Liposome-containing polymer films and colloidal assemblies towards biomedical applications

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**Introduction**

In the last decade or two, liposomes have attracted significant attention as potential drug carriers from researchers interested in the field of nanomedicine. These thermodynamically stable supramolecular structures are formed spontaneously when amphiphilic lipids are brought into contact with an aqueous phase. Once formed, liposomes are typically between 20 nm to 10 μm in size with the phospholipid bilayer membrane being approximately 5 nm in thickness.¹ The unique architecture of liposomes allows for the loading of hydrophobic and hydrophilic therapeutic molecules; their charge and surface properties can be tuned to enable stability during storage, control over the drug-release rate, and to meet specific therapeutic needs.²

These nanocarriers are also biocompatible, since they are typically made from lipids commonly found in biological systems and biodegradable via the usual metabolic pathways. As such, the high biocompatibility and versatile nature of liposomes have made these nanocolloids key components in many biomedical research topics such as biomimetic chemistry, and have been used as subunits in multilayered polymer thin films (e.g. in ref. 3) and in colloidal assemblies for biomedical applications.⁴

In order to provide a succinct overview on the recent developments in the use of liposomes as nanocontainers in the fabrication of subcompartmentalized functional layer-by-layer (LbL) surface coatings and multicompartmentalized colloidal assemblies (Scheme 1), this minireview is organized as follows. First we outline several subcompartments, namely, cyclodextrins (CDs), block copolymer micelles (BCMs) and with focus on liposomes within polymer thin films as examples of successful
proof-of-concepts in surface mediated drug delivery (SMDD). Each of the subunits will be reviewed in terms of its assembly and characterization towards SMDD. We will highlight those composite coatings that are successfully used to deliver (active) compounds to adhering cells, and emphasize the few films which have been tested in vivo and in vitro. Secondly, we will outline and highlight several recent advances in colloidal assemblies containing liposomes as nanocontainers. We will discuss the assembly approaches employed to fabricate these biomimetic carriers and provide examples of successful proof-of-concepts of these colloidal assemblies as biomedical platforms.

**Planar coatings**

Planar films consisting only of polymers are themselves able to control cellular responses (e.g., cell adhesion and differentiation) or can be loaded with active cargo (e.g., proteins or genes) towards SMDD. However, trapping a small molecular payload or cargo relying on its 3D structure such as enzymes, remains challenging. Trapping larger carriers such as drug deposits to address this aspect has turned out to be a successful approach, especially when fundamentally different building blocks are employed, yielding subcompartmentalized multilayered hybrid films.

**General overview**

Cyclodextrins (CDs). CDs, cyclic oligosaccharides consisting of six (α), seven (β) or eight (γ) glucose units within the ringed structure results in a unique cup like morphology with a polar exterior and a hydrophobic interior, allowing for the encapsulation of small hydrophobic molecules in an inclusion complex e.g., to enhance therapeutic effects or to reduce toxic side effects of drugs. Therefore, modified CDs have been incorporated into multilayered films, and these coatings can be engineered to be, for instance, responsive to solvent change, or degradable by exposure to reducing conditions. There are also a limited number of reports where the biological evaluation of the CD-containing films was considered, including the assembly of pure anti-inflammatory films, anti-inflammatory/microbicidal films, films with antitumoural activity, and films for growth factors or gene delivery.

Block copolymer micelles (BCMs). BCMs, assembled from amphiphilic block copolymers, are powerful drug deposits within polymer films which can either be glassy (i.e., hard and relatively brittle) or responsive to environmental stimuli. There are only single digit studies on BCM-containing films which demonstrated drug delivery ability in vitro by delivering small hydrophobic cytotoxic cargo, with so far no in vivo studies.

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Brigitte Städler obtained her PhD degree at ETH Zürich, Switzerland followed by postdoc time at the University of Melbourne, Australia. She has been head of the Laboratory for Cell Mimicry at iNANO, Aarhus University, Denmark since 2010. Her research group focuses on the development and characterization of smart, nature-inspired assemblies towards their use in therapeutic cell mimicry as an alternative approach to treat chronic medical conditions in a sustained manner.
Miscellaneous. In this section, examples of other types of drug deposits that have been trapped in polymer films with the aim to be used in SMDD will be briefly reviewed, although only little biological evaluation has been reported. Cubosomes, cubic lipid mesophase nanoparticles, are one of the examples as an alternative to liposomes. Polymersomes, assembled from amphiphilic synthetic block copolymers, have been entrapped in planar polymer films consisting of poly(vinyl pyrrolidinone) and tannic acid by Lomas et al., and in surface-adhering physical poly(vinyl alcohol) hydrogel films by Hosta-Rigau et al. In the latter case, the viability of the cells adhering to these coatings containing polymersomes loaded with a hydrophobic cytotoxic drug was significantly reduced as compared to films with empty polymersomes. Further, hydrophobic nanodomains have been trapped in polymer films with demonstrated control of the release of encapsulated paclitaxel. In a recent study by the Lalavle group, vascular endothelial growth factor loaded degradable nanoparticles were embedded within polyelectrolyte multilayers (PEMs). Enhanced proliferation and nitric oxide secretion has been demonstrated for human umbilical cord vein cells adhering to films containing the drug loaded particles.

Liposome-containing films. Liposomes are by far the most explored drug deposits in polymer films probably due to their biocompatibility, ease of preparation via self-assembly and their inherent capability to entrap both hydrophilic cargo in their lumen and/or hydrophobic cargo in their lipid membranes.

Assembly. The challenge of stably trapping intact liposomes without their displacement or rupture in polymer films has been addressed in different ways. Katagiri et al. were the first ones to combine liposomes and polymer films in 2002 by employing silica-coated liposomes. Alternatively, intact liposomes coated with poly-\(\nu\)-Lysine were incorporated into polymer films, including their use as microreactors. Furthermore, liposome trapping approaches without the need for stabilization prior to their immobilization within the polymer coatings employ electrostatic interactions or non-covalent anchoring via cholesterol- or oleic acid-modified polymers. In the latter case, cholesterol turned out to be a more efficient anchor than oleic acid or electrostatic interactions only to assemble (multiple layers of) liposomes in polymer films.

Control over retention and release of cargo entrapped within the liposomes is of paramount importance for applications in SMDD. To this end, mechanisms to trigger the release of their cargo include temperature or electrochemistry.

Drug delivery applications. To the best of our knowledge, we were the first ones in 2011 who employed liposome-containing films in vitro towards SMDD by immobilizing zwitterionic liposomes (\(L\) and \(L\)) for fluorescently labeled liposomes) onto a poly-\(\nu\)-lysine (PLL) pre-coated glass slide and subsequently capping with a poly(dopamine) (PDA) layer. PDA, deposited by oxidative self-polymerization of dopamine, is a versatile building block in hybrid coatings with many unique properties including the possibility to control its thickness via the deposition conditions or the blending of DA with a (functional) polymer during assembly. We demonstrated, that the cell mean fluorescence (CMF) of adhering cells, due to their association with fluorescent lipids trapped in the liposome membrane, could be controlled via varying the thickness and type of capping layer. With the aim of facilitating the assembly, a mixture of liposomes and dopamine (\(F_{10M:10L}\); mixture of fluorescent liposomes (\(F\)) and \(x\) mg mL\(^{-1}\) dopamine) was successfully used to assemble liposome-containing films with more sustained delivery of fluorescent cargo to adhering cells over 24 h depending on the assembly conditions (e.g., dopamine concentration). Further, we delivered the small hydrophobic and cytotoxic compound thiocarboline (TC) trapped in the lipid membrane of the liposomes from these films to adhering myoblast cells and found a significant decrease in cell viability, demonstrating the potential of these coatings in SMDD.

Additionally, liposome-containing multilayered polymer films were considered, and the response of adhering mammalian cells was assessed. Graf et al. used pH-sensitive, negatively charged liposomes loaded with calcine trapped within polymer layers, and demonstrated the uptake of the triggered release of the cargo by adhering myoblasts. We recently employed PLL/PEMs (PEMs: (PLL/poly(methacrylic)-co-(cholesterol methacrylate) (PMA\(_{c}\)) or (poly(allylamine) (PAH)/poly(styrene sulfonate (PSS))\(_{c}\)) films, and evaluated the response of adhering myoblasts and hepatocytes. The key findings were that (i) the CMF of the hepatocytes, due to their association with fluorescent lipids, was higher than for myoblasts adhering to the same film for the same time, (ii) the fluorescence was evenly distributed across the cell interior of the hepatocytes, while there was an accumulation of fluorescence in the myoblasts in the proximity of the nuclei (Fig. 1a), and (iii) the cell viability of adhering myoblasts was found to be more reduced than for the hepatocytes when TC was incorporated into the liposomes. In a follow up, we combined the sequential deposition of polyelectrolytes with PDA-based building blocks and assembled PLL/PEMs/F\(_{10M2.5}\) films. An interesting finding was that there was no significant difference in the amount of adsorbed L depending on the terminating polymer in the separation layers for PMA\(_{c}\) or PSS, but the CMF of myoblasts adhering to films equipped with \(F\) was significantly lower in the latter case (Fig. 1b). This demonstrated that the access of the cells to the underlying liposomes could be controlled via the film assembly without the need for an active trigger.

In an alternative approach, we assembled liposome-containing surface adhering PVA hydrogels – lipogels. We demonstrated that cells could adhere to the lipogels when PLL or PDA was present and that the cells could associate with entrapped fluorescent lipids. Further, paclitaxel as a small hydrophobic cargo entrapped within the liposomes was successfully delivered to the adhering cells from the lipogels, demonstrated by the reduced cell viability compared to cells grown on lipogels loaded with empty liposomes.

Recently, DeMuth et al. reported the assembly of polymer/liposome coated poly(\(\nu\)-glutamic acid) (PLGA) microneedles for vaccination purposes. In particular, liposomes loaded with an antigen, an adjuvant and a fluorescent tracer for the lipid walls were embedded within multilayers of biodegradable poly(\(\beta\)-amino ester) (PBAE) (Fig. 2a1). The microneedles were injected
into the cutaneous tissue of mice since it contains a high number of antigen presenting cells (APCs) (Fig. 2a2). The liposome containing films were then transferred from the surface of the microneedles into the mice tissue and, upon degradation of the PBAE, the liposomes were taken up by the APCs, liberating the encapsulated antigens and adjuvants (Fig. 2a 3 and 4). A significant enhanced humoral immune response due to the transcutaneous vaccination was observed. Fig. 2b shows a SEM micrograph of the liposome coated microneedles.

### Colloidal assemblies

Subcompartmentalized hybrid films in colloidal form are relevant in therapeutic cell mimicry. This concept is inspired by the hierarchical structure of biological cells, which can perform multiple encapsulated reactions in a temporally and spatially controlled manner in parallel or as a cascade.

### General overview

The assembly of subcompartmentalized colloidal systems has attracted considerable interest in the last few years. Conceptually, single and multiple composition systems can be distinguished, depending on whether the subunits and the carrier are assembled from the same or different type of components. The former case includes vesosomes, polymersomes within polymersomes, or polymer capsules within polymer capsules. The latter case includes polymer capsules containing cubosomes, polymersomes, liposomes (termed capsosomes) or polymer capsules together with liposomes. Although for many of these examples only the assembly has been reported, it shows nonetheless the potential of compartmentalized systems. However, within the scope of this minireview, only concepts involving liposomes and subunits as drug deposits will be further discussed. Therefore, approaches involving (metallic) nanoparticles e.g., for light triggered activity or imaging will not be considered and the reader is referred to the recent review by Zasadzinski et al.

### Liposome-containing colloidal carriers

#### Assembly

Vesosomes, liposomes within liposomes, is a single component system first reported in 1997. The initial rather complex assembly approach has been replaced with a simplified protocol based on the trapping of small liposomes due to the conversion of cochleate cylinders of dioleoylphosphatidylserine sheets into micron-sized liposomes. Also, interdigitated lipid–ethanol sheets which close on themselves when the temperature is increased, have been employed to entrap small liposomes. Vesosomes have demonstrated superior stability and control over cargo retention and release in biologically relevant fluids.
Capsosomes are assembled in a similar manner as earlier described for liposome-containing planar polymer films but using sacrificial colloidal template particles. Cholesterol-based liposome anchoring to the polymer film is again a core concept for the assembly. The polymer carrier capsules have predominantly been assembled by using not only degradable PVP/thiolated PMA (e.g., in Chong et al.\textsuperscript{83}) but also non-degradable PAH/PSS.\textsuperscript{84} In the former case, the type of the used crosslinker, to stabilize the thiol to yield stable PMA hydrogel capsules under physiological conditions, depends on the encapsulating cargo. The oxidation agent chloramine T\textsuperscript{85} has proven to be a valid approach for capsosomes carrying hydrophobic cargo in the lipid membrane of the subunits,\textsuperscript{75,85} while more benign techniques were developed either based on a thiol–disulfide exchange\textsuperscript{54,83,87} or by using small crosslinking molecules\textsuperscript{75,85–90} when enzymatic cargo was present. Stability and cargo retention are important properties for capsosomes. In the former case, PEGylation of the outer membrane of the carrier capsules not only improved their fouling characteristics, but also reduced the enzymatic degradation of the liposomal subcompartments by phospholipase A\textsubscript{2}.\textsuperscript{90} In the latter case, the lower limit of the size of cargo for stable encapsulation was found to be between 400 and 550 Da,\textsuperscript{91} with good long-term stability for larger molecules like enzymes.\textsuperscript{53,67}

**Activity.** This paragraph reviews those assemblies, which have demonstrated functionality relevant for biomedical applications i.e., for intracellular drug delivery or for encapsulated catalysis towards therapeutic cell mimicry.

**Intracellular drug delivery.** Admittedly, the assemblies discussed here are rather large for intracellular drug delivery, and their use for intravenous administration is rather limited. Nonetheless, their interaction with cells/tissue is an interesting aspect to consider in the right settings.

Fusogenic vesosomes were considered for transcutaneous immunization and evaluated in cell culture and in vivo.\textsuperscript{92} The antigen tetanus toxoid (TTx) was encapsulated in the liposomal compartments. They found a significantly increased level of anti-TTx antibodies in vivo when TTx was topically delivered via the fusogenic vesosome system as compared to cationic unilamellar liposomes or the free antigen. This study demonstrated the potential of liposome based single component systems in drug delivery.

Although intracellular drug delivery was never the core application considered for capsosomes, assessing the interaction of capsosomes with the cell becomes valid when considering other administration pathways than intravenous injection. The ability to encapsulate small lipophilic cargo in the subunits was confirmed using TC\textsuperscript{86} and taxol (TX, a mitotic inhibitor),\textsuperscript{75} and the amount of encapsulated cargo was confirmed to be dependent on the number of liposome deposition steps. The reduction in viability of cells upon exposure to capsosomes loaded with the cytotoxic compounds TC or TX was confirmed, while pristine capsosomes did not exhibit intrinsic toxicity, making them promising candidates for biomedical applications.

**Encapsulated catalysis.** The performance of encapsulated catalysis in subcompartmentalized systems is a promising way to mimic (simple) metabolic activities. Among the first ones to explore this concept using liposomes was the Vogel group. Bolinger \textit{et al.} employed surface-tethered large unilamellar liposomes with entrapped small unilamellar liposomes to demonstrate consecutive enzymatic reactions in a single nanoreactor.\textsuperscript{93}

Encapsulated enzymatic reactions within capsosomes were used with the aim to mimic metabolic activities. Initially, the enzyme β-lactamase was employed to convert the substrate nitrocefin into its hydrolyzed product, using the surfactant Triton X to lyse the liposomes and to start the reaction.\textsuperscript{94} In follow up work, we confirmed that the reaction kinetics correlates with the amount of encapsulated enzymes, and that temperature can be used to trigger the reaction allowing for the recycling of the capsosomes.\textsuperscript{95,96} However, most biologically relevant reactions are more than single-step conversions but most often involve multi-step cascade reactions running simultaneously within the same carrier. To date, one of the most advanced synthetic assemblies relies on the immobilization of different enzymes at different positions of the carrier \textit{i.e.}, in the void, inside the membrane, and on the surface.\textsuperscript{95,96} With the aim to further push the complexity of capsosomes, triggered peptide cargo release using encapsulated enzymatic catalysis was demonstrated, by enzymatically converting glutathione disulfide into its reduced form, glutathione which was able to

Fig. 3 Advanced capsosomes: schematic illustration (left) and fluorescent microscopy image (right) of microreactors containing liposomes and hydrogel capsules as subunits. Enzymatic conversion rates of heterogeneous microreactors containing β-lactamase loaded liposomes in the presences (closed symbols) and absence (open symbols) of Triton X. Reprinted with permission from ref. 76.
cleave the disulfide bond that linked the peptide to a polymer carrier.28 The assembly of a heterogeneous microreactor containing liposomes and hydrogel capsules as subunits has been reported (Fig. 3, top).29 The functionality of these heterogeneous microreactors has been demonstrated by controlled degradation of the polymeric subunits and the preservation of the activity for liposome encapsulated β-lactamase (Fig. 3, bottom).

Conclusions/future perspective

In this minireview, we summarized and highlighted the recent progress of employing drug deposits, namely, CDs, micelles and liposomes, assembled on 2D planar surfaces and 3D colloidal carriers. Focus was put on liposomes since this type of drug deposit is particularly interesting due to its distinguished advantages over CDs and micelles, including the facts that liposomes can be carriers for hydrophilic and hydrophobic cargo, a diverse range of lipids is commercially available or their use in drug delivery has accumulated broad fundamental and applied knowledge. Although there is enormous potential for the translation of these substrates containing nanocontainers towards biomedical applications, the number of in vitro studies reported is scarce with even rarer in vivo studies being reported. Further, long-term effects and sustained delivery has also yet to be realized for many systems. The field is still in its infancy but the potential is tremendous, reflected by the growing number of scientific reports each year. The focus will have to shift from introducing different assembly concepts to implementing functions into the films such as controlled loading of drug deposits and desirable sustained cargo release to adhering cells, imposing a positive therapeutic effect. We expect that in the next few years, there will be rapid progress and development in this field particularly in performing in vitro and in vivo studies and their wide implications in medical therapies. Hence, we hope that this minireview will inspire multidisciplinary research interest in this fascinating topic and to bring these substrates a step closer to clinical trials.

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Notes and references

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