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Polymeric Vesicles: From Drug Carriers to Nanoreactors and Artificial Organelles

PASCAL TANNER, PATRIC BAUMANN, RAMONA ENEA, OZANA ONACA, CORNELIA PALIVAN,* AND WOLFGANG MEIER*

Department of Chemistry, University of Basel, Klingelbergstrasse 80, CH-4056 Basel, Switzerland

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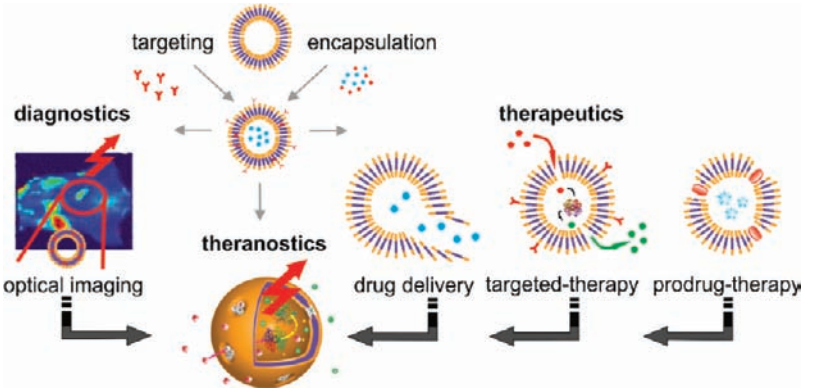

One strategy in modern medicine is the development of new platforms that combine multifunctional compounds with stable, safe carriers in patient-oriented therapeutic strategies. The simultaneous detection and treatment of pathological events through interactions manipulated at the molecular level offer treatment strategies that can decrease side effects resulting from conventional therapeutic approaches. Several types of nanocarriers have been proposed for biomedical purposes, including inorganic nanoparticles, lipid aggregates, including liposomes, and synthetic polymeric systems, such as vesicles, micelles, or nanotubes.

Polymeric vesicles—structures similar to lipid vesicles but created using synthetic block copolymers—represent an excellent candidate for new nanocarriers for medical applications. These structures are more stable than liposomes but retain their low immunogenicity. Significant efforts have been made to improve the size, membrane flexibility, and permeability of polymeric vesicles and to enhance their target specificity. The optimization of these properties will allow researchers to design smart compartments that can co-encapsulate sensitive molecules, such as RNA, enzymes, and proteins, and their membranes allow insertion of membrane proteins rather than simply serving as passive carriers. In this Account, we illustrate the advances that are shifting these molecular systems from simple polymeric carriers to smart-complex protein–polymer assemblies, such as nanoreactors or synthetic organelles.

Polymeric vesicles generated by the self-assembly of amphiphilic copolymers (polymersomes) offer the advantage of simultaneous encapsulation of hydrophilic compounds in their aqueous cavities and the insertion of fragile, hydrophobic compounds in their membranes. This strategy has permitted us and others to design and develop new systems such as nanoreactors and artificial organelles in which active compounds are simultaneously protected and allowed to act in situ. In recent years, we have created a variety of multifunctional, proteinpolymersomes combinations for biomedical applications. The insertion of membrane proteins or biopores into the polymer membrane supported the activity of co-encapsulated enzymes that act in tandem inside the cavity or of combinations of drugs and imaging agents. Surface functionalization of these nanocarriers permitted specific targeting of the desired biological compartments.

Polymeric vesicles alone are relatively easy to prepare and functionalize. Those features, along with their stability and multifunctionality, promote their use in the development of new theranostic strategies. The combination of polymer vesicles and biological entities will serve as tools to improve the observation and treatment of pathological events and the overall condition of the patient.

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Introduction

The design of new diagnostic and therapeutic strategies strives to improve the pathways of medicinal administration, specificity of response, detection limits, or required doses, and to even combine multifunctional systems for greater patient-oriented treatment. In this effort, the stringent demands placed on medical science today are shifting the focus to the creation of hybrid systems at the nanoscale that combine a variety of synthetic materials advantageously, including the addition of biological entities that can act in an extremely specific manner. Such systems offer the possibility of overcoming the complex challenges of improving both detection sensitivity and drug efficacy, and thus patient status.[1]

Various types of polymers with well-defined compositions, architectures, and functionalities have been prepared using methods such as atom transfer radical and ring-opening polymerization.[2] A reasonable understanding of the mechanisms and kinetics of these methods of synthesis has helped scientists design versatile architectures and correlate molecular structure with mesoscopic properties. For therapeutic applications in particular, it is necessary to provide safe, functional carriers for efficient transport and protection of active molecules.

The ability of nature to create functional and dynamic superstructures of tunable conformation and size has been mimicked synthetically by the design of compartments whose membranes are based on amphiphilic block copolymers. Generally speaking, amphiphilic block copolymers composed of a hydrophobic and a hydrophilic domain can be regarded as higher molar mass homologues of conventional lipids or surfactants that form the same kind of self-assembled superstructures as these in selective solvents.[3] However, as a result of their higher molar mass, they frequently form larger aggregates with lower dynamics. For example, membranes of amphiphilic block copolymers may be up to 10 times thicker than those of natural bilayer-forming phospholipids. As a result, they frequently display considerably higher mechanical stability than conventional lipid bilayers.[4]

Here, we will focus on hybrid systems based on polymeric vesicles as compartments for "active" compounds encapsulated inside and/or inserted within the membrane. We will present the development of hybrid systems ranging from simple nanocarriers, as seen in conventional drug-release approaches, to more challenging nanoreactors and artificial organelles.[5] Our interest is in showing new concepts that may lead to medical applications must combine various types of molecular entities. These must also integrate multifunctionality to allow a specific biological response in terms of the detection and treatment of a pathological condition.

Generating polymeric vesicles to serve as carriers for drugs/markers ranging from small molecules to enzymes must take into account a complex scenario based on various requirements that include biocompatibility, biodegradability, nontoxicity, specificity of biological compartments and the particular application. In this respect, the chemical nature of copolymers, the methods to generate vesicular assembles with desired properties, and their combination with "active compounds" represent the basis for developing new hybrid systems. The aim in this case is to shift from conventional therapeutic and diagnostic approaches to a combined, or theranostic, strategy.

Generating Polymeric Vesicles

The synthesis of macromolecules to generate specific, supramolecular architectures and properties, such as membrane permeability, responsiveness to stimuli, and the ability to incorporate membrane proteins, has proven possible.[6,7] These goals have been achieved by changing the chemical nature and length of each polymer block, the hydrophobic/hydrophilic ratio,[8,9] and the experimental conditions (temperature, pH, light, or reductive conditions).[10−12]

In particular, the following approaches have been used to generate polymeric vesicles for diagnostic and therapeutic applications: (i) self-assembly of amphiphilic copolymers, and (ii) layer-by-layer assembly of polymers.[13,14]

Polymeric vesicles with dimensions in the nanometer region, called polymersomes, are generated by self-assembling amphiphilic copolymers in dilute aqueous solutions, analogously to lipids.[15,16] These consist of spherical, closed-block-copolymer membranes with diameters in the range of 50 nm to approximately 10 μm, depending on the chemical constitution and the size of polymer blocks, the preparation method, as well as reaction conditions. These polymer vesicles are significantly more stable to lysis by classical surfactants than liposomes, while preserving low immunogenicity.[3,16] Polymersomes are usually prepared using various techniques: direct dissolution of copolymers, addition to aqueous media of the copolymer previously dissolved in an organic solvent, or hydration of a copolymer film.[15]

Polymersomes can be tuned to specific sizes for particular routes of administration, improved mechanical stability, and stimuli responsiveness, and can be functionalized for

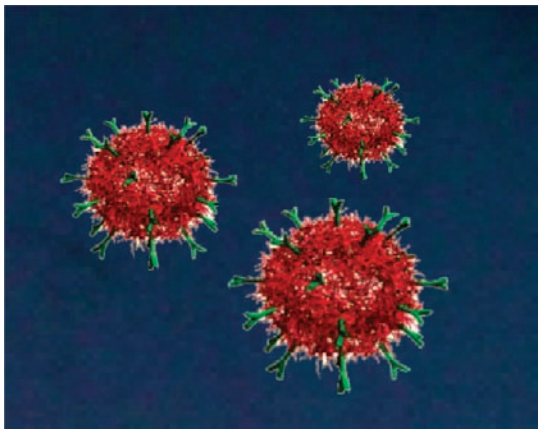

FIGURE 1. Poly(*N*-vinylpyrrolidone) capsules assembled by the layer-by-layer technique and functionalized with NHS-PEG-Az linkers (succinimidyl ester group−poly(ethylene glycol)−acid). Adapted with permission from ref 68. Copyright 2010 American Chemical Society.

targeting approaches.[6,17] A key feature of polymersomes is the membrane, because it serves to partition aqueous volumes with different compositions and concentrations, based on its selective permeability to hydrophobic and hydrophilic molecules.[4] Different studies have contributed to highlighting the fact that high molecular weights of polymers are related to thicker membranes up to 30 nm[18] as well as to a decrease in membrane fluidity.[19] In addition, they preserve the encapsulated compound for longer periods, because their diffusion coefficients ($D \sim 0.1\ \mu m^2/s$ and less) are at least 1 order of magnitude lower than those of lipid membranes. The flexibility of the membrane is essential when insertion of membrane proteins becomes necessary for the passive diffusion of various compounds or for the development of nanodevices.[20] Polymersomes have the advantage of allowing for the simultaneous encapsulation of hydrophilic components in their aqueous cavities and insertion of hydrophobic molecules within their membranes.[21]

An alternate approach to designing nanocarriers is based on layer-by-layer (LbL) assembly technology (Figure 1). The assembly of LbL polymer capsules entails the sequential adsorption of interacting polymers onto sacrificial template particles, followed by template removal.[13] The flexibility of this approach in terms of chemical nature and the properties of polymers/template particles is limited only by the specific requirements related to therapeutic applications, such as biodegradability and biocompatibility. LbL technology confers the advantage of generating capsules with excellent structural stability[22] and allows the functionalization of nanoparticles smaller than 100 nm while restricting aggregation.[13] The number of polymer layers is expected to act as a tool to control payload retention, degradation profiles, and drug release rates.[13,23] However, the thickness of the polymer wall can affect cross-transport and therefore decrease the efficacy of active compounds encapsulated inside. The biggest drawback of the LbL strategy is the time-consuming sequential polymer deposition cycles and the purification steps. In addition, designing LbL capsules for drug targeting approaches still remains one of the core challenges in the field. The intrinsic limitations of LbL capsules explain why they have been mainly used only as conventional carriers for drugs or fluorescent agents.

Cytotoxicity and Release from Polymeric Vesicles

A basic issue in medicinal applications using synthetic polymer vesicles is the avoidance of nonspecific, systemic, or off-target toxicity. Inherent cytotoxicity represents a severe limiting factor for the utility of a potential candidate carrier and dramatically decreases the number and combination of copolymers that can serve to generate appropriate vesicles.[24] The selection of *"safe"* building blocks (biocompatibility, water solubility, immunogenicity) is one of the critical steps in macromolecular design of a therapeutic/diagnostic candidate. The first detailed studies on such were carried out with polymersomes made of poly(ethylene glycol)−poly(lactic acid)/poly(ethylene glycol)−poly(caprolactone) mixtures loaded with doxorubicin and paclitaxel,[25] where the efficiency of the loaded polymersomes was twice that of the free drug in tumor cells.

A key factor in conventional drug delivery is the release profile of the payload. This mainly depends on the polymer itself (concentration, molecular weight, charge, etc.). For example, the polymer density in the membrane is often used to control the release of a loaded compound, while the molecular weight, proportional to its inherent viscosity, has to be limited to favor the release. Moreover, the degradation rate of the polymers affects the release of the loaded compounds. These critical aspects made it necessary to introduce new concepts, such as nanoreactors and synthetic organelles.[5]

In terms of supramolecular structures, there are various properties that play a role in the loading efficacy of the carrier, such as size, morphology, polydispersity, and adhesion properties. For example, vesicle morphology is an important factor in interaction with cells: a pronounced difference in morphology leads to a different cell response and mechanism of internalization.[26] These factors together constitute a complex scenario that has to be considered

in order to optimize vesicles for specific and efficient medical applications.

Polymer Vesicles in Therapeutics

Since the first reports on block copolymers and their self-assembly into polymer vesicles were brought to attention, increasing heed has been given to their medical applications, in particular as candidates for drug/protein delivery purposes. Encapsulation of drugs,[27−29] DNA,[30] and proteins[31−33] inside polymersomes for delivery applications was achieved years ago. Hydrophilic drugs can be easily encapsulated in the aqueous core of polymersomes. Now, more attention is being paid to designing polymers that allow for the entrapment of RNA,[34] water insoluble drugs[35] or the simultaneous loading of hydrophilic and hydrophobic drugs[21,36] with high efficiency. In therapeutic applications, high encapsulation efficiency is desirable. This can be achieved by complexation of the encapsulated compound[37] and by optimization of the copolymer block lengths.[38] For example, an encapsulation efficiency of 88% has been achieved for insulin when this was complexed with sodium deoxycholate to generate an ion-pair complex inside poly(ε-caprolactone)−poly(ethylene glycol)-based polymersomes.[39]

There are intrinsic drawbacks to conventional drug delivery approaches: (i) difficult control of the release profile, especially for time-release effects, (ii) possible release of the payload into biological compartments other than those desired, and (iii) difficult control of polymer degradation. These drawbacks made it necessary to introduce new concepts such as allowing active compounds to act in situ, combining multiple, active compounds in the same carrier and making cascade reactions possible. Such circumstances require a shift from passive drug carriers to smart polymeric vesicles that act as compartments for nanoreactors, or to synthetic organelles.

Some years ago, we introduced the concept that we now refer to as polymer nanoreactors. These are polymersomes with encapsulated enzymes that, in their entirety, act as nanometer-sized reaction compartments (Figure 2a).[40] Polymer vesicles made of poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA) block copolymers serve to build nanoreactors by shielding the enzymes from proteolytic attack and simultaneously facilitating their activity in situ. This avoids the necessity of releasing the enzyme to act, and therefore the previously mentioned drawbacks. Achieving in situ

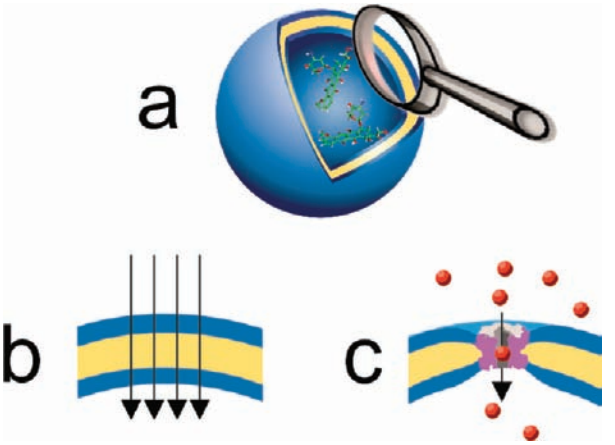

FIGURE 2. Schematic representation of (a) polymersome containing an active compound inside its aqueous cavity, (b) selectively permeable polymeric membrane, and (c) polymeric membrane with inserted channel protein.

reactions requires selectively permeable polymer membranes in order to exchange substrates and products with the environment. Typically, these can be created by using appropriately designed block copolymers that form nanoporous membranes[41] or by inserting channel proteins into the vesicle walls (Figure 2).

In the case of polymersomes with permeable membranes, the substrate penetrates the vesicle wall and is converted by the encapsulated enzyme, as reported for a number of model enzymes: lipase B,[42] glucose oxidase,[41,43] and trypsin.[44,45] We introduced the concept of antioxidant nanoreactors by using polymeric vesicles with an oxygen-permeable membrane to encapsulate superoxide dismutase, well-known as an antioxidant enzyme (Figure 3a).[46] Only a few enzyme molecules inside the cavity were necessary to efficiently detoxify superoxide radicals present in the vesicle environment. We improved the antioxidant nanoreactor by modifying the molecular properties of polymersomes to achieve high encapsulation efficiency and increased membrane permeability.[38]

A drawback to a nanoreactor with a permeable membrane is that the product accumulates inside the vesicle and can inhibit the enzyme or oxidize the polymer, resulting in vesicle rupture and enzyme release.[43] Various chemical modifications are required, such as designing polymers that are oxidized by one of the reaction products (H_2O_2), resulting in the destabilization of polymersomes and release of the products.[43] Of course, upon vesicle destabilization, the enzyme may also be released, which imposes a maximum concentration limit in the H_2O_2 such that it will not destroy the vesicles.

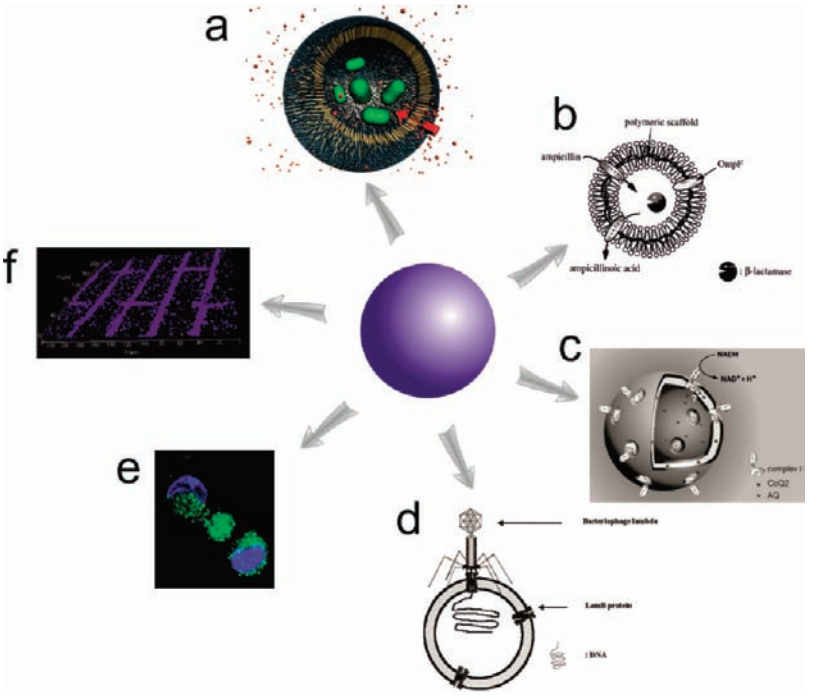

FIGURE 3. Schematic representation of various approaches that we designed for therapeutic and diagnostic applications. (a) Antioxidant nanoreactor. (Reprinted with permission from ref 46. Copyright 2008 American Chemical Society.) (b) Reconstitution of channel proteins in polymer membrane. (Reprinted with permission from ref 48. Copyright 2001 American Chemical Society.) (c) Electron-transfer nanodevice. (Reprinted with permission from ref 60. Copyright 2010 WILEY-VCH Verlag GmbH & Co. KGaA.) (d) Virus-loaded polymersome. (Reprinted with permission from ref 47. Copyright 2002 National Academy of Sciences, U.S.A.) (e) Visualization of nanoreactors uptake and function in cell lines. (Reprinted with permission from ref 44. Copyright 2008 American Chemical Society.) (f) visualization of nanoreactors immobilized on surfaces. (Reprinted with permission from ref 54. Copyright 2010 WILEY-VCH Verlag GmbH & Co. KGaA).

Remarkably, despite their thickness and mechanical stability, synthetic block copolymer membranes of PMOXA-PDMS-PMOXA allow for a functional insertion of membrane proteins that enable controlled permeability (Figure 3b), as proved by reconstituting various bacterial channel proteins, such as LamB, OmpF, maltoporin, and aquaporin.[47−51] Using this concept, various enzymes (β-lactamase,[52] acid phosphatase,[53,54] horseradish peroxidase,[55] and purine-specific nucleoside hydrolase[51]) have been encapsulated and their substrates/products have been transported through the polymersome wall with the help of channel proteins. So far, nanoreactors that encapsulate enzymes and include reconstituted channel proteins have been demonstrated to be suitable candidates for prodrug therapy, as shown by nucleoside hydrolase encapsulation, where a prodrug (2-fluoroadenosine) converts to a cytotoxic molecule 2-fluoroadenine.[51]

A design that permits control of vesicle wall permeability using channel proteins confers the great advantage of possible tuning, for example, by genetic engineering.[55−57] A similar attempt was undertaken using synthetic pores.[58] The pores had a diameter of 13.9 and 14.5 Å, comparable to the cross section of the OmpF channel protein, but only ions or protons were transported. Bigger molecules require pores with larger diameters and, in this case, the question that arises is: How stable will these large pores be in the polymersome membrane?

It has been possible to perform coupled cascade reactions by incorporating different enzymes in the inner cavity and within the membranes of vesicles.[42] The cascade reaction inside the polymersome increased the in situ complexity of the system considerably. Additionally, copolymer biomembranes have been functionalized by means of bacteriorhodopsin and cytochrome c oxidase, which are capable of energy transduction,[59] or by reconstitution of NADH-ubiquinone oxidoreductase to mediate electron transfer inside the membrane (Figure 3c).[60] Also, gamma-phages have been shown to bind to LamB receptors within the walls of block copolymer vesicles and inject their DNA into the polymer particles (Figure 3d).[47] These studies represent a necessary foundation that permits an increase in the engineered complexity inside protein−polymersome assemblies in order to overcome the challenges of pathological events. If the polymer nanoreactors are designed in such a manner that

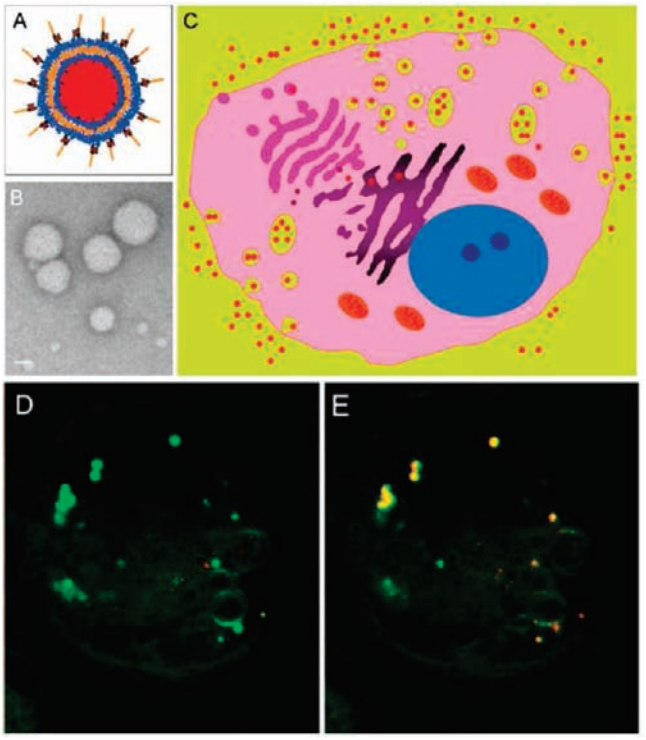

FIGURE 4. (A) Schematic drawing of a functionalized polymer vesicle and (B) the corresponding transmission electron microscopy image (scale bar: 50 nm). (C) Cross section of a schematic cell incubated with functionalized polymer vesicles (red dots). (D) THP-1 cells, where lysosomes are stained green, incubated 5 min in the presence of functionalized polymeric vesicles loaded with sulforhodamine B. (E) Lysosomes turn to yellow after an additional 5 min incubation, due to the overlap of red (functionalized polymer vesicles) and green (lysosomes). Reprinted with permission from ref 44. Copyright 2008 American Chemical Society.

their structural integrity inside the cells is preserved over extended periods of time, de facto, they would serve as artificial organelles (Figure 4).[44]

Functionalization of polymeric vesicles with specific ligands that target receptors on cell surfaces allow their selective targeting toward biological cells (Figure 5).[17,53] Targeting the polymersomes to a desired cell type and location inside the body is of essential importance for drug delivery applications.[61,62] Nanoreactors made of PMOXA-PDMS-PMOXA have already been functionalized with poly G ligands and delivered selectively inside various cell lines (Figure 3e).[28,44,53] The nanoreactors were stable and retained their activity for several days in serum.[44] Low cytotoxicity and inflammatory response ex vivo and in vivo have been confirmed after incubation with hepatocytes for 4 days.[63]

Encapsulation of active compounds inside layer-by-layer assembled capsules has been extensively studied and described in literature,[64,65] and we will focus only on aspects relevant to therapeutic application. After numerous studies of fluorescent dyes and soluble drugs encapsulated in LbL vesicles, research is now heading toward encapsulation of hydrophobic drugs[66] or DNA.[67] Functionalization of the polymers that form the layers of the capsules is another aspect in focus in the field. Capsules formed from poly(methacrylic acid) and poly(*N*-vinylpyrolidone) have been covalently coupled with a functionalized antibody and administered to cancer cells.[68] LbL capsules made of various polymers have been extensively studied as models, but studies pertaining to their cellular uptake, cytotoxicity, degradation, and biodistribution are limited.[69] Poor control of their permeability limits therapeutic applications, as in the case of encapsulated urease that retained only 13% of its activity, due to the difficulties of substrate penetration.[70] To the best of our knowledge, there are currently no complex nanoreactors or synthetic organelles based on LbL capsules because of the procedure necessary for their preparation, which might induce deactivation of sensitive enzymes.

A new approach to form self-assembled polymer capsules from oppositely charged polymers has been recently described.[71] The self-assemblies have been named PICsomes and were able to encapsulate fluorescent water-soluble molecules[71,72] and proteins.[73] Release studies indicated that the permeability of the vesicles is increasing with time and thus further cross-linking to improve stability is required. The cross-linked PICsomes had long lifetime in the bloodstream comparable with the one of polymersomes, but no release studies for the latter have been done.[72] It would have been of interest for encapsulation efficiencies to be calculated as the authors present the PICsome concept as being cost-effective; this depends also on the capacity of the PICsomes to entrap molecules during the encapsulation procedure.

Polymer Vesicles for Diagnostics

As a prerequisite for the development of multifunctional systems for theranostic strategies, we will briefly present specific aspects related to diagnostic requirements, conditions, and challenges. Various "active" compounds are available, depending on the physical phenomenon used for detection: fluorescent dyes, inorganic compounds, or metal complexes. Polymeric vesicles in combination with these active compounds open new perspectives in terms of decreasing the concentration of the imaging agent below the limit associated with side effects, protecting the payload

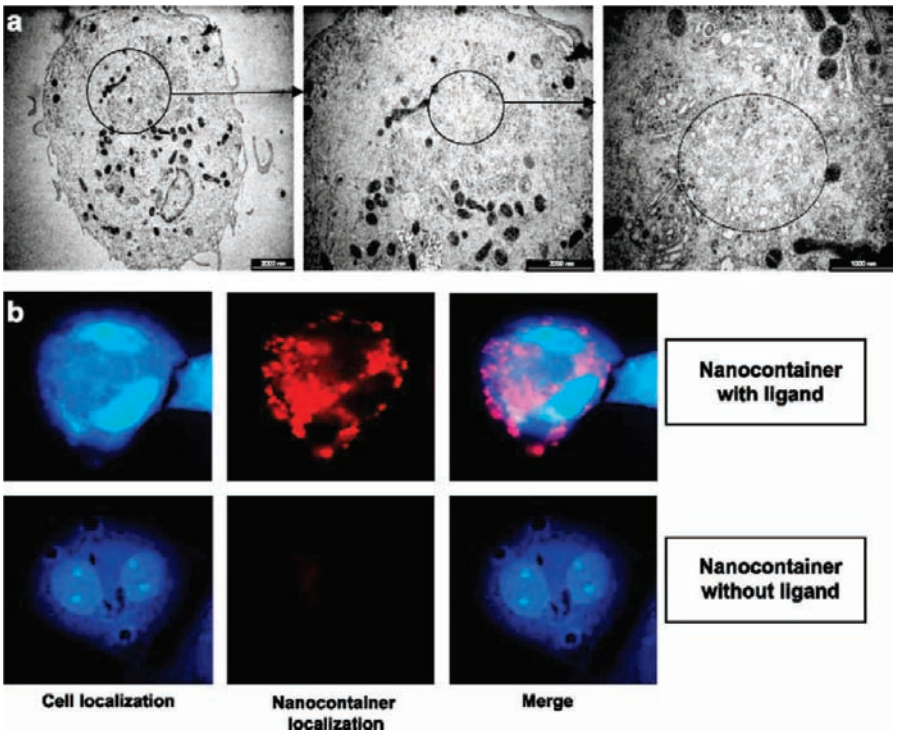

FIGURE 5. (a) TEM shows functionalized nanocontainers in THP-1 cells. (b) Fluorescently labeled nanocontainer found at the surface and inside macrophages. Reprinted with permission from ref 53. Copyright 2005 Elsevier B.V.

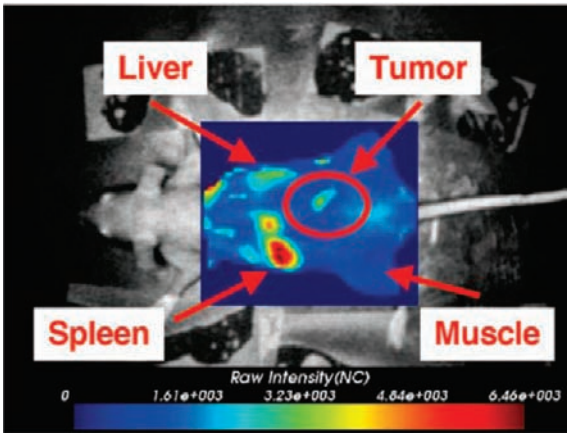

FIGURE 6. Images of a tumor-bearing mouse (in the prone position) taken 6 h after tail-vein injection of nontargeted quantum dots containing polymersomes. Reprinted with permission from ref 77. Copyright 2009 by John Wiley & Sons, Inc.

from degradation, or targeting to a particular area.[74] In order to be used for in vivo diagnostic approaches, markers containing polymer vesicles must fulfill general requirements of biocompatibility and biodegradability as well as specific requirements of high sensitivity and specificity to detect particular changes generated by the pathological condition.[75]

Polymersomes, as compartments for specific detection, offer both their inner cavity for encapsulation and their membranes for insertion of signal generating compounds. Appropriate fluorescent dyes such as Nile Red (peak emission 603 nm), which do not overlap in terms of absorbance with other molecules under biological conditions, were used inside polymersomes.[76] Encapsulation inside polymersomes represents the ideal way to prevent toxic effects of particular active compounds, for example, of quantum dots (Figure 6).[77] In a model system, quantum dots such as PbS or TiO_2 were enclosed within oleic acid and encapsulated in poly(styrene-*b*-acrylic acid) polymer vesicles[78] for use in detection via a noninvasive technique.

Ultrasound and magnetic resonance imaging are detection methods for which the quality of the image is significantly improved by using various contrast agents. The insertion of superparamagnetic iron oxide nanoparticles within the membrane of polymer vesicles significantly enhances sensitivity, in both ultrasound and in vivo MRI experiments.[79] To improve water exchange, which is essential for improving MRI image quality, the contrast agent can be trapped by chelation inside a polymersome with a porous membrane.[80] A step further is taken by the simultaneous use of multiple, active compounds in the same vesicle (Figure 7).

An elegant approach was introduced by encapsulation of acid phosphatase to produce in situ Gd nanoparticles.[81] This nanoreactor combined an improvement in detection with a

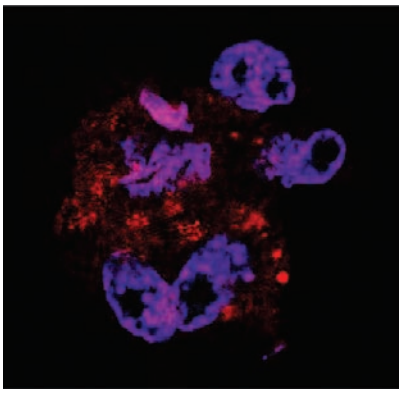

FIGURE 7. Confocal microscopy imaging of THP-1 macrophages treated with fluorescence-labeled polymer vesicles. Reprinted with permission from ref 81. Copyright 2009 CLINAM.

limitation of side effects, due to Gd encapsulation inside the cavity. We expanded the use of nanoreactors to exploit them in diagnostics by encapsulating acid phosphatase, which converts a nonfluorescent substrate to a fluorescent product. The nanoreactors, immobilized on a glass surface, showed preserved activity of the encapsulated enzyme (Figure 3f).[54]

Polymeric vesicles obtained via the layer-by-layer technology have been used in combination with fluorescent molecules, for example, a pH-sensitive dye that allowed specific detection of cancer cells.[82] However, the use of layer-by-layer generated vesicles in diagnostics is still limited, due to their intrinsic drawbacks related to membrane permeability.

Polymeric Vesicles for Theranostic Applications

The combination of drugs and markers/contrast agents inside of polymeric vesicles allows the design of multifunctional systems that operate in the form of theranostic-nanoagents.[83] Different diagnostic techniques, such as optical, magnetic resonance, or ultrasound imaging can be combined with drugs/enzymes in order to formulate an efficient theranostic strategy. Bifunctional compounds have been encapsulated in polymersomes of different block copolymers.[84−87] These biodegradable polymersomes have great potential for site-specificity, where they would release encapsulated drugs simultaneously with fluorescence detection. Difficulties in application may occur because of the reduced signal intensity of in vivo fluorescent detection in tissue.

Polymersomes have been simultaneously loaded with superparamagnetic iron oxide (SPIO), an MRI contrast agent, and doxorubicin (DOX), an anticancer drug (Figure 8).[88]

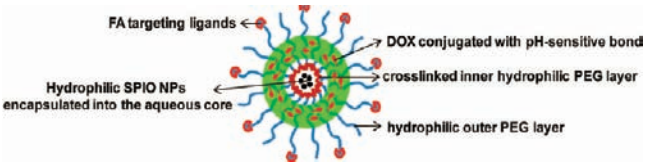

FIGURE 8. Schematic illustration of a tumor-targeting multifunctional polymer vesicle for targeted cancer chemotherapy and imaging. Reprinted with permission from ref 88. Copyright 2010 American Chemical Society.

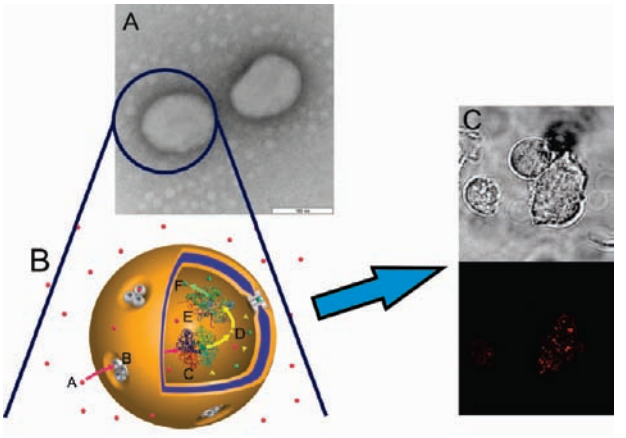

FIGURE 9. (A) Transmission electron micrograph of PMOXA-PDMS-PMOXA vesicles containing a combination of antioxidant enzymes. (B) Schematic representation of enzymatic cascade reactions inside polymersomes. (C) In vitro uptake and activity in THP-1 cells. Reprinted with permission from ref 90. Copyright 2011 WILEY-VCH Verlag GmbH & Co. KGaA.

Readily taken up by HeLa cells, they improved MRI imaging. However, cytotoxicity, even though lower than that of the free drug, is still a limitation. Similarly, poly(*g*-benzyl L-glutamate)-*block*-hyaluronan polymersomes, loaded with a radioactively labeled docetaxel, successfully accumulated at the tumor site.[89]

We designed a combined system to fight oxidative stress and simultaneously visualized its activity in cells (Figure 9).[90] A combination of enzymes acting in tandem inside polymersomes detoxified both superoxide radicals and related H_2O_2, ending in a completely safe scenario by generating water and O_2. Incorporation of the channel protein, OmpF, in the polymer membrane enabled the co-substrate of the second enzyme to penetrate the cavity, where it was converted to a highly fluorescent product. This simple fluorescent method enables a following of the efficacy of the detoxification process in real time and in various cell lines.

All these systems, which combine active compounds with detection agents inside polymeric vesicles, represent the first step toward theranostic applications.

Conclusion

In this Account, we have shown that different functional hybrid systems can be created. These include nanoreactors and artificial organelles based on polymeric vesicles and active compounds for efficient biomedical strategies. The selection of polymers for vesicle preparation must take into account the complex requirements of biological conditions for innocuous, specific transport to the areas with precise pathological events. Polymeric vesicles feature the advantages of greater stability than lipidic carriers, the most investigated platform for medical purposes, while preserving their low immunogenicity. They span a wide range of properties in terms of size, membrane permeability and target specificity. In particular, polymeric vesicles generated by self-assembly of amphiphilic copolymers (polymersomes) offer both their inner cavity and the membrane itself for encapsulation/insertion of highly sensitive molecules, such as DNA, RNA, enzymes, and membrane proteins. The large variety of combinations of active molecules serves to design nanoreactors or artificial organelles with the specificity and multifunctionality necessary to cope with pathological events in terms of detection and treatment. Nanoreactors offer the advantage of simultaneous protection from proteolytic attack, degradation, and toxicity and allow in situ complex reaction schemes for sensitive, rapid, and specific reply to pathological conditions. In addition, they allow for the development of theranostic approaches by combining therapeutic compounds with markers/contrast agents in a single active system. Even if these systems are still at the level of models and in vitro or in vivo experiments, their improved properties in terms of stability, detection sensitivity, and multifunctionality make them excellent candidates for new strategies in the medical domain.

BIOGRAPHICAL INFORMATION

Pascal Tanner received his M.S. degree in Nanoscience from the University of Basel. He is currently a Ph.D. student at the University of Basel, with PD Dr. Cornelia Palivan.

Patric Baumann received his M.S. degree in Nanoscience from the University of Basel. He is currently a Ph.D. student at the University of Basel, with Prof. Wolfgang Meier.

Ramona Enea received her Ph.D. degree in Macromolecular Chemistry in September 2007 from the Technical University Iasi, Romania. She is since a postdoctoral fellow in the Department of Chemistry at the University of Basel. Her efforts are focused on interactions between self-assembled structures and living systems.

Ozana Onaca received her Ph.D. degree in Biochemical Engineering at the Jacobs University Bremen in 2007. After postdoctoral work, she is since 2010 an Ambizione fellow of the Swiss National Science Foundation at the University of Basel. Her research interest focuses on genetic engineering of membrane proteins and reconstitution in polymeric membranes.

Cornelia Palivan studied Physics at the University of Bucharest and received, after a two year research stage at the University of Geneva, her Ph.D. degree in Atomic and Molecular Physics in 1995. She is currently Privat Dozent in Physical Chemistry and Senior Group Leader at the University of Basel. She received several awards for her research. Previously focused on structural characterization of metal complexes, she enlarged her domain by combining metal proteins with amphiphilic copolymers to develop hybrid materials.

Wolfgang Meier studied Chemistry at the University of Freiburg and received his Ph.D. degree in Macromolecular Chemistry in 1992. His Habilitation followed at the University Basel in 1998. In 2001, he was appointed as a Professor at the International University of Bremen, and since 2003 he is Professor of Chemistry at the University of Basel. He received several awards for his research. His main research interests are in the field of hierarchical self-assembly of functional polymers, and polymer–protein hybrid materials.

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FOOTNOTES

*To whom correspondence should be addressed. E-mail: cornelia.palivan@unibas.ch (C.P.); wolfgang.meier@unibas.ch (W.M.).

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