from gold functionalized capsules occurs, at least partially, due to the laser/nanoparticle interaction. Still, there are no reports on the mechanico-chemical effects of pulsed lasers on polymeric microcapsules. Therefore, it cannot be excluded that the observed release may be partially due to laser ablation, independently from the gold nanoparticles within the shell. It would be necessary to elucidate whether remote release of encapsulated substances is truly an effect only due to the presence of nanoparticles in the aforementioned experiments, for...
instance by using the same microcapsule designs as Angelatos et al. 135 or Kefet et al. 37 but impregnating them with a low surface coverage of gold nanorods 128 or nanoassemblies of gold nanoparticles 147, both of which have strong intrinsic absorption in near-IR, instead of a densely packed shell of gold nanoparticles. If the opening observed in the aforementioned reports is only due to the laser/plasmon heat contribution, one could theoretically expect that the presence of a single gold nanorod or nanoassembly per capsule should be sufficient to open the capsules (covered in sections 3.4 and 3.5, respectively). Yet, using CW irradiation is still preferable for remote release applications that require greater control over permeability in a non-destructive manner.

3.4. Gold nanoparticles aggregates

In order to significantly reduce the irradiation intensity requirements for optical release from polymeric microcapsules and obtain more sensitive films with controllable properties, emphasis must be put on the organization of the films. Carefully selected thin film designs could allow one to control the wall permeability and replace current destructive approaches to optically address microcontainers with reversible encapsulation and release protocols, as illustrated in Fig. 3.1 127. Controlling the distribution of gold nanoparticles is one of these methods by which clusters or aggregates of nanoparticles have properties that differ from single nanoparticles in two aspects: (1) nanoparticle assemblies tend to absorb light at longer wavelengths than their individual entities 147,149, (2) the surface area of nanoparticle assemblies is much greater than of single nanoparticles 127, 147. Lu et al. also devised a method to reverse the aggregation of gold nanoparticles along with the infrared absorption of gold aggregates being reversed 148. However, controlling the distribution of nanoparticles in solution while maintaining stable optical properties is not a simple task. To obtain some degree of controlled interaction between nanoparticles one must bring the nanoparticle close enough for their electronic shells to interacts and stabilize in this manner while preventing that the whole system goes uncontrolled forming very large aggregates. Polyelectrolytes can be used for this purpose 150, 151. Skirtach et al. observed that optical properties and stability of different types of gold nanoparticles (core-shell and DMAP-stabilized) in presence of oppositely charged polyelectrolytes greatly vary. After the addition of a proper polyelectrolyte to a solution of gold nanoparticles, clusters of gold nanoparticles form. The aggregation of nanoparticles can be stopped along with the corresponding changes in absorption spectra, by transferring the solution of growing gold complexes to a suspension of LbL coated microparticles to which the aggregates adhere, stopping their growths. This method referred to as “arrested aggregation” provides a convenient way to visualize the assembly behavior of nanoparticles since the particles do not detach from the LbL coating 147. Increased sensitivity to near-IR irradiation was observed in gold-doped microcapsules templated on CaCO₃ microparticles 152. This effect was attributed to the porous nature of CaCO₃ which favors interactions between neighboring gold nanoparticles, influencing their absorption at longer wavelengths. Bédard et al. recently demonstrated the advantageous optical properties of assemblies of gold nanoparticles and their use to sensitize microcapsules to near-IR irradiation (Fig. 3.3) 147. In this case, aggregation of the nanoparticles was obtained by adding an aliquot of citrate stabilized gold nanoparticles into a stirring aliquot of salt solution. A change in color from red to blue-gray resulted within seconds as single nanoparticles form dimers, and dimers join single nanoparticles or other dimers to larger aggregates. Volodkin et al. recently used a similar strategy to open liposome/nanoparticle assemblies 152.

Fig. 3.3. Release of encapsulated material using a CW near-IR laser diode. Capsules impregnated with homodispersed nanoparticles before (A) and after (B) laser irradiation did not release their cargo (C). Capsules impregnated with aggregates of nanoparticles before (D) and after (E) irradiation released their cargo. The threshold intensity at which 50 % of the microcapsules released their content was approximately 40 mW. From [147]. Reprinted with permission of AAAS.

Mixing growing nanoparticle assemblies with microcapsules allows controlling the average size of the assemblies and thus, their main absorption region. Two types of capsules containing the same encapsulated molecule and differing only in the distribution of the gold nanoparticles in their shell were subjected to laser irradiation: only the ones with assemblies of nanoparticles released their content 147. This is because, as previously explained, singly distributed nanoparticles do not absorb significantly in the IR region, but in the proximity of other nanoparticles the plasmon interacts and couples producing an absorbance band at longer wavelengths 149. Theoretical simulations revealed that in small aggregates at low laser intensity, the average temperature increase around the nanoparticles could easily reach 10 K against less than 1 K for a single particle 147. A few Kelvin may
appear insignificant but at room temperature, 10-15 K is sufficient to raise the temperature of a (PDA/DMAC/PSS)₉ polymer shell from ambient temperature to above the glass transition of the polymer complex (Tg ~ 37°C for the molecular weight of polymers used in this study) ²⁹. It is believed that upon exceeding this glass transition the shell area surrounding the nanoparticles softens due to a local increase in polymer entropy, which in turn leads to the diffusion of encapsulated substances down its concentration gradient. Using this approach, one can both minimize the amount of nanoparticle and significantly decrease the laser intensity required needed to release encapsulated substances from polymeric capsules.

3.5. Gold nanorods

Skirtach and coworkers recently reported on milder approaches to light addressable nanoparticles using gold nanorods ¹²⁷, ¹²⁸. The synthesis and optical properties of nanorods are described in great details in the literature ¹⁵³–¹⁵⁶. Like gold nanoparticles, gold nanorods absorb light, but the mean absorbance region of nanorods is situated in the near-IR region. For colloidal gold particles, this absorption peak is largely dependent on surface properties, but additionally in the case of nanorods; on their length to width ratio (i.e. aspect ratio). Selecting the synthetic conditions, one can produce gold nanorods with the dimensions that correspond to a specific absorption window. Gold nanorods differ from the assemblies of nanoparticles in the same two aspects as assemblies differ from single nanoparticles: (1) their molar absorptivity in the near-IR is greater, and (2) the surface coverage of gold nanorods is much lower than aggregates of gold and are instead comparable to homodistributed gold nanoparticles. These two points are particularly important if one wishes to obtain a controlled response that can be precisely tuned.

Just as discussed at the end of the previous section, the key to control permeability with great precision and minimal irradiation intensity is not the quantity of nanoparticles used but in the magnitude of their absorption in the IR region (i.e. large molar absorptivity). During irradiation, one locally melts the polymer complexes around the nanorods, lowering the density of the matrix and increasing the permeability of the heated region (Fig. 3.4). Because gold nanorods of similar proportions possess a very narrow absorption region in comparison with nanoparticle aggregates, the range of laser intensity needed to open “pores” in the capsules should be comparatively narrow. The IR laser power range where gold aggregate-functionalized capsules could be opened spans 50 mW because the aggregates’ size distribution in a shell is broad and larger aggregates absorb more IR than smaller ones ¹⁴⁷. In addition, the small size of nanorods leads to the opening of small pores during irradiation of the microcapsules. Roughly identical in size, these pores would be significantly smaller than holes created from the destructive opening methods and definitely smaller than the pores created by aggregates of nanoparticles. The direct consequence of smaller pores is the much lower diffusion coefficient for a substance to permeate the membrane. As a result, gold nanorods are a great candidate sensitizing material to optically control the permeability of thin membranes. Skirtach et al. recently demonstrated for the first time that capsules could be resealed after pore opening, partially releasing their content during the process ¹²⁷. Repeating this a second time, it was possible to release a fraction of the capsule’s cargo. In this case, resealing is the result of the heated pore area of the capsule membrane cooling below the glass transition (Fig. 3.4, top panel). Nanorods also display interesting self-assembly possibilities that modify their optical and electronic properties ¹⁵⁷. In summary, the small size and strong absorption of gold nanorods makes it possible for shells impregnated with them to partially release encapsulated content during illuminations with an IR laser.

![Fig. 3.4. Schematic of remote release from microcapsules containing aggregates of gold nanoparticles or gold nanorods (top row). The partial release observed for microcapsules containing gold nanorods (bottom row) support the theory that under mild laser-induced heating, the nanoparticles produce just enough heat to locally melt a small volume of the polymer matrix around them, opening a pore. Switching off the laser allows for the melted volume to cool down sealing this pore. From [127]. Reprinted with permission of AAAS.](image)

To summarize Section 3, various metal nanoparticles can be used in composite polymeric microcapsules to yield interesting light addressable release properties. TiO₂ nanoparticles offer an option to damage capsules chemically using UV, while silver and gold nanoparticles induce similar responses thermally using visible light. Incorporating aggregates of metal nanoparticles in capsules improves the latter’s absorption at longer wavelengths, which in combination with inherent localization effects can be used to open “large” pores in...
capsules at much lower laser intensities than necessary for microcapsules functionalized with non-aggregated nanoparticles. Gold nanorods on the other hand, offer even higher absorption in the near-IR than aggregates of gold nanoparticles without localization effects, thus enabling opening “small” pores with well defined sizes in the microcapsule wall.

4. Functional Dyes for Remote Capsule Activation

Dyes are colored compounds that absorb or emit light in the visible range. Those which emit do so in various manners including phosphorescence and fluorescence. Functional dyes are those molecules that display properties beyond color. Photochromic dyes, such as reversacol for instance, reorganize and become colored or change color only when exposed to light. Photocatalysts such as chlorophyll-type molecules or materials found in photosynthetic cells lower the activation barrier of certain reactions when irradiated at the proper wavelength. These optically addressable functionalities and many others could be harvested to introduce new properties to thin films. Many functional dyes are designed to possess a high optical stability (i.e., fastness) making them all the more suitable for light addressable applications. With their versatility as a whole, stability and small size, it is hoped that composite films of dyes and polyelectrolytes offer a more precise approach to optically control the properties of thin films. The effects of laser irradiation on capsules containing fluorescent and functional dyes are discussed in this section.

4.1. Fluorescent dyes

Fluorescent dyes are regularly used to visualize polymeric microcapsules. However, it is difficult to obtain shells with monomeric dyes as building blocks since they tend to neutralize rather than reverse the electrostatic overcompensation that drives the LbL assembly. For this reason, fluorescent labels may be inserted in the polymeric shells in their monomeric form after capsule preparation, or covalently bound onto a polymer constituent of the shells. Alternatives to organic dyes for visualization of microcapsules are inorganic fluorophores such as quantum dots. The effects of photobleaching light sensitive molecules within LbL microcapsules have been briefly investigated. A fluorescent near-IR dye (IR 806) was absorbed on the surface of PAH/PSS based microcapsules. The shells were found to become severely damaged or deformed when irradiated with CW near-IR at moderate intensity (830 nm, 60 mW) while capsules containing no IR 806 remained unaffected by the laser. Although these results are encouraging and useful as a proof of concept, the use of a simple fluorescent dye to damage microcapsules comes at the cost of photobleaching the dye, which is both irreversible and constitutes a source of active degradation products such as radicals, which are not desirable for many applications (e.g., drug delivery) and are reminiscent of the arguments used concerning the limitations of pulsed UV laser sources.

4.2. Porphyrinoid dyes

Porphyrin and phthalocyanine dyes are members of the larger group of porphyrinoid conjugated cyclic systems of methine groups, which contain 4 and 8 aza (-N=) groups within the ring system, respectively. The structure of two water soluble dyes, porphyrin tetrasulphonate and phthalocyanine tetrasulphonate are illustrated in Fig. 4.1. Porphyrins are related to the largest group of naturally occurring dyes (chlorophyll) and have strong absorbance regions between 400-500 nm. At the opposite side of the visible spectrum, a sub-class of synthetic functional dyes named phthalocyanines possesses a primary absorption peak (Q band) between 650-730 nm. Both in nature and in technological applications, porphyrinoids have the ability to lower the energy of important redox reactions, acting as photocatalysts. The optical activity of most porphyrins and phthalocyanines are highly concentration dependent as they tend to aggregate in aqueous solution. The aggregation state of porphyrins could also be controlled in solution by light and within LbL films by adjusting the pH. The synthesis, optical properties and applications of films containing porphyrin and phthalocyanine dyes has been reviewed. The broad spectroscopic, catalytic and self-assembly properties of porphyrin-type dyes makes them an interesting substance to functionalize LbL capsules and perhaps, induce optical responses with very low energy requirements.

A method to incorporate reasonable quantities of a porphyrin dye and induce its catalytic properties was recently reported, where a non-metallic tetrasulfonated porphyrin was incorporated in PAH/PSS microshells. In order to significantly increase the amount of porphyrin in the multilayer complex, the dye was premixed with the oppositely charged polycation PAH before adding the resulting mixture to PSS coated templates. This premixing strategy resulted in a fivefold increase in porphyrin content on flat substrates in comparison to adding the dye on PAH coated substrates alone. However, premixing was not advantageous when using a strong polyelectrolyte such as PDADMAC and very little porphyrin seemed to form polyion complexes, presumably because the interaction with the oppositely charged polyelectrolyte was much more favorable than with the dye molecule. The optical response to the dye was less than anticipated as damages due to the laser illumination were only observed in presence of an oxidizer (15% hydrogen peroxide). Other dyes are currently under investigation to further improve optical response of shells containing...
photodynamic agents. While it was demonstrated in Section 3 that nanoparticles are most appropriate to trigger an optical response in polymeric microcapsules, metallic nanoparticles induce highly energetic and very rapid changes to the microcapsule wall nonetheless. These can be undesirable for materials that are thermally sensitive (e.g. proteins) and that cannot handle large concentration of a release material (e.g. drugs). Controlling the permeability of microcapsules using dyes (by driving an optically addressable pH change within the shell for example) consists in a promising strategy to obtain sustained release of encapsulated materials which has not been possible so far by the other means discussed in the previous sections.

![Diagram of Tetrasulfonated porphyrin and phthalocyanine](image)

Fig. 4.1. Tetrasulfonated porphyrin (left) and phthalocyanine (right).

5. Light Addressable Microcapsules for Cell Studies

The use of polymeric microcapsules for encapsulation and delivery of substances to living cells and tissues is an important area of application that requires the combined efforts of physicists, chemists, material scientists and biologists alike. Amongst the various microdelivery technologies that already exist (e.g. organic micelles, inorganic shells and emulsion-emulsion capsules), polymeric microcapsules are particularly interesting due to the versatile construction and by their relative stability. Living organisms are very sensitive to extreme environments, and the use of an indirect activation method to address technologies alien to living cells is most appropriate. Light, magnetism and sonochemistry are the most suitable candidates for such a task.

5.1. Cell studies

Polymeric shells that can be remotely opened by optical means to release encapsulated substances are interesting materials for in vivo studies. Such systems offer a protective environment to the encapsulated material allowing the investigator to store substances inside a living organism until the proper conditions are met to release the substance in question. This is advantageous in vitro since the cells or tissue can be given the necessary time to restore an equilibrium state after being put in presence of capsules. In vivo, however, serious concerns arise as very little is known on the fate of such complex systems. Should a system perform well the task of recognizing a cell, being internalized and releasing its content in the appropriate cellular compartment, the question as to what happens precisely to the capsule construct is ill understood. Even less is known about the fate of such capsules in higher organisms in which microcapsules are exposed to metabolic processing. The best studied system for intracellular studies was gold containing polymer microcapsules. A brief summary on light addressable microcapsules studies in presence of living cells is presented here. We recommend reference [10] for a complete review on biomedical applications of polymeric microcapsules.

Sukhorukov et al. were the first to report on the uptake of polymeric capsules by cells. Parak et al. pushed their research further in this direction and studied the cellular uptake and toxicity of microcapsules to living cells in great details. It was shown that shells of capsules based on PAH/PS or PDADMAC/PS induced no cytotoxic response in tumor cells. However, certain nanoparticles used to sensitize the capsules to light, such as 5 nm gold could be a toxicity concern. Gold nanoparticles were reported to be toxic due to (1) aggregation, (2) surface chemistry, and (3) size. However, Hauck et al. recently reported that PDADMAC/PS coated gold nanorods have little or no effect on cell viability.

The capsules studied by the joint efforts of the Sukhorukov and Parak groups were constructed from non-degradable polyelectrolytes. For drug delivery purposes degradability is often required. This aspect of biodegradability was assessed by the group of De Geest in vitro and in vivo. Capsules consisting of biopolymers such as polysaccharides and polypeptides showed to be prone to enzymatic digestion upon internalization by cultured cells. De Kok et al. injected such microcapsules subcutaneously in mice and tissue sections taken at different time intervals allowed to investigate the fate of the injected capsules as a function of time. Initially the capsules behaved as a porous implant which becomes gradually infiltrated by inflammatory cells, emerging from the border of the injection spot. Two weeks post injection, the injection volume was completely infiltrated by inflammatory cells and the capsules were found largely deformed inside cells. Thirty days post injection, no intact capsules could be observed anymore and only debris of broken capsules was visible.

Besides release from capsules governed by enzymatic digestion of the polyelectrolyte shell, another remote release strategy of interest is to release active species only at specific sites or upon a specific trigger. Pioneering work on this subject has been done by Skirtach et al. who
demonstrated that polymeric capsules functionalized with gold nanoparticles could, once internalized by cultured cells, be remotely opened by IR laser irradiation without impairing the cell viability \(^\text{31}\). In a further study, Muñoz-Javier et al. investigated the intracellular laser activated opening of capsules more in detail \(^\text{177-179}\). It was found that high laser powers lead to cell death while moderate laser powers could induce release of the capsules’ content into the cytosol of the cells. Fig. 5.1 shows two internalized capsules loaded with fluorescently-labeled dextran before (left) and after (right) remote opening of the microcontainer at relatively low laser intensity. It can be seen that the dextran spreads throughout the cytosol, but is unable to spread through the nuclear membrane.

![Remote release of encapsulated dextran](image)

Fig. 5.1. Remote release of encapsulated dextran (red) inside living cells from gold functionalized microcapsules using a near-IR laser at 830 nm. Before release, the microcapsules (indicated by two arrows) are found to be undamaged and internalized in the cell. After irradiation with the laser, the dextran was released and spread throughout the cytosol of the cell without penetrating the nuclear membrane. From \(^\text{179}\). Reprinted with permission of AAAS.

5.2. Intracellular fates of optically released materials

This ability of polymeric microcapsules to release their payload into the cellular cytosol points out an important property of laser activated capsules. In general, phagocytosed species end up in endo/lysosomal compartments where they are broken down by enzymes and, if necessary, exocytosed by the cell \(^\text{184}\). However, for several classes of drug molecules, which cannot readily permeate through the cell membrane, the aim is not only to reach the endo/lysosomes but the cytosomal space as well. This is the case in gene therapy where polynucleotides such as siRNA, mRNA and DNA should be delivered into the cellular cytosol and should even be transported to the cell’s nucleus in the case of DNA. Yet, conquering the endo/lysosomal membrane is not that straightforward. Several strategies have been developed in order to improve the release of encapsulated material from the endo/lysosomes into the cellular cytosol. Common methods involve using membrane-disruptive peptides, lipids or polymers, or use the buffering capacities of polymers such as PEI to cause osmotic bursting of the endo/lysosome. These methods often suffer from low efficiency and toxic side effects. Recently, other means involving light irradiation such as the use of photosensitizers have been explored. The latter can accumulate in the endo/lysosomal membrane and form oxygen radicals upon light irradiation causing the endo/lysosomal membrane to rupture. Although highly efficient these photosensitizers are often toxic and should be administered separately from the drug. This could cause difficulties in an in vivo setting where the minimal dose of photosensitizing dye necessary to open the endo/lysosomal membrane without affecting other cellular activities is a very challenging task. Also these small molecular weight compounds will drain easily from the administration spot while microparticulate drug formulation will have a much longer residence time in the tissue where they are administered. Taking these considerations into account allows us to define some potential solutions using remotely activated polymeric microcapsules. Firstly, these capsules allow the simultaneous delivery of photosensitive species (e.g. gold nanoparticles) and drug molecules (i.e. encapsulated within the capsules’ cavity). Moreover, due to their size an efficient targeting to phagocytizing cells can be obtained and surface functionalization of the capsule wall with specific ligands (e.g. antibodies) is easily performed, which allows to enhance their uptake by specific tissues or cells. Wang et al. recently introduced capsules loaded with Hypocrellin B (HB), a photosensitizer used in so-called photodynamic therapy to treat diseases such as some forms of cancer and viral infections \(^\text{174}\). Upon exposure to light, HB generates singlet oxygen (\(\text{^1O}_2\)), which is cytotoxic and induces cell death. However, HB displays no cytotoxicity in the absence of light irradiation. To allow the not water-soluble HB to enter living cells, it was loaded in polymeric microcapsules by non-specific interactions applying a solvent exchange step using ethanol as a solvent for HB. Upon incubation with living cells, the HB loaded capsules were efficiently taken up and neither capsules nor HB loaded capsules appeared to be cytotoxic for the cells. However, upon irradiation with 488 nm light, cell viability dropped by 70 %. In this in vitro setting, light addressable microcapsules showed promising promises for the specific delivery of drugs molecules that need a protective container on the way to their target cells or tissue.

6. Conclusions

Microcapsules of polymers with optically addressable properties are being actively investigated and much attention is being put at their potential as drug delivery vehicles. Polymer based microcapsules were developed
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and investigated mostly for near-UV response. Shells that contain UV chromophores sensitive to photodegradation can damage to the capsule over prolonged exposure to UV light. Incorporating TiO₂ nanoparticles in the microcapsule shell is a much more efficient way to release encapsulated material. Microcapsules containing stable azobenzene chromophores, however, can be used to change the permeability of the microshells and even encapsulate substances. Functional dyes such as porphyrin photocatalysts can be effectively used to damage microcapsules upon visible light exposure. The use of more reactive phthalocyanine dyes is expected to be useful in the development of microcapsules with slow sustained release upon irradiation. Silver and gold nanoparticles can be used to destructively release encapsulated materials using low energy irradiation sources due to their ability of absorb photons and convert them to heat, affecting materials around them. However, the irradiation requirements for remote opening by IR can be significantly decreased by modifying the distribution or shape of the nanoparticles used to impregnate polymeric microcapsules. This is partially because the absorbance of gold assemblies and gold nanorods are located in the IR instead of the visible range, thus making the heat production more efficient. Additionally, the heat produced by gold nanorods result in very small pores in the capsules that are sealed when irradiation stops. This effect makes it possible to reversibly change the permeability of polymer microcapsules with great sensitivity. Finally, the current accomplishments in using light addressable microcapsules in living cells and in vivo was reviewed.

Much is still expected from polymeric microcapsules and new developments on their remotely addressable properties are currently actively investigated. This review merely highlights the combined efforts on the topic of light addressable microcapsules done in large part by half-a-dozen research groups around the globe in the past five years. Lately, much focus has been committed to the study of light addressable microcapsules in living cells and tissues. An increase in collaboration with pharmacists, biologists and health professionals has significantly improved our understanding in this respect. However, much of the reported work done in presence of living material involves synthetic parts that cannot be degraded by cells. At present, destructive strategies to release substances from microcapsules are the norm. However, many developments are still needed in order to achieve capsule constructs possessing the degree of permeability control needed for biomedical applications. Maximizing the function of such containers for biomedicine requires the production of microcontainers that are fully biodegradable (1) with relatively small diameters (2) and possessing reversible and/or selective permeability (3). The permeability control aspect is most challenging, requiring a precise control over the pore size and the time pores are open across the thin capsule wall. Both of these aspects could be solved using small, highly light sensitive materials in low concentration such as particle aggregates, gold nanorods or functional dyes.

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References

4. G. B. Sukhorukov; E. Donath; S. Davis; H. Lichtenfeld; F. Caruso; V. I. Popov; H. Mohwald, Polymers for Advanced Technologies 1998, 9, (10-11), 759-767.
15. A. N. Zelikin; Q. Li; F. Caruso, Chemistry of Materials 2008, 20, (8), 2655-2661.
192. 
121. H. C. Leventis; I. Streeter; G. G. Wildgoose; N. S. Lawrence; L. Jiang; T. G. J. Jones; R. G. Compton, Talanta 2004, 63, (4), 1039-1051. 
126. G. B. Sukhorukov; D. G. Shchukin; W. F. Dong; H. Moehwald; V. V. Lulevich; O. I. Vinogradova, Macromolecular Chemistry and Physics 2004, 205, (4), 530-535. 


134. M. Malcher; D. Volodkin; B. Heurtault; P. Andre; P. Schaaf; H. Moehwald; J. C. Voegel; A. Sokolowski; V. Ball; E. Boulimedais; B. Frisch, Langmuir 2008, 24, (18), 10209-10215.


185. B. G. De Geest; R. E. Vandenbroucke; A. M. Guenther; G. B. Sukhorukov; W. E. Hennink; N. N. Sanders; J. Demeester; S. C. De Smedt, *Advanced Materials* 2006, 18, (8), 1005-1009.