The fabrication of functional hybrid materials composed of nanoscale building blocks represents one way in which nanotechnology can contribute to biology and medicine. For example, the synthesis of three-dimensional hollow sphere structures has attracted enormous interest in recent years for a variety of different applications, ranging from drug delivery systems and targeted gene therapy to biosensor devices. Hereby, the fundamental challenge is to fabricate biocompatible multifunctional vehicles whose properties can be tuned at the nanoscale. In this context several systems have been suggested and also demonstrated, such as liposomes, block copolymers, and dendrimer polymers. Polyelectrolyte multilayer capsules, e.g. polymer walls around cavities, have also been used in such systems for several reasons. Firstly, they can be synthesized under mild conditions by using numerous different materials. Secondly, their functional properties can be well-defined by embedding different nanoscale building blocks (as colloidal inorganic nanoparticles or biomolecules) within and on top of their wall. Thirdly, they can efficiently host (biological) macromolecules within their cavity for numerous biomedical applications. Finally, they can be composed of biocompatible materials for the delivery of encapsulated materials into cells.

In this review, the main concepts involved in the fabrication and functionalization of polyelectrolyte multilayer capsules are described and some suggestions for their possible intracellular use in both therapeutic and diagnostic applications are presented.

Synthesis of polyelectrolyte capsules
Polyelectrolyte capsules are fabricated by layer-by-layer adsorption (LbL). This technique is based on the consecutive assembly of oppositely charged synthetic polymer layers around a preformed charged spherical core. At the end of the LbL adsorption process, the cores can be successfully removed to obtain hollow and stable capsules whose inner cavity and polymer wall can be loaded and functionalized, respectively, with a variety of substances such as molecular dyes, drugs, nanoparticles and biomolecules, which retain their distinctive properties after the embedding procedure (Fig. 1). The resulting hollow capsules usually have a wall thickness of between a few tens and several hundreds of nanometers and a diameter ranging from tens of nanometers to several micrometers depending, on the size of the original core.

Self-assembly of the subsequent layers is governed by the strong electrostatic interactions that occur between the oppositely charged
layers in solution, resulting in overcharging of films after deposition of each layer. Based on this phenomenon, the fabrication of multilayered polymer capsules is a highly versatile procedure, since it depends on the capability of a charged molecule to be consecutively adsorbed onto the top of an oppositely charged surface/layer. Thus, the two fundamental components for capsule fabrication are the core templates and the polyelectrolyte pairs (Fig. 1).

An ideal template has to be stable under the LbL process, soluble in mild conditions, and completely removable from the inside of the capsules, without affecting the morphology and stability of the multilayer assembled on top of it. In recent years, numerous materials have been employed as sacrificial templates, such as (i) polystyrene latex, (ii) melamine formaldehyde, (iii) SiO₂, (iv) carbonate particles (MnCO₃, CaCO₃, CdCO₃), and (v) biological cells. Such materials can be dissolved by solvents like (i) tetrahydrofuran (THF), (ii) hydrochloric acid (HCl), (iii) hydrogen fluoride (HF), (iv) ethylene diamine tetraacetic acid (EDTA), and (v) saline solution (e.g., sodium hypochlorite solution), respectively. However, advantages and disadvantages are observed in each of the dissolution procedures and great efforts are currently being focused on the development of new cores that could merge all the above requirements within a unique material.

The capsule wall composition also plays a crucial role for the fabrication of functional capsules, as their permeability/porosity strongly depends on the chemical nature and the molecular weight of the employed polyelectrolyte pairs. The majority of polyelectrolyte capsules described in the literature are composed of pairs of synthetic anionic poly(sodium) styrene sulfonate and cationic poly(allylamine) hydrochloride. Although these multilayers are known to have numerous advantages, for therapeutical purposes there is a special interest in using more biocompatible materials, which are potentially biodegradable under physiological conditions. Enzymatically degradable multilayer capsules have already been described.

The mechanical/elastic properties of polyelectrolyte capsules are influenced by several parameters, such as the chemical nature of the polymers used (which can cause weak or strong intermolecular interactions within the multilayer) and the molecular composition of the inner part of the capsules. In particular, atomic force spectroscopy studies have shown that capsules with elasticity ranging within 0.05–10 GPa can be obtained depending on the composition, treatment, and filling of the capsule.

Several methods, such as electrophoretic mobility and light scattering measurements are typically used to follow the layer growth of polyelectrolytes onto the spherical templates, while for their structural characterization, both electron and atomic force microscopy are commonly employed.

Finally, confocal laser scanning microscopy analysis is widely used for real-time observation of core dissolution in vitro to characterize the permeability properties of these capsules, their cellular uptake, and the subsequent intracellular release of the encapsulated materials.

**Embedding functionalities into the walls of polyelectrolyte capsules**

The distinct advantage of using the capsule-based systems described above lies in the potential of combining a variety of substances.
with different functionalities in one unique system. Modification of capsule walls by colloidal nanoparticles and biological macromolecules is described further in this section. Fig. 2 shows how, based on the principle of the LbL assembly, different types of charged nanoparticles can be simultaneously embedded in the capsule wall by employing electrostatic forces between the nanoparticles and the oppositely charged layer.

By adding luminescent colloidal semiconductor nanoparticles such as CdTe and CdSe quantum dots, the capsules can be easily detected by measuring their fluorescence signal with noninvasive optical techniques. The use of fluorescent nanoparticles as fluorophores for bio-labeling applications presents two main advantages compared with commercial organic fluorophores. First, they are characterized by nearly continuous excitation spectra with narrow emission bands, located at different wavelengths depending on the nanoparticle size. This allows for simultaneous excitation of probes of different colors by light of a single wavelength. Secondly, their reduced photobleaching makes them suitable for measurements over long periods of time.

On the other hand, the Cd-based quantum dots, which are still most frequently used, can cause cytotoxic effects by the release of Cd ions.

By embedding magnetic nanoparticles such as Fe₃O₄ into the capsule walls, the movement of capsules in a desired direction can be controlled by applying an external magnetic field gradient. In addition, heating of the nanoparticles upon application of radio frequency fields increases the permeability of the capsule walls.

Noble metal nanoparticles such as gold and silver are known to strongly absorb light. Upon irradiation, the major part of the absorbed light energy by each nanoparticle is transformed into heat. The collective effect of several nanoparticles in a unique system is to amplify the heating effect, resulting in an increase in temperature in the surrounding areas. In this way, by embedding metal nanoparticles into the walls of polyelectrolyte capsules, the integrity/permeability of the wall of individual capsules can be selectively perturbed.

Another possibility for polyelectrolyte capsule functionalization is through conjugation of biological molecules to their walls. Biomolecules could be added to the outermost layers of the capsule walls by electrostatic adsorption or covalent binding, depending on their charge features (Fig. 3). Such biofunctionalized capsules might fully mimic the features of bioactive molecules, thus improving the biocompatibility of the capsules. Using this concept, the interaction of capsules with living cells could be greatly improved. Furthermore, based on the molecular recognition system of cells, specific interactions between capsules and cells can be achieved by adding ligand molecules to the capsule surface to specifically bind to receptor molecules present on cells.

Loading of biomolecules inside the cavity of polyelectrolyte capsules

One important feature of capsules lies in the possibility to load desired cargo molecules into their cavity. Encapsulation allows for protection, both of the cargo materials from inactivation and dilution effects inside living cells, and the cell itself from the possible toxic effect of the cargo. In recent years, numerous approaches have been envisaged to entrap different types of materials, such as small crystals, fluorescent dyes, drugs, and biomolecules (e.g., DNA, small-interfering RNA (siRNA) and enzymes) within capsules.

One strategy employs the charged properties of enzyme crystals and of cells to use them as templates onto which oppositely charged polyelectrolyte pairs can be directly adsorbed. Remarkably,
the functional properties of the enzymes trapped in the capsule cavities are preserved during the LbL assembly process. The activity of coated enzymes is fully retained after exposure to proteases, whereas uncoated enzymes are deactivated by more than 90% in a short period of time, which demonstrates that the cargo inside the capsule cavity is protected from enzymatic degradation. Also, cells embedded in polyelectrolyte walls are able to maintain their viability, functionality, and normal exchange of nutrients and waste. However, one limit of the method lies in the impossibility of synthesizing monodisperse particles with regular shapes since the final embedded product strongly depends on the template.

Another method exploits the possibility to load materials into the assembled polyelectrolyte capsules immediately after the dissolution of the core (post-loading method). In particular, the wall permeability of capsules can be reversibly switched from the opened to the closed state through variation of pH value or through variation of the solvent polarity. The capsules can be loaded at low pH or in the presence of ethanol (open state), as in this state the polyelectrolyte network of which their walls are composed is swollen and thus permeable, so that molecules can diffuse in. After increasing the pH value or dispersing the suspension in the original medium (without ethanol), the polyelectrolyte network shrinks, the walls are no longer permeable, and thus the loaded materials are retained inside the cavity (closed state). One of the main advantages of encapsulating molecules using the post-loading method relies on the suitability of commercially available monodisperse templates which allow for the synthesis of monodisperse polyelectrolyte capsules. Obviously, materials sensitive to pH changes are not suitable for loading under these conditions. However molecules that are too big to diffuse through the walls in closed state can be loaded.

An alternative to the above mentioned procedures has recently been proposed. It is based on the coprecipitation of the cargo onto porous templates, such as carbonate crystals, followed by the multilayer assembly of polyelectrolyte pairs (pre-loading method). Some limits of this method are the difficulty in obtaining colloidal crystals of low dispersity in the desired size range and in the unavailability of commercially synthesized carbonate templates. Nevertheless, these cores can be easily dissolved in mild conditions (e.g. by using EDTA or low pH) without affecting the encapsulated materials.

Interaction of capsules and living cells

Cells can incorporate objects ranging from the molecular up to the micrometer scale by processes including pinocytosis, endocytosis, and phagocytosis. Surprisingly, uptake by living cells of several polyelectrolyte capsules as big as 5 µm has been demonstrated by different groups and to date it is a fact that capsules of different sizes, ranging from the nanometer to the micrometer size, are spontaneously ingested by a huge variety of cells. Currently, there are numerous studies concerning the cellular internalization mechanism of capsules. In most cases, phagosomal/endosomal/lysosomal perinuclear compartments were suggested as final locations of the capsules, similar to that which is claimed for colloidal nanoparticles. De Geest et al. suggested a lipid raft-mediated uptake mechanism based on the previous results by Rejman et al., who revealed size-dependent internalisation of ligand-devoid particles.

Although strictly speaking the detailed uptake process of polyelectrolyte capsules by cells remains to be clarified in future studies, some important parameters that regulate this process have recently been elucidated. For instance, the overall charge of capsules has been demonstrated to play a role for capsule uptake. As for
smaller colloidal nanoparticles, charged capsules are ingested faster than uncharged ones, and positively charged capsules are found to be ingested more than negatively charged ones75. At any rate, adsorption of cell medium proteins to the capsule surface tends to smear out differences in surface chemistry for long incubation times. Atomic force microscopy measurements of adhesive forces between capsules and cell membranes show that the uptake of polyelectrolyte capsules strongly correlates with the adhesion of capsules to the outer cell membrane75. In addition, it is frequently observed that capsules are deformed upon the incorporation process due to the mechanical stress caused in the intracellular space. Deformation naturally depends on the structure of the capsule walls. Recently, heat-shrunken capsules that do not lose their cargo, even upon compression inside cells, have been demonstrated72. The capsule uptake rate of cells can also be regulated by using the molecular recognition system of cells. By attaching specific ligands to the capsule surface (Fig. 5), the capsule uptake rate of target cells, which are expressing the corresponding receptors, can be increased36. On the other hand, non-specific uptake of capsules by cells can be also reduced by immobilizing polyethylene glycol (PEG) on their surface76.77.

As ‘foreign objects’, capsules may well induce harmful effects on living cells. Toxicity can originate from two different sources, from the actual polyelectrolyte capsules and from the functional nanoparticles embedded in the capsule walls. Initial studies have suggested that capsules alone do not exhibit acute cytotoxic damage on cell cultures, but rather that the nanoparticles with which the capsules are functionalized are potentially cytotoxic, as for example when Cd-based semiconductor nanoparticles embedded in the capsule walls corrode and release toxic cadmium ions78. On the other hand, both magnetic and gold nanoparticles are relatively harmless to the cells79,80. Cytotoxicity assays in vitro have been employed to confirm the innocuousness of polyelectrolyte-based systems81, although more advanced studies involving animal tissue also demonstrate that plain unfunctionalized capsules can cause local inflammations10. Despite the impact this would have on medical applications, the cytotoxicity effects of capsules have not yet been fully investigated.

Polyelectrolyte capsules as carrier systems for local delivery of cargo

One of the possible contributions of nanomedicine would be in building biocompatible multifunctional carrier systems that are able to navigate within living organisms using remote guidance and activation for the local release of their cargo. Such carrier systems are used to improve cargo stability, to sustain and control their release rates, to increase
the membrane of target cells. Functionalized with ligands that bind to corresponding receptors localized in the membrane of target cells.

Fig. 5 (a) Magnetic targeting of capsules for local accumulation. Capsules functionalized with magnetic NPs (black) are trapped in a magnetic field gradient created with a permanent magnet. Fluorescent NPs (green) in the capsule walls permit visualization by optical techniques. (b) Specific uptake of capsules by cells via molecular recognition. The surface of the capsules is functionalized with ligands that bind to corresponding receptors localized in the membrane of target cells.

Target delivery

Drug targeting (as an important example for targeted cargo delivery) is defined as selective drug delivery to specific physiological sites, organs, tissues, or cells, where a drug's pharmacological activity is required. Target delivery technologies have been applied to drugs whose designated site of action is difficult to reach and also to cytotoxic compounds which must not reach sites other than the therapeutic sites in order to prevent cytotoxic side effects. There is currently no applied pharmaceutical treatment available that can be applied locally in a controlled way, thereby shielding healthy cells from drug interaction. Magnetic drug targeting has been suggested as a method to locally accumulate drugs. It has recently been shown in vivo that it is possible to direct drugs bound to magnetic nanoparticles to tumor tissue using magnetic field gradients. The same concept can be applied for polyelectrolyte capsules whose walls are functionalized with magnetic nanoparticles (Fig. 5a). Specific accumulation of magnetic nanoparticle-functionalized polymer capsules in a magnetic field gradient, followed by capsule internalization by breast cancer cells has been recently demonstrated in vitro. By labeling the walls of the capsules with fluorescent nanoparticles (in addition to the magnetic nanoparticles), high local concentration and thus their drastically increased internalization along the field gradient could be easily observed with a fluorescence microscope. Although this method works reliably in cell cultures, it has to be pointed out that due to the technical challenge of focusing magnetic field gradients, this method does not work on a single cell level, but that accumulation of capsules can be enhanced at certain regions within the cell culture/tissue.

Naturally, the classical concept of targeted drug delivery via receptor-ligand interaction can also be applied to the capsules (Fig. 5b). For this purpose, ligands that specifically bind to receptors expressed by target cells are immobilized on the capsule surface, resulting in an increase in the uptake rate of capsules by target cells compared with surrounding cells. Unfortunately, all the complications and technical problems associated with molecular recognition-based drug delivery also apply to ligand-modified capsules. In particular, improved surface chemistries of the capsule walls (such as PEG) are certainly required in order to obtain sufficient retention time (the time for which the capsules are circulating in the blood stream until they are cleared from it by the organism). Nevertheless, these capsule-based systems are presented as drug carriers that allow for at least two parallel ways of targeting: (i) local accumulation of capsules near the target tissue by attraction of magnetic nanoparticles in the capsule walls with magnetic field gradients and (ii) enhanced uptake by target cells due to ligands attached to the surface of the capsule walls, which specifically bind to their receptors present on the outer membrane of the target cells. In this way, the combination of two strategies within one carrier system is expected to lead to improved targeting and thus less unwanted delivery of drugs to surrounding tissue.

Controlled release of cargo

Targeted cargo delivery, as described in previous section, can be combined with remotely controlled cargo release. Remote activation is based on an external physical stimulus such as light, ultrasound, or radio frequency, which acts on colloidal nanoparticles present in the walls of the capsules and leads to rupturing/permeation of the walls of the capsules so that the cargo is released from the cavity to the environment. Irradiation of noble metal and magnetic particles with light and radio frequency waves, respectively, leads to local heating as described earlier and subsequent disintegration of the capsule walls. Alternatively, sonication of capsules functionalized with colloidal
nanoparticles in their walls leads to mechanical disintegration of the capsule walls. In this way, the cargo can be released from the cavity of the capsules following an external trigger. Ultrasound and radio frequency fields can penetrate tissue well, but they are complicated to focus. For this reason they are favorable for cargo release from capsules deep inside tissue. Visible light, on the other hand, is strongly absorbed by tissue but can be easily focused to micrometer size spots. For this reason, controlled opening of individual capsules inside single cells and subsequent release of their cargo to the cytosol is possible (Fig. 6)\textsuperscript{87}. Short irradiation (<10 ns) with moderate light intensities (~35 mW) is sufficient for opening capsules without damaging cells and tissues\textsuperscript{85,88}. While moderate irradiation disintegrates the walls of capsules, releasing molecules from their cavity, higher doses of irradiation can increase the local temperature so far as to destroy surrounding cells, an effect known as hyperthermia. Local accumulation of heatable capsules (i.e. with noble metal and magnetic nanoparticles in their walls which are responsive to light and radio frequency exposure, respectively) in cancerous tissues could be used for induced heating mediated by the nanoparticles involving subsequent tumor deterioration\textsuperscript{89}. As only the temperature of cells close to the particles is raised, the heated cells would be selectively killed, without exposing the entire organism to elevated temperatures\textsuperscript{90–93}.

In summary, nanoparticle functionalized capsules offer the potential to combine all of the above listed concepts into one single system for drug delivery (Fig. 7). Magnetic nanoparticles in the capsule walls allow direction and accumulation of capsules at the designated target region upon application of external magnetic field gradients. Ligands immobilized on the capsule surface, which are specific for receptor molecules present on target cells, would permit specific and enhanced cellular uptake via receptor-ligand binding. Fluorescent semiconductor nanoparticles in the capsule walls allow monitoring of the transportation and uptake process using fluorescence microscopy. Capsules inside target cells could be opened with a laser pointer by heating gold nanoparticles in the capsule walls which then releases the cargo molecules. Combination of these methods is expected to lead to greater specificity in drug delivery. This procedure could be a great advantage to anticancer therapy, as selective toxicity in tumor cells could be achieved via local chemotherapeutic damage. Nevertheless, to be applicable in clinical practice, several additional hurdles will have to be overcome. First of all, to reach a specific tissue, intravenous administration is often required. Therefore, the capsules should be limited in size as they could obstruct the smallest blood capillaries. From the circulation point of view, the upper limit for an ideal vehicle size should not exceed 200 nm\textsuperscript{94}. Secondly, due to their polyionic nature, the capsules tend towards protein adsorption, potentially leading to capsule aggregation in the blood capillaries.

Besides the potential use for targeted and controlled release of drugs, cargo delivery with capsules can be seen in the more general concept of triggered local delivery. Caged Ca is introduced into chelating complexes from which it can be released by light-illumination. Noble-metal functionalized capsules could serve the same purpose, although they would allow for light stimulated release of macromolecules from the cavity of the capsules. Stimulated local release of enzymes\textsuperscript{63,70} and DNA\textsuperscript{95} would be of particular relevance. Release of enzymes could, for example, trigger the conversion of prodrugs within cells into active drugs\textsuperscript{96,97}. Also short oligonucleotide sequences, such as si-RNA, could be introduced as cargo inside the capsule cavities for gene delivery applications\textsuperscript{98}. In particular, such capsules could provide an easy way to improve the current transfection methods used in molecular biology as they would lead to more efficient translational arrest of specific transcripts from RNA inside living cells. In this role,
the incorporation of single-stranded antisense oligonucleotides, linear double-stranded and plasmid DNA into polymer nanospheres has been demonstrated\(^99\)–\(^{101}\).

**Polyelectrolyte capsules as carrier systems for local sensing**

Sensing based on optical measurements is very attractive as it allows the transduction of chemical concentration information into optical signals which can be quantified. Fluorescent indicators are a class of fluorophores whose spectral properties are sensitive to a substance (the analyte) of interest\(^{102}\). Numerous indicators are commercially available for a variety of analytes, including Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^{-}\), H\(^{+}\), Na\(^{+}\), and O\(_{2}\)\(^{-}\)\(^{103}\). Such analyte-sensitive fluorophores can be integrated into polymer capsules\(^{64}\). Due to the size-dependent permeability of the walls of the polymer capsules, analyte-sensitive fluorophores of high molecular weight can be maintained in their cavity, whereas analyte molecules of low molecular weight can diffuse in and out freely. A microcapsule-based pH-sensor system using the seminaphthorhodafluor dye (SNARF-1) has already been described\(^{64}\) [Fig. 8a]. Whereas capsules in the alkaline cell medium are fluorescent in the red, capsules which have been incorporated into cells inside acidic compartments are fluorescent in the green [Fig. 8a]. Such pH-sensitive capsules present an interesting tool for high throughput quantification of cellular uptake\(^{85,104}\). Besides the fact that naturally incorporated capsules are trapped inside intracellular compartments and thus are not in contact with the cytosol – a problem which will be discussed later – there are several advantages to embedding analyte-sensitive fluorophores into capsules. Firstly, long term measurements could be achieved, as the fluorophores inside the capsules are protected against enzymatic degradation and the cell is protected from the free fluorophore. Secondly, as many fluorophores are embedded in each capsule, there is a high local fluorophore concentration, which enhances their sensitivity [Fig. 8c].

Most importantly, in contrast to alternative technologies such as the probes encapsulated by biologically localized embedding (PEBBLE) system\(^{105,106}\), capsule-based sensors do, in principle, allow for multiplexed measurements. This is based on the fact that capsules can be functionalized with fluorescent molecules at two distinct positions, in their walls and in their cavities. In this approach, fluorophores sensitive to different analytes are loaded into the cavities of different capsules and the walls of each capsule are fluorescently labeled with a barcode [Fig. 9a]. The color of the capsule wall would allow for identification of each capsule and thus provide the information for which analyte this particular capsule is sensitive. The local analyte concentration could be derived upon recording the fluorescence resulting from the cavity. As fluorophores for sensing different analytes are locally separated by embedding them in different capsules, spectral overlap between the different fluorophores is no longer a problem. Presumably, this concept would allow for the detection of multiple analytes in parallel in the cytosol of single cells [Fig. 9b].

However, as mentioned, the delivery of capsules to the cytosol remains a fundamental problem for multiplexed measurement of different analytes inside cells. Though capsules can be directly introduced to the cytosol by electroporation\(^{9}\), delivery to the cytosol via active capsule incorporation by cells would be preferable. However, as mentioned in earlier, capsules taken up by cells are stored inside intracellular compartments. One way of direct delivery
to the cytosol might be the modification of the capsule surface with virus-derived ligand molecules\textsuperscript{107}, such as TAT-peptides which have been successfully used for the delivery of nanoparticles into cells\textsuperscript{108}. An alternative strategy is presented in Fig. 10. By repeated coprecipitation, double-shell capsules can be synthesized\textsuperscript{109}. The inner capsule could now be a sensor capsule with a fluorescent barcode in the walls and analyte-sensitive fluorophores in the cavity, while the wall of the outer capsule could be functionalized with noble metal nanoparticles. Light-illumination of such double-shell capsules inside intracellular compartments would lead to local heat generation in the metal particles. Therefore, it can be speculated that the heat would be sufficient to rupture and permeate the outer wall of the capsules as well as the membrane of the vesicular compartment in which the double-shell capsule is trapped. In this way, the intact inner sensor capsule would be released into the cytosol. Although this concept still has to be proved experimentally, there are experimental data that support the idea. Indeed, light-illumination of single metal particle-functionalized capsules not only permeates the capsule walls, but also the surrounding membrane of incorporated capsules, as demonstrated upon release of fluorescent cargo from the capsule cavity to the cytosol\textsuperscript{87}. Although vesicular membranes around the capsules must have been locally disintegrated, cells have been demonstrated to tolerate this treatment.

**Conclusion**

Multifunctional polyelectrolyte capsules fabricated by the LbL assembly technique possess remarkable properties, even though they are held together by electrostatic attraction. In particular, this very general assembly mechanism allows for the integration of virtually all different types of charged nanoscale objects into and on top of their walls. Introduction of nanoscale objects introduces functionality (such as fluorophores, magnetic particles, and local heat sources) and specificity (such as biomolecular ligands). The cavity of the capsules can be filled with cargo, which is to be released at designated targets or with active molecules, as for example for multiplexed sensing. Compared with other carrier systems, capsules can be functionalized at two distinct compartments, walls and cavities, which introduces flexibility for the interference-free introduction of multiple functionalities.

It has to be clearly pointed out that the individual concepts outlined in this article for use with capsules have previously been suggested for other types of carrier systems. Magnetic drug targeting, receptor-ligand based targeting, hyperthermia, and photo-induced release of cargo are concepts well known in medical research. The novelty of the capsules as a carrier system lies in the possibility of combining all these different strategies into one single object and thus provide true multifunctionality.

Most of the concepts mentioned in this article have been experimentally demonstrated on cell cultures and the experimental data can be found in the references. In particular, a proof of principle for a microcapsule-based system for biospecific target delivery and for local analyte detection in small volumes in cell cultures has been successfully realized by several groups. Though several groups have started with experiments on animals, enormous hurdles still have to be overcome before applying polymer capsules to clinical practice. Of particular importance will be the prevention of capsule aggregation in blood vessels, the control of capsule clearance by the organism, and the synthesis of highly biocompatible capsules.\textsuperscript{11}

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