Nanoparticle-triggered release from lipid membrane vesicles

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Research Paper

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Abstract

Superparamagnetic iron oxide nanoparticles are used in a rapidly expanding number of research and practical applications in biotechnology and biomedicine. We highlight how recent developments in iron oxide nanoparticle design and understanding of nanoparticle membrane interactions have led to applications in magnetically triggered, liposome delivery vehicles with controlled structure. Nanoscale vesicles actuated by incorporated nanoparticles allow for controlling location and timing of compound release, which enables e.g. use of more potent drugs in drug delivery as the interaction with the right target is ensured. This review emphasizes recent results on the connection between nanoparticle design, vesicle assembly and the stability and release properties of the vesicles. While focused on lipid vesicles magnetically actuated through iron oxide nanoparticles, these insights are of general interest for the design of capsule and cell delivery systems for biotechnology controlled by nanoparticles.

Introduction

Encapsulating drugs and vaccines has been shown to be highly beneficial for multiple reasons. A vesicle surrounded by an amphiphilic membrane allows incorporation of hydrophobic and amphiphilic drugs into the membrane while hydrophilic drugs can be stored in the aqueous interior [1]; the encapsulated cargo is protected from enzymatic degradation [2]. Furthermore, the surface functionality of the delivery vehicle can be modified to target them to certain locations [2–5]. Decreased degradation and in particular a high degree of localization of released cargo allows for use of much lower overall concentrations of the drug compared to systemic release. With this decrease in the injected and freely available drug dose, the risk of adverse side effects, e.g. toxicity or immune system reaction, is lowered [2,6]. Targeted release systems should ideally also allow for external control over timing and dose of the released cargo at the target location. There are also several biotechnological applications where externally controlled triggered release vehicles might serve as useful tools. Delivery of compounds, more efficient transfection mechanisms, poration of cells and even artificial organelles incorporated into cells are all possible applications that go hand in hand with a greater understanding of membrane release mechanisms.

Nanoparticles are increasingly researched as functional components in hybrid materials, where the integration of the nanoparticle with its matrix is key. An emerging such set of applications is the use of nanoparticles to control permeability of membranes and in particular vesicles. Many nanoparticles have unique properties allowing them to localize response, e.g. in the form of heat, to optic, electric and magnetic fields. They can therefore be used to control the properties of drug delivery structures, such as stealth liposomes, on the nanoscale; such systems and their applications have received much attention and been the subject of several recent reviews [7–9]. However, such applications put tremendous demand on nanoparticle stability at physiological conditions, close control over nanoparticle size and controlled surface presentation of functionalities.

Controlling the release rate of compounds from the vesicle in response to a change in the local environment actuated by a

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nanoparticle provides a great opportunity to optimize systems for biomedical release applications. Importantly, such constructs can circumvent the typical problem of having to balance low passive permeability (leakage) against high release efficiency for thermally activated release vehicles. The enabled externally triggered release at a controlled location and time point allows the use of very potent drugs, since the interaction with the right target is ensured. In extension, by varying the trigger particle, it can enable the sequential release of multiple drugs at controlled dosage; a major goal of personalized or nanomedicine could thereby be reached. The focus of this short review is in particular the magnetically triggered release from liposomes containing nanoparticles; what is known about the assembly of such vesicles and their optimization.

Stabilization of core–shell nanoparticles: Example of superparamagnetic iron oxide nanoparticles

Although a variety of inorganic, metal and oxide, nanoparticles are considered and tested for biomedical and biotechnological applications, superparamagnetic iron oxide nanoparticles have distinct advantages such as very low toxicity [10,64,65] and interaction with magnetic fields that penetrate biological environments [8]. Therefore, iron oxide nanoparticles are used in a rapidly expanding number of research and practical applications in the biomedical field [11]

Nanoparticles will rapidly aggregate without a dispersant shell through interactions between themselves or through interactions with biological molecules. The result of aggregation is disruption of structure, loss of function and precipitation. For the grafted dispersant shell to prevent aggregation it has to fulfill a minimum of important criteria: (i) be densely grafted; (ii) have sufficient thickness to screen interactions with the core; and (iii) be irreversibly grafted to the inorganic core surface also during extreme dilution and heating during actuation [11]. Grafting of dispersants refers to their binding to the inorganic NP surface. By covalently binding a high-affinity anchor to the end of a linear dispersant molecule a well-defined adsorption geometry to the nanoparticle surface can be achieved that fulfills these requirements, which are tantamount to colloidal stability. The resulting core–shell nanoparticles can be divided into four components: core, anchor, spacer and optional surface functionalities (Fig. 1). Each of these components can independently be adjusted through modular build-up starting from dispersants grafted to the nanoparticle interface. The resulting defined geometry renders such nanoparticles very versatile for a multitude of applications [11–13]; it is now realized to be especially crucial for the application to nanoparticle-actuated capsules [8].

Nanoparticle actuated vesicle release systems

The assembly and characterization of drug delivery systems has been the subject of many studies with an increasing number dealing with smart, bioinspired, nanoscale carriers [14,15] This tremendous scientific interest is closely related to commercial demand for stable, smart, nanoscale capsules that can easily and cost effectively be assembled in a versatile way with controlled release properties. Recent advances in application of nanoparticles to control drug release from vesicles build on new tools for controlling the architecture, colloidal stability and functionality of iron oxide, but also gold, nanoparticles.

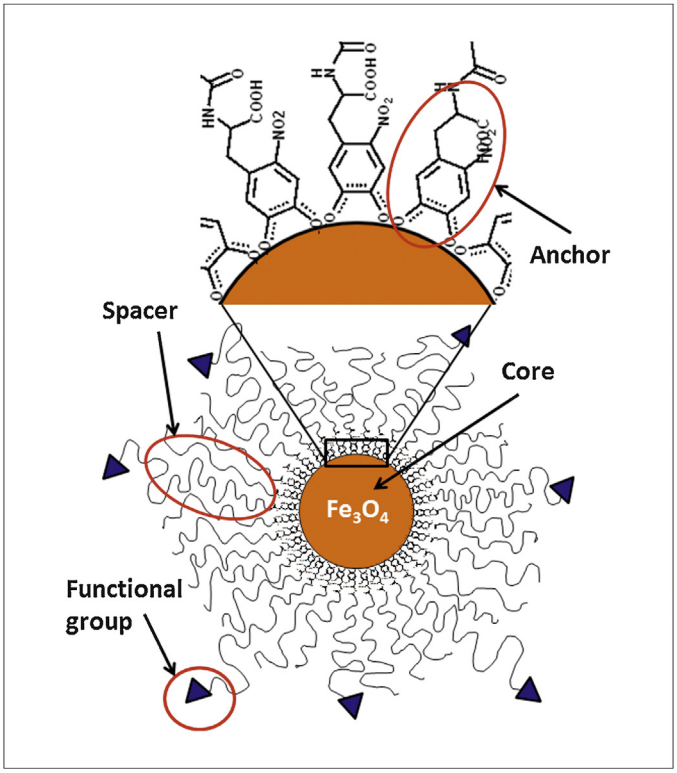

FIG. 1

Nanoparticles stabilized with irreversibly binding low M_W dispersants result in a defined core–shell structure. Such NPs can be divided into four components: core, anchor, spacer, and end-functional group, which can be optimized for the desired function in parallel. The trigger function in the core can therefore be made independent on the vesicle interaction of the shell.

Using nanoparticles as actuators to control release in drug delivery vehicles require embedding of the nanoparticle into the drug containing structure. Particularly elegant constructs are nanoscale vesicles that contain nanoparticles as part of the structure (Fig. 2). Superparamagnetic nanoparticles have some advantages for delivery applications in dense biological media compared to the more commonly proposed use of plasmonic metal nanoparticles. Magnetic fields penetrate and are relatively benign to tissue, but can interact strongly with magnetic nanoparticles even smaller than 10 nm [8]. Light with longer wavelength than near infrared light cannot penetrate into tissue or other biological samples; metallic nanoparticles resonantly interacting with NIR light require at least one dimension larger than 100 nm; such large size severely impacts their application, but can be circumvented by combining the metal nanoparticle with a sufficiently dense dielectric layer, either as a core or as coating that shifts the resonance [16,17]. Magnetically labeled drug delivery vehicles further can be used as magnetic resonance imaging contrast agents for theranostic applications where imaging/diagnostics and triggered release are combined [11].

One of the most common ways to control the release rate of compounds from delivery vehicles is to make use of a thermally driven change in permeability or solubility. For example, lipid vesicles display highly increased permeability to small compounds around the membrane melting temperature (T_m) [18,19]. Also amphiphilic block copolymers as well as hydrogel particles can

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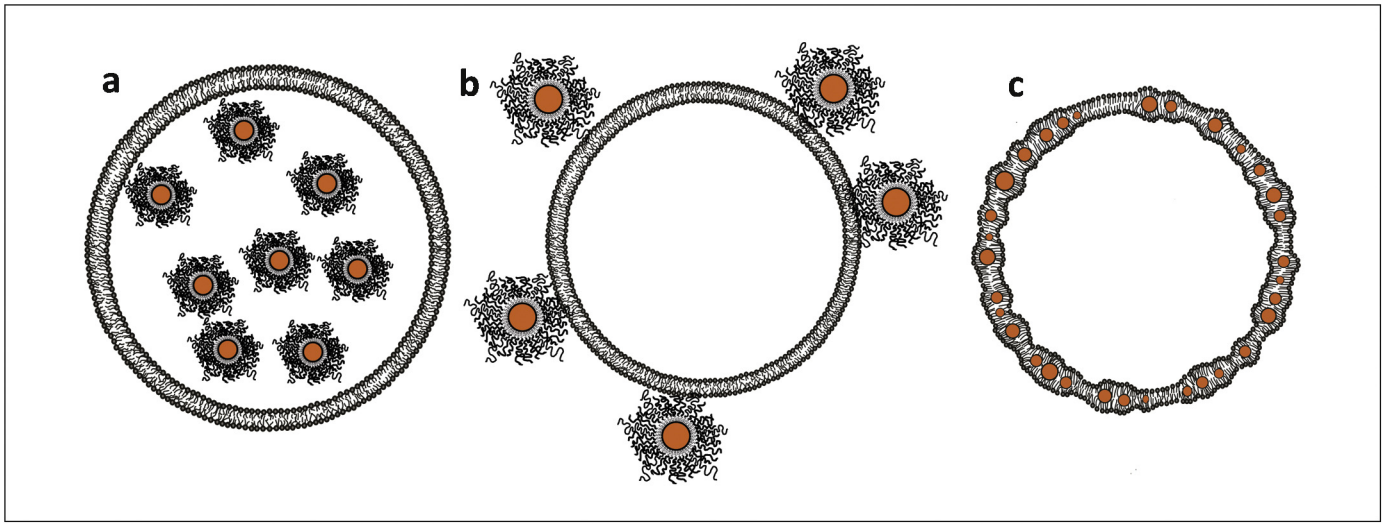

FIG. 2

Nanoparticle functionalized liposomes. Nanoparticles used as actuators to trigger cargo release can be located (a) in the liposome lumen, (b) at the liposome membrane surface and (c) within the hydrophobic region of the liposome membrane.

be engineered to have an LCST close to the physiological range at which their hydration and structure change dramatically. Nanoparticles have been integrated in thermoresponsive vesicles to trigger a thermal transition by external means for localized drug delivery. Thus, the requirements on drug delivery vehicles of low leakage at body temperature, high cargo release efficiency and minimal harm to surrounding tissue by global heating can now be optimized in parallel and simultaneously met. This is achieved through that the temperature controlled transition of the vesicle permeability can be placed at a temperature far higher than body temperature. Superparamagnetic nanoparticles locally generate heat through Brownian and Néel relaxations when they are subjected to an alternating magnetic field (AMF) in the sub-megahertz range [20]. Through magnetic heating nanoparticle local temperatures far exceeding the body temperature can be reached. Magnetically responsive nanoparticles can be as small as a few nanometer in diameter [10] and can therefore be encapsulated into the nanoscale structures of drug delivery vehicles. Nanoparticles of such small size even allows for inclusion in the lipid membranes of liposomes [21–24].

As mentioned, an efficient permeability barrier that can be modified for use in vesicular drug delivery vehicles is the cell mimetic membrane which has a hydrophobic membrane core surrounded by hydrophilic faces; drug delivery liposomes are smaller than 100 nm and comprise synthetic or natural lipids (Fig. 3). The biocompatibility and ease to assemble and surface modify liposomes render them especially attractive for biomedical applications [6,25–27]. Incorporation of PEGylated lipids into the liposome bilayer not only increases their blood circulation time in vivo [28], but was also shown to significantly reduce the leakiness of liposomes if stored at temperatures below their T_m [21]. Liposomes have been functionalized with nanoparticles localized in their lumen, inside the membrane or attached to the membrane surface (Fig. 2) to actuate release from liposomes with a T_m set higher than body temperature. The different strategies lead to differences in design criteria and release properties which will be discussed in the following sections.

Nanoparticles in the liposome lumen or associated with the membrane

Liposomes loaded with iron oxide nanoparticles in their lumen (Fig. 2a) [29–34] or in which iron oxide nanoparticles were synthesized in the lumen through aqueous precipitation methods using Fe^{3+} and Fe^{2+} salts are known for more than 20 years [35]. The nanoparticles often interact strongly with the membrane and, in more careful designs, are synthesized to decorate the vesicle surface in a specific way (Fig. 2b) [36–40]; the most common such designs have been nonfunctionalized nanoparticles or nanoparticles functionalized with ascorbic or citric acid, which also show membrane interaction.

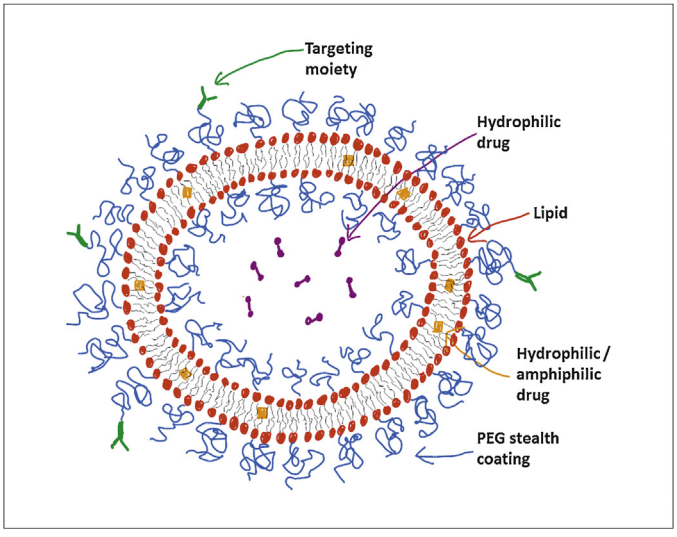

FIG. 3

Schematic of drug delivery liposome. The basic constituents of a drug delivery liposome are lipids forming a liposome membrane in the 100 nm size range, thereby encapsulating small hydrophilic drugs in the lumen or hydrophobic and amphiphilic drug in the membrane. Optionally the lipids can tether PEG to increase in vivo circulation time and specific binding groups to target the release site.

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Many studies have demonstrated AMF triggered content release from liposomes loaded with iron oxide nanoparticles in their lumen or bound to the membrane surface [1,29–34,36,37,41]. Superparamagnetic iron oxide nanoparticles as small as 5 nm in diameter were shown to convert energy from an AMF into heat sufficiently effectively to trigger content release [10,21]. The cargo release efficiency depends on the total concentration of iron oxide nanoparticles contained in the liposome dispersion, which is proportional to the heat conversion. However, the release efficiency has also been shown insensitive to the ratio of iron oxide nanoparticles localized in the liposome lumen to nanoparticles freely dispersed in solution [32]; this is a consequence of the unchanged specific absorption rate (SAR) of iron oxide nanoparticles upon encapsulation in the liposome lumen [34] and the fast heat dissipation caused by the high thermal conductivity of water [32]. Therefore, the temperature in the bilayer is the same as the bulk water temperature [32]. For most applications the difference between encapsulated and free nanoparticles is important, and any paper reporting on release efficiency should carefully characterize the true encapsulation or membrane-bound fraction of nanoparticles. In an in vivo application the drug delivery vehicles and free nanoparticles will be quickly diluted and not co-localized; the cargo release efficiency will decrease dramatically if the majority of iron oxide nanoparticles are freely dispersed. It is even questionable that the membrane can be heated sufficiently through the few encapsulated nanoparticles to provide effective release.

Interestingly, it was recently shown that cargo release from liposomes encapsulating uncoated 6 nm $CoFe_2O_4$ nanoparticles could also be triggered with low frequency magnetic fields [36]. Release through low frequency magnetic fields does not rely on heating. The mechanism is likely of mechanical origin, and therefore circumvents some of the potential problems with AMF induced release, such as non-specific heating of surrounding tissue.

Iron oxide nanoparticles loaded into the liposome lumen of the vast majority of the reported magnetoliposomes agglomerated due to poor steric stabilization [1,32–34,37]. The phosphate of the phospholipid headgroup strongly interacts with bare iron oxide. Uncoated iron oxide nanoparticles or sterically stabilized with reversibly physisorbed dispersants such as starch or dextran therefore interact with and might disrupt the liposome membrane [42,43]. The nanoparticle encapsulation efficiency has been shown to decrease if nanoparticles agglomerate prior to or during encapsulation. For example, starch coated iron oxide nanoparticles were reported primarily to be located outside the liposomes [34]. The influence of nanoparticles interacting strongly with the lipid membrane on the liposome permeability is not quantitatively known but several examples from the literature indicate that it is substantial. It has been shown that 12–50% of the agglomerated carboxydextran functionalized iron oxide nanoparticles initially encapsulated in the lumen of soy phosphocholine liposomes leaked out within 48 h after liposome preparation [43]. This indicates that liposomes ruptured within 48 h. Increasing the loading of dextran coated iron oxide nanoparticles in the liposome lumen resulted in a reduced temperature at which increased liposome permeability was observed [1]. The conclusion is that to gain control over the concentration of encapsulated nanoparticles and to retain the liposome membrane properties over time upon loading iron oxide nanoparticles in their lumen, it is crucial to sterically stabilize nanoparticles with dispersants that adsorb *irreversibly* to the nanoparticle surface.

Nanoparticles incorporated in the membrane

Successful incorporation of nanoparticles directly into the vesicle membrane (Fig. 2c) provides them with the same interaction with the environment as a stealth liposome. Nanoparticles incorporated in the membrane have the advantage of directly transferring the locally generated heat to the membrane itself. Accordingly, externally triggered release from vesicles incorporating nanoparticles in the membrane has been shown more efficient compared to release from vesicles containing nanoparticles in the lumen [21,23]. However, a major challenge to obtain this advantage is to incorporate nanoparticles without compromising membrane integrity and cause leaking of encapsulated compounds.

Liposome membranes are only 4–6 nm thick, matching the maximum thickness of a lipid double layer. Theoretical studies revealed that it is energetically favorable to embed hydrophobic uncharged nanoparticles with diameters below 6.5 nm into liposome bilayers whereas larger hydrophobic nanoparticles are surrounded by a phospholipid monolayer resulting in micelles [44,45]. Only nanoparticles with hydrophobic shells insert into the hydrophobic core of the membrane, as demonstrated for liposomes prepared with hexanethiol and mercaptosuccinic acid (MSA) coated Au nanoparticles respectively [23].

The theoretical prediction that individually stabilized nanoparticles larger than 6.5 nm in diameter cannot be incorporated into liposome membranes is in good agreement with experimental studies. *Tri-n*-octylphosphine oxide (TOPO) stabilized CdSe quantum dots (QDs) with a diameter of 5 nm could be incorporated into giant 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine liposomes, while 8 nm QDs could not [22]. Agglomerates of hydrophobic nanoparticles would be larger than 6.5 nm, which leads to membrane disruption and micelle formation. Thus, irreversible steric stabilization of hydrophobic nanoparticles is absolutely necessary for embedding them in the hydrophobic part of the phospholipid bilayer. That only individually stabilized nanoparticles insert into the liposome membrane was experimentally observed in several studies [21,46]. A recent deviation from these results was reported by Petri-Fink et al.; they showed that deformable nanoparticle aggregates of small nanoparticles could form large micellar caps embedded in liposome membranes [47]. Clusters as large as 60 nm of nanoparticles 5–8 nm in diameter were embedded in this way. The method of assembly through surfactant dialysis with clusters that could optimally deform during assembly explains the different conclusion from this study. Although, resulting in advantage for the nanoparticle loading it is less clear how this method can be combined with effective drug loading of the vesicles. That improved nanoparticle loading into the membrane also of individually stabilized nanoparticles can be achieved through co-assembly of lipids and nanoparticles is also indicated by that a higher loading into unilamellar large liposomes can be achieved through solvent inversion and evaporation than by extrusion [48].

How nanoparticles organize in the membrane and affect the organization of the lipids is still contested in the literature and might be dependent on the detailed architecture of the core–shell

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nanoparticles and the method of vesicle preparation. On one hand, close-packing of nanoparticles, attributed to a reduction of the energy related to the imposed membrane curvature and reduced packing density of the lipids, has been observed [49]. On the other hand, well-dispersed nanoparticles in the membrane have also been observed [21]. The difference between these two results might stem from the use of much smaller nanoparticles in the first study (nanoparticle diameter ~2 nm) than in the second study (nanoparticle diameter ~5 nm). A strong size dependence on nanoparticle membrane inclusion during electroswelling of lipid/quantum dot mixtures has been demonstrated for particles as small as 3 nm [50]. This implies different energetics for electroswelling, since these nanoparticle sizes are known to spontaneously insert into rehydrated lipid membranes. Structurally, SAXS analysis of lipid membranes with embedded 2.5 nm hexanethiol stabilized Au nanoparticles did not reveal any changes in the ordering of the lipids in the bilayers [51]. A change in lipid ordering should also affect the phase transition, but no significant change in the T_m of liposome bilayers was observed after incorporating palmityl-nitroDOPA stabilized 5 nm core diameter iron oxide nanoparticles in the liposome membranes [21]. Relatively disperse sterylamine coated nanoparticles in LUVs were also reported by Bothun et al. [24]. They further reported effects on lipid organization and T_m that depended on nanoparticle–lipid ratio. However, a very large excess of free sterylamine ligand that produced similar changes on its own puts a conclusive argument for nanoparticle effect on lipid ordering still out of reach. Bothun et al. also provide an estimate of the capillary and vdW forces expected to arise between hydrophobically stabilized nanoparticles in a membrane based on a model for hydrophobic protein interactions in membranes [24]. It is found that steric stabilization through hydrophobic dispersants >1 nm will prevent aggregation through vdW interactions and that capillary forces will be longer range repulsive or attractive depending on if the lipid membrane is in the gel or liquid crystalline state. The strength of the latter interaction increases with nanoparticle size. The observations of nanoparticle clustering in fluid phase liposomes and dispersion in gel phased liposomes seems generally to agree with previous reports [21,49,51]. There are also indications that nanoparticle loading could be higher in polydisperse lipid mixtures in the liquid crystalline phase than for lipid membranes of a single species [50]. The nanoparticle loading in the membrane was low in the above reported studies. An alternative explanation to the rather unexpected absent effect on lipid ordering could therefore be that a too small (local) fraction of the lipids was affected to be measurable by the used techniques.

Paasonen et al. were the first ones to show triggered release of liposomes containing nanoparticles in the lipid bilayer using hexanethiol stabilized 2.5 nm Au nanoparticles [23]. Triggered cargo release from liposomes containing superparamagnetic nanoparticles in the membrane interior by applying low [52] and high frequency AMFs [21,46] was only demonstrated recently. However, the significant passive leakage observed for most of these liposomes reduces their applicability [46,52,53]. It was shown that the stability of the nanoparticle coating is central to reduce passive leakage [21]. A loss of membrane structure and an increase in permeability is observed if the nanoparticle shell is not irreversibly linked to the core. For example, nonleaking liposomes were observed for nanoparticles stabilized with palmityl-nitroDOPA [21], while liposomes containing oleic acid stabilized nanoparticles in the membrane showed membrane deformation and rapid passive leaking [21,46,52]. The much higher stability of the nitroDOPA-anchored compared to carboxyl-anchored dispersants [12] made it possible to control cargo release by pulsing the applied AMF [21]. However, it has also been shown that a higher loading per liposome can be achieved for less colloidally stable hydrophobic nanoparticles, and that the nanoparticles aggregating in the shell can suppress dye leakage at high membrane concentration [46,54].

There is still some controversy about the exact mechanism responsible for the enhanced permeability of the liposome membrane subject to actuation by membrane incorporated nanoparticles. Release triggered by low frequency AMFs applied to membrane associated nanoparticles [36,37] clearly relied on mechanical membrane distortion. For high frequency AMFs, local heat generation leading to a membrane phase transition has been claimed to cause the increased membrane permeability [21,46]; however, the local heat mechanism has not been directly demonstrated and it has been argued that bulk heat diffusion is too fast for local heating to be possible [9]. It should be noted that the permeability change has been observed at global temperatures lower than the T_m of the lipid composition [21,46] and with the liposome structure remaining intact through repeated actuations [21]. Regardless of the mechanism, these results demonstrate that magnetically triggered release from liposomes incorporating nanoparticles *within* the membrane can be performed with low leakage and high dosed efficiency, while minimally effecting the surrounding environment.

Next steps

The study of the fundamental properties of nanoparticle actuated liposomes and other vesicles is still in its infancy. The same is true for their application to drug delivery with many emerging directions in which the future development is taking place.

Full use of magnetic vesicles for drug delivery would combine magnetically triggered release with other known properties of liposomes such as stealth coatings and targeting. Both have already been combined in vitro, e.g. folate receptor targeted liposomes [55], PEGylated liposomes [21] and intracellular release of drugs using liposomes with membrane-associated magnetic nanoparticles [54]. The latter study showed that triggered liposome drug release can achieve cancer cell death without hyperthermia effects [54].

Lipid vesicles have the limitation of a severely restricted thickness of its hydrophobic volume [9]. As reported above, only nanoparticles smaller than 5–6 nm can be inserted into the membrane of vesicles that demonstrated the desired properties for drug delivery vesicles; so far the number of particles that could be stably inserted also seems strongly limited [21,51] unless they are controllably aggregated [47]. However, the interaction with magnetic fields, the most important property for applications, scales with the volume of the particles and therefore very strongly with size. The principle of amphiphilic membrane vesicles has, however, been extended to amphiphilic block copolymers [56]. Large nanoparticles can be fitted into the membranes of polymersomes since the thickness of the hydrophobic region can be increased

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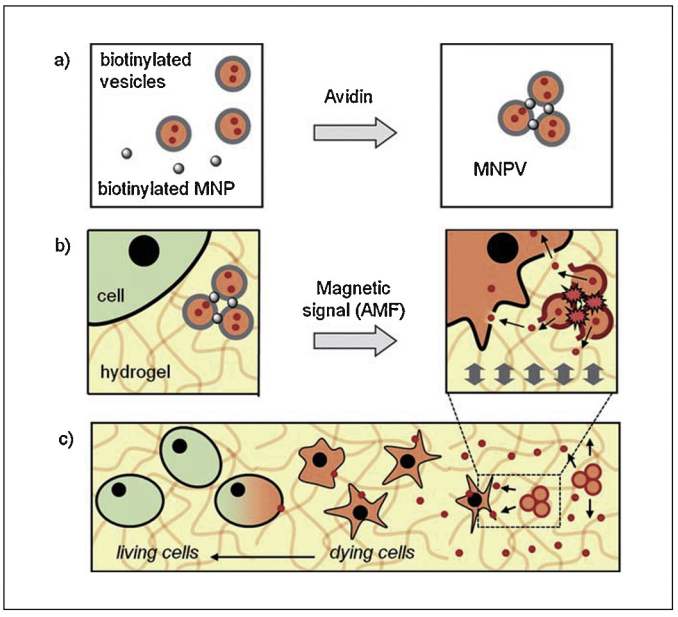

FIG. 4

Triggered release from liposomes can be combined with other drug delivery matrices such as hydrogels and polyelectrolyte multilayer capsules. A demonstrated example combines nanoparticle aggregated liposomes within an alginate matrix [60]: (a) Magnetic nanoparticle-vesicle assembly (MNPV) formation by mixing biotinylated vesicles and magnetic nanoparticles with avidin. (b) An alternating magnetic field (AMF) initiates release of chemical messengers from MPNVs, which in turn induce responses from cells within the hydrogel. (c) Schematic representation of AMF-induced and spatially controlled release of a cytotoxic agent from MNPVs. Reproduced from Ref. [60] with permission from The Royal Society of Chemistry.

compared to a lipid membrane. By introducing a fraction of thermally responsive block copolymers into the membrane it has already been demonstrated that plasmonic heating of microscale polymersomes can be used to trigger release [57].

In a variety on lipid drug delivery vesicles, red blood cells have been investigated in combination with nanoparticle actuators. Magnetic labeling of red blood cells has been achieved using iron oxide nanoparticles [58]. Recently, it was shown using membrane-associated plasmonic particles and laser actuation, that release through membrane permeabilization could be achieved from red blood cells [59]. From these and other recent studies, it seems likely that the knowledge gained from tailoring the interaction of nanoparticles with synthetic membranes can be used increasingly to also develop new methodologies for biotechnological applications. Nanoparticles can be envisioned as externally controllable gateways built into cell walls allowing for magnetically triggered poration or for example as on/off switches for artificial organelles enzymatically producing biochemicals on demand.

An additional emerging use of magnetically responsive vesicles is their embedding in matrices to create multifunctional smart materials. A recent example is the use of micron-sized biotinylated lipid vesicles crosslinked with avidin-coated iron oxide nanoparticles embedded in an alginate hydrogel (Fig. 4) [60]. Ni ions in the vesicles were released from the hydrogel upon application of an alternating magnetic field, which resulted in apoptosis of fibroblasts cultured on the hydrogel. Triggered release could not be achieved when the nanoparticles were not associated directly with the membrane but contained in the alginate matrix [61]. A more structured approach to embedding triggered liposomes in larger particles and vesicles is to embed them into polyelectrolyte membranes and capsules [62,63], which has been proposed as a way to create cell-mimicking multifunctional vesicles. In these concepts, different types of liposomes could be triggered using different triggers to achieve a cocktail or sequence of release responses. These developments still await a combination with the refined, magnetically triggered constructs described in this review.

Conclusions

The increasingly demanding and expanding range of requirements imposed on superparamagnetic iron oxide nanoparticles intended for biomedical applications require close control over nanoparticle size, structure and surface properties. This is only possible if iron oxide nanoparticles are stabilized with optimized dispersants that consist of an irreversibly binding anchor covalently linked to an organic spacer long and dense enough to provide steric stability. Control over the release from liposomes functionalized with nanoparticles requires this exquisite control over the colloidal stability of the nanoparticles *within* the vesicular structure. The properties of the nanoparticle shell determine the nanoparticle location within a vesicle and thereby its structural and colloidal stability. Hydrophobic nanoparticles smaller than 6.5 nm in diameter self-assemble into the hydrophobic part of liposome bilayers; larger or aggregated nanoparticles form micellar structures which under certain conditions can incorporate into liposomes. Hydrophilic nanoparticles can be localized in the vesicle lumen. Hydrophilic nanoparticles interact nonspecifically with the liposome membrane when their shells are not dense, stable and sterically stabilizing; membrane interactions lead to passive leakage and reduced control over actuation.

The efficiency of release has been shown to be highest for vesicular structures where nanoparticles are incorporated into the vesicle membrane and actuates the permeability through *localized* heating that does not have to be transferred through the bulk. Liposomes incorporating nanoparticles in the lumen heat the bulk liquid; therefore, the entire environment has to reach the temperature of actuation to achieve permeability change and release. Control over both timing and dose has been demonstrated for optimized nanoparticle-actuated vesicles and there are an increasing number of smart material applications in which they have been incorporated and tested. In summary, proof-of-principle examples of magnetically triggered vesicles have been published, but much work remains to understand mechanisms of assembly and actuation to optimize them for applications. Already at the present level of understanding and control, however, it is evident that magnetically triggered vesicles and cells can be used as future tools in biotechnology to move and release biological compounds directly, as artificial organelles or through poration.

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