We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Lipobeads

Sergey Kazakov

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.70056>

Abstract

A combination of lipid bilayer and cross-linked polymer network is the logical step in development of polymeric and liposomal nanoscopic systems to provide the natural level of functionality. From liposomal systems, lipobeads borrow the well-developed methods for preparation, diversity of lipids to control the properties of a lipid bilayer, biocompatibility of the lipid bilayer, possibility to vary size and morphology (passive targeting), availability of the external surface for attachment of various ligands (active targeting), encapsulation efficiency of both hydrophilic and hydrophobic molecules. Mechanical stability of the lipid bilayer and environmental responsiveness of the whole structure are the properties that hydrogel core brings to the new construct. The reports reviewed in this chapter demonstrate that lipobeads of nanometer and micrometer sizes can be prepared in different media, retain their stimuli responsiveness under physiological conditions, exhibit both reversible and irreversible aggregation, can be loaded with both small and high molecular weight molecules. As a platform for drug delivery systems, lipobeads have already been loaded with chemotherapeutics, malaria vaccine, and dermatological agent providing different controlled release profiles without leakage. Consecutive multistep triggering, new schemes of drug release, combined drug delivery, vesobeads, proteo-lipobeads, enzyme-containing lipobeads, and hemi-lipobeads are the directions for their future development.

Keywords: liposomes, nanogels, supramolecular assembly, lipobeads, preparation, properties, application

1. Introduction: why lipobeads?

In accord with our understanding of complex biological mechanisms prevailing *in situ*, the arrangement of a lipid bilayer/hydrogel assembly—the lipid vesicles filled with polymeric

networks (**Figure 1**) achievable experimentally in laboratory—could be a logical step in the development of polymeric and liposomal-beaded nanoscopic systems.

In the last several decades, different terminology, such as supramolecular biovectors (SMBVs), lipid-coated microgels, lipobeads (LB), gel-filled vesicles, lipogels, gel core liposomes, microgel-in-liposomes, hydrogel-supported lipid bilayer, and nanolipogels particles (nLG), has been utilized to describe these lipid membrane/hydrogel structures. In this chapter, we use the term “lipobeads” for the spherical bipartite structures made of a hydrogel core enclosed within a lipid bilayer.

Actually, the bicompartimental structure of lipobeads mimics natural arrangements of living cells. Just look at the cell envelopes for all three main domains of life (*eubacteria*, *archaea*, and *eukaryotes*). They represent a successive organization of the macromolecular networks (cytoplasm, cell wall, capsule, etc.) and lipid bilayers (cell bilayer membranes and internal membrane system), which Nature uses to provide workability, multifunctionality, and dynamism of living cells of different types.

Evidently, the first synthetic lipid vesicles filled with hydrogel (lipobeads) were reported in 1987, when a successful polymerization within liposomes had been accomplished [1] and microspherules of agarose-gelatin gels filled with gold particles had been mixed with liposomes in the course of their preparation [2]. In 1989, a concept of supramolecular biovectors (SMBVs) was filed as a patent application [3]. The SMBV system was prepared from polysaccharide gel fragments obtained by disruption of a gel of chemically cross-linked maltodextrins and subsequently phospholipidated. In 1994, the SMBVs were reported as new carriers of active substances, such as interleukin-2 (IL-2) [4]. In 1995, lipobeads with Ca-alginate hydrogel core were obtained as a byproduct of a method for the preparation of Ca-alginate hydrogel nanoparticles using the internal compartment of liposomes [5]. In 1996, the spherical hydrogel/lipid bilayer constructs for the first time were named as “lipobeads,” and it was shown that a lipid bilayer was formed on the surface of hydrogel polymer beads upon the addition of phospholipids, if their surface had been modified with the covalently attached fatty acids [6]. Lipobeads with an environmentally sensitive hydrogel core prepared by hydration of lipid films with microgel suspension were described as drug delivery systems in 1998 [7]. In the early 2000s, photopolymerization within liposomes was used for preparation of the so-called synthetic polymer complements with imprinted recognition sites [8] and the environmentally responsive nanogels [9]. The latter work contributed toward the characterization of the compatibility of nanogels

Figure 1. Schematic of a spherical lipid bilayer/hydrogel assembly (lipobead).

and phospholipid bilayer leading to spontaneous phospholipidation of nanogels [10]. Further studies on lipobeads development were devoted either to new methodologies including different compositions of hydrogel core or different agents which could be loaded into the lipobeads.

The chapter has a comprehensive view on (1) synthetic feasibility, functionality and characterization of lipobeads (Sections 2 and 3) and (2) their potential applications as precursors for novel encapsulated and combined drug delivery systems, as microbiochemical reactors, and as an experimental model to study the origin of life (Section 4). It is a useful source of references for the researchers from both academia and industry, who deal with the aforementioned areas of applications. It may be predicted that in the future, the demand in this information will rise dramatically because of a growing interest, especially, in the encapsulated drug delivery systems with tiny bioscopic mechanisms of drug release.

2. Strategies of lipobeads preparation

Two general methods available to date for preparation of artificial bilayer-coated hydrogel particles (lipobeads, giant, or nano) are sketched in **Figure 2**.

The first one (**Figure 2A**) uses the liposomal interior as a chemical reactor for the formation of hydrogel by polymerization [1, 8–22]. In this method, lipid bilayer should be sealed enough to retain the concentration of pregel components and strong enough to withstand the steps preventing polymerization in the surrounding medium.

The second one (**Figure 2B**) is based on the formation of lipid layers around hydrogels after microgel/liposome mixing. In this case, lipid bilayer adsorption on the surface of hydrogel particles prepared separately was promoted via Coulombic attraction between the charged microgels and oppositely charged lipids [7, 23, 24], hydration of lipid films with micro- or nanogel suspension [2, 3], introduction of hydrophobic anchors at the microgel surface around which adsorbing lipids may assemble [6, 25–28], centrifugation of microgels onto a lipid film [29], microfluidic flowing [21], and emulsification [30–32].

In both cases, it is the stability, fluidity, and permeability that are the main properties of a lipid bilayer, which should be governed in the course of lipobeads engineering.

2.1. Effectors of the lipid bilayer fluidity, stability, and permeability

Stability and permeability of a lipid bilayer depend on its fluidity, which can vary with temperature and phospholipid composition. Bilayers undergo a change from liquid to gel (solid) state at the so-called lipid (or order-disorder) phase transition temperature (T_t) characteristic to a phospholipid used (**Figure 3**).

Therein, the shorter the length of hydrocarbon chains, the lower is the T_t , a long hydrocarbon chain at sn-1 position and a short chain at sn-2 position on glycerol exhibit a lower T_t than that of a phospholipid with the opposite arrangement, presence, and position of double bonds in the hydrocarbon chain makes T_t lower than that of the saturated analogue; a bulky

Figure 2. Two methods for lipobeads preparation: (A) formation of hydrogel core by polymerization within vesicle interior and (B) mixing of separately prepared lipid vesicles and hydrogel particles (microgels or nanogels).

Figure 3. Structure of lipid bilayer below and above the order-disorder transition temperature.

head group confers the lipid a lower T_t than it would be with a smaller head group, the phospholipids with negatively charged head groups favor a lower T_t than that of an uncharged phospholipid. Ionic conditions can modulate this effect, for example, presence of cations in the surrounding water increases T_t .

In the liquid-crystalline "disordered" state, the membrane is fluidic, namely: (i) both alkane chains and head groups of phospholipids are more flexible than in the solid "ordered" state, (ii) the area lipids occupy becomes greater by changing from a $0.48 \text{ nm}^2/\text{head group}$ to $0.7 \text{ nm}^2/\text{head group}$, that is, bilayer expands, (iii) lateral diffusion of phospholipids in the plane of the bilayer and rotation of lipid molecules around C—C bonds accelerate, and (iv) transverse "flip-flop" migration of lipids from one monolayer to the other side of the bilayer becomes more probable.

The factors affecting the lipid phase transition temperature are crucial for lipobeads engineering. Indeed, one can expect the lipid bilayer to be more elastic (favorable for formation of unilamellar membrane) and less sealed (unfavorable for gelation within liposomal interior) above T_t than below T_t . It is known that stability and permeability of naturally occurred membranes can be varied by balancing composition of cholesterol and alcohols. Particularly, cholesterol strongly interacts with phospholipids and inhibits the passive permeability of lipid membrane to water and small electrolytes and nonelectrolytes. The extent of “sealing” directly depends on the amount of cholesterol present, up to moderate levels of cholesterol. However, at very high levels of cholesterol, pure cholesterol phase separates out and leads to an increased leakage through interfacial lipids and unstable aggregates of cholesterol. On the contrary, the insertion of anesthetic molecules, such as alcohols, into the membrane increases the membrane fluidity at a given temperature by depression of the lipid order-disorder transition temperature. Additionally, sphingolipids are commonly believed to protect the cell surface against harmful environmental factors by forming a mechanically stable and chemically resistant outer leaflet of the plasma membrane.

2.2. Polymerization within liposomal nano-/microreactors

In general, preparation of lipobeads using vesicle interior as microreactors includes a number of crucial steps, as shown in **Figure 2A**.

First of all, encapsulation of hydrogel-forming components into liposomes can result from hydration of lipid cast film formed upon solvent evaporation [1, 2, 5, 9, 11, 13, 14, 16, 32], electroformation [12, 22, 33, 34], or rapid phase evaporation [19, 20]. The size of liposomes ensures the final size of lipobeads. Depending on the size, lipobeads can be classified into two groups: nanolipobeads (<1000 nm) and giant lipobeads (>1 μm). Nanolipobeads should be used in realistic drug delivery systems, whereas giant lipogels permit a direct study of structural functionality of the hydrogel/lipid bilayer assemblies, cargo loading, and release mechanisms [12] using optical microscopy.

For 100-nm lipobeads, the liposomal formulations have to be sonicated [1, 9] or extruded through a nanopore filter of a needed pore size [5, 8, 11, 14, 16, 19, 32]. Another approach to control the size of pre-lipobeads is based on hydrodynamic focusing of a stream of the liposome precursor solution by the flow of a hydrogel-forming solution within a microfluidic device [21]. Although the microfluidic-directed approach and electroformation are very elegant methods, they are hardly suitable for a scaled production of lipobeads with regards to pharmaceutical applications.

If the ultimate goal was to engineer the giant lipobeads greater than 1 μm , the gentle hydration of a phospholipid cake [17, 36] or a hybrid agarose/lipid film [unpublished], electroformation [12, 22, 33, 34], emulsification [32], and reverse phase evaporation [1, 13, 30] were used without any agitation of the lipid formulations.

Typical lipid formulations for preparation of lipid vesicles filled with the hydrogel-forming solution are shown in **Table 1**.

Lipid formulation			Method for prelipobead preparation	Size	Ref.
Main phospholipid	T_t	Other components			
EPC	−10°C	Cholesterol	Reverse phase evaporation, sonication	~600 nm	[1]
		—	Lipid film hydration, sonication	~150 nm	[9]
		—	Detergent removal or extrusion	~200 nm	[13]
		—	Emulsification	1–40 μ m	[32]
		Cholesterol	Reverse phase evaporation	~1–2 μ m	[30]
		—	Lipid film hydration, extrusion	~100 nm	[14]
		DOPC/cholesterol		~100 nm	[19]
		NH ₂ -PEG-DSPE/cholesterol		~120 nm	[11]
		Cholesterol		0.2–1 μ m	[8]
		—		100–200 nm	[37]
DOPC	−22°C	—	Electroformation	10–30 μ m	[12]
				0.2–100 μ m	[22, 34]
DPPC	42°C	—	Lipid film hydration, extrusion	~800 μ m	[5]
		Cholesterol/DCP	Hydrodynamic focusing in microfluidic device	150–300 nm	[21]
SOPC, neutral	6°C	DOTAP, positively charged	Lipid film hydration, extrusion	~350 nm	[16]
Soybean polar lipid extract: PC (45.7%), PE (22.1%), PI (18.4%), PA (6.9%), others (6.9%)	?	—	Lipid film hydration	10–50 μ m	[17]
HSPC	52°C	Cholesterol	Lipid film hydration	2–200 μ m	[36]

Abbreviations: DCP: dihexadecyl phosphate, DOPC: 1-2 dioleoyl sn-glycero 3-phosphocholine, DOTAP: dioleoyl trimethylammoniumpropane (positively charged), DPPC: 1,2-dipalmitoyl-snglycero-3-phosphatidylcholine, DSPE: 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, EPC: Egg chicken 1- α -phosphotidylcholine, HSPC: Hydro Soy 1- α -phosphotidylcholine, PA: phosphatidic acid, PC: phosphocholine, PE: phosphatidylethanolamine, PEG: polyethylene glycol, PI: phosphatidylinositol, SOPC: 1-stearoyl-*sn*-glycero-3-phosphocholine.

Table 1. Lipid formulations used for preparation of lipobeads by polymerization within lipid vesicles.

Second, when a suspension of vesicles filled with the hydrogel-forming solution is prepared, it is important to prevent cross-linking or polymerization outside those vesicles. This has been done by several methods, such as a 5- to 20-fold dilution [9, 11, 12, 14, 16, 22, 33, 34], gel

filtration [1, 21], centrifugation and dialysis [5, 13, 20, 30], or introduction of polymerization scavengers (e.g., ascorbic acid [19]) into the extravesicular space. In addition, hydrogel-forming solution as well as cross-linker and initiator can be microinjected directly into the internal compartment of a giant unilamellar phospholipid vesicle (GUV) [17].

The third step is gelation of the hydrogel-forming solution entrapped within the closed lipid bilayer. Thermal and ionotropic cross-linking are the examples of physical cross-linking reactions. Some hydrogel cores were made of polysaccharides when temperature changes [2, 5, 32]. Indeed, agarose [5] and κ -carrageenan [32] are the temperature-sensitive polysaccharides which structure in aqueous solutions undergoes a transition from a random-coil conformation to the cross-linked double helices upon cooling. Agarose is not biodegradable, but its combination with gelatin can bring biodegradability [38]. Gelatin is a thermoresponsive protein, forming a reversible cross-linked network by cooling a water-based solution of the polymer below 35°C. The hydrogel can be liquefied by heating it to physiological temperatures. Interestingly, κ -carrageenan, an anionic polysaccharide carrying one sulfate group, can be cross-linked both thermally (upon cooling) and ionotropically in the presence of divalent or monovalent cations [38]. Similar to alginate, the degradation of carrageenan hydrogels is driven by the exchange of ions with the surrounding fluids. In the course of ionic cross-linking within interior of vesicles, the sections of the polymer backbone carrying the charge bind with ions of opposite charge. For example, when multivalent cations (e.g., Ca^{2+}) were added to a water-based alginate [5] or poly(ethylene dioxythiophene)/poly(styrene sulfonate) [17] solutions, they bound adjacent polymer chains forming ionic interchain bridges that caused a cross-linking. The pH-driven cross-linking inside liposome was carried out by lowering the pH of aqueous solution of poly(acrylic acid) carrying carboxyl groups [20, 30].

The greatest portion of works on gelation within liposomal reactor used photopolymerization to generate a strong covalently cross-linked hydrogel [1, 9, 11, 14, 16, 22, 33, 34, 39]. The monomer and cross-linker depends on the hydrogel core properties required for different applications, as shown in **Table 2**.

Finally, the formulation has to be washed from unreacted chemicals using centrifugation and/or dialysis. In the course of this step, the required medium external to the lipobeads can be introduced. For example, lipobeads could be dispersed in distilled water [13, 30, 36], buffers with pH varied from 7.0 to 7.8 [1, 8, 9, 11, 14, 16, 17, 19–21, 30], or aqueous solutions of sucrose [12, 24, 33, 34]. If necessary, the prepared lipobeads can be dried by gentle evaporation in temperature gradient to be stored at 4°C.

2.3. Hydrogel/liposome mixing

From the general scheme of hydrogel/liposome mixing (**Figure 2B**), one can conclude that hydrogel particles and liposomes should be prepared separately, therein the final size of lipobeads will be defined by the size of hydrogel particles prepared before mixing with liposomes. It has been demonstrated microscopically [40, 41] that hydrophobic modification of the nanogels is not required for spontaneous formation of the bilayer on their surface. Together with the other studies [42], these findings presume that hydrogel/lipid bilayer is an energetically favorable structure.

Monomer.	Cross-linker	Initiator	Prevention of macroscale gelation	Property of hydrogel core	Ref.
PAAm	MBA	ACVA + TEMED	GPC	Not specified	[1]
PSA	BAA	ACVA	GPC	Not specified	[8]
Anchored and nonanchored PNIPA-VI	MBA	DEAP	Dilution 25-fold	Temperature and pH sensitivity	[9]
Anchored PNIPA	TEGDM	DEAP	Dialysis	Temperature sensitivity (probably)	[13]
PNIPA or PAAm	MBA	DEAP	Dilution 20-fold	Temperature sensitive	[14]
PNIPA	MBA	DEAP	Gel filtration	Temperature sensitivity	[21]
dex-HEMA	HEMA	IC2959	Dilution 10-fold	Enzymatically degradable (dextranase)	[16]
PAA	MBA	IC2959	Radical scavenging by AA	pH sensitivity (probably)	[19]
PNIPA	MBA	DEAP	Dilution	Temperature, pH, pI sensitivity	[22, 33, 34]
PLA-PEG-PLA	Diacrylate	IC2959	Dilution 5-fold	Biodegradable	[11]
PAAm	MBA	DEAP	Dilution 20-fold by glucose solution (2.8 M)	Enzymes entrapment, storage, protection, and release	[37]

Abbreviations: ACVA: 4,4'-azobis(4-cyanovaleric acid); BAA: bis-acrylamido acetate; DEAP: 2,2'-diethoxyacetophenone; dex-HEMA: dextran hydroxyethyl methacrylate; GPC: gel permeation chromatography; IC2959: Irgacure 2959; MBA: *N,N*-methylenebisacrylamide; PAA: polyacrylic acid; PAAm: polyacrylamide; PEG: polyethylene glycol; PLA: polylactide; PNIPA: poly(*N*-isopropylacrylamide); PSA: sorbitol acrylate; TEMED: *N,N,N',N'*-tetramethylethylenediamine; TEGDM: tetraethylene glycol dimethacrylate; VI: 1-vinylimidazole.

Table 2. Composition and properties of hydrogel core of lipobeads prepared by polymerization within lipid vesicles.

Lipid formulations used for preparation of lipobeads by hydrogel/liposome mixing are systemized in **Table 3**. Phospholipid with different T_i were employed to prepare conventional liposomes mostly by the lipid film hydration followed by sonication or extrusion in a variety of buffers at pH 7.0–7.6 and deionized water. Nonetheless, the experiments on giant lipobeads show [36, 41] that injection of ethanol solution of phospholipid into hot water is a promising method for preparation of lipidic formulations, which may allow one to exclude the time-consuming steps of lipid film formation and hydration and reduce the time for the scaled fabrication of lipobeads from days to hours.

There are only a few reports (**Table 4**) that deal with nanogels to prepare lipobeads on the nanometer scale: one group employed a high pressure homogenizer to crash bulk polysaccharide hydrogel down to nanosized particles [39, 42], the other group used nanogels extracted from liposomal reactors [9, 10, 26, 27]. In principle, emulsion polymerization

Lipid formulation			Lipid vesicles preparation	Medium (pH)	Ref.
Main phospholipid	T_i	Other components			
DOPC	-22°C	Cholesterol/triolein	w/o emulsion, organic solvent evaporation	5% dextrose	[2]
DOPG	-18°C			HEPES (pH 7.4)	
EPC	-10°C	Cholesterol	Injection of ethanol solution of PL in water, homogenization	PBS (pH 7.4)	[3, 4, 40]
DPPC	42°C				
EPC	-10°C	–	Lipid film hydration, sonication or extrusion	HEPES (pH 7.4)	[6]
DPPC/DPPG	42°C/41°C	–		Tris (pH 7.0)	
SOPC/DOPG	6°C/-18°C				[7]
EPC/DMPE	-10°C/50°C	Cholesterol		HEPES (pH 7.0)	[44]
EPC	-10°C	PS/cholesterol		HEN (pH 7.4)	[45]
DMPC/DPPC	24°C/42°C	–		PBS (pH 7.4)	[36]
EPC	-10°C	PS/cholesterol		HEN (pH 8.0)	[46]
PE	63°C	Olein oil/cholesterol		HEPES (pH 7.4)	[47]
SOPC	6°C	DOPA(-)		Water	[23]
SOPC	6°C	DOTAP(+)			
DOPE	-16°C	DOTAP(+)			
POPC	-2°C	–		TRIS (pH 7.2)	[28, 48]
DOPC	-22°C	DOTAP(+)		HEPES (pH 7.6)	[24]
DOPG	-18°C				
HSPC	52°C	–	w/o/w microemulsion	Water	[32]
HSPC	52°C	Cholesterol	Injection of ethanol solution of PL in hot water, sonication	Water	[37, 42]

Abbreviations: DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine, DMPE: 1,2-Dimyristoyl-sn-glycero-3-phosphoethanolamine, DOPA: dioleoyl glycerol phosphate (negatively charged), DOPC: 1-2 dioleoyl *sn*-glycero-3-phosphocholine, DOPE: dioleoyl glycerol phosphoethanolamine (neutral), DOPG: dioleoylphosphatidylglycerol, DOTAP: dioleoyl trimethylammoniumpropane (positively charged), DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine, EPC: Egg chicken 1- α -phosphotidylcholine, HSPC: Hydro Soy 1- α -phosphotidylcholine, PE: phosphatidylethanolamine, PL: phospholipid, POPC: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, PS – phosphatidylserine, SOPC: 1-stearoyl-*sn*-glycero-3-phosphocholine.

Table 3. Lipid formulations used for preparation of lipobeads by hydrogel/liposome mixing.

enables preparation of hydrogel particles with a diameter less than 150 nm. However, there is a problem of complete removal of the residual materials. In the absence of an added surfactant, the method is called precipitation polymerization. With the latter two methods, the lipobeads of 1- μ m diameter are produced [2, 7, 23, 25, 28, 31]. To prepare giant lipobeads with a diameter up to 100 μ m, the inverse suspension polymerization (ISP) method is commonly applied [6, 24, 35, 43–45].

Liposomes can be brought into contact with hydrogel particles by mixing hydrogel particles and liposomes, addition of hydrogel particles into dried lipid film before hydration or

Hydrogel bead composition	Method of bead preparation	Size	Property of hydrogel beads	Ref.
Agarose-gelatin cross-linked by cooling	Emulsification	~1–5 μm	Degradable	[2]
Cross-linked polysaccharide fragments	Extrusion in high-pressure homogenizer	30–60 nm	Temperature, pH, pI: core/bilayer interactions	[39]
Acylated PVA cross-linked by freeze-thaw	ISP	1–100 μm	–	[46]
PMAA-NPMA cross-linked by MBA	ISP	~6 μm	pH sensitivity	[7]
Anchored PDMAA cross-linked by E-BIS	ISP	5–600 μm	– Antibody-antigen interaction [45]	[35, 43–45]
dex-HEMA-MAA (–) or dex-HEMA-DMAEMA (+)	Emulsification	2–5 μm	Degradable	[23]
Acylated PVA cross-linked by freeze-thaw	ISP	~80–100 μm	–	[46]
Anchored PNIPA/P(NIPA-AA) core-shell cross-linked by MBA	Precipitation polymerization	~0.3–2 μm	Temperature, pH sensitivity	[25, 28]
Sodium hyaluronate-PEGDA	Emulsification	1–15 μm	Drying/wetting	[31]
PAAm-allylamine (+) or PAAm-AMPS (–) cross-linked by MBA	ISP	1–100 μm	Electrostatic liposomes/MG interactions	[24]

Abbreviations: AA: ascorbic acid, AMPS: 2-acrylamido-2-methyl-1-propanesulfonic acid, dex-HEMA: dextran hydroxyethyl methacrylate, DMAEMA: dimethylaminoethyl methacrylate, E-BIS: *N,N'*-ethylene-bis(acrylamide), ISP: inverse suspension polymerization, MAA: methacrylic acid, MBA: *N,N*-methylenebisacrylamide, NPMA: 4-nitrophenyl methacrylate, PAAm: polyacrylamide, PDMAA: polydimethylacrylamide, PEGDA: poly(ethylene glycol) diglycidyl ether, PMAA: polymethacrylic acid, PNIPA – poly(*N*-isopropylacrylamide), PVA: polyvinyl alcohol.

Table 4. Composition and properties of hydrogel particles for lipobeads prepared by hydrogel/liposome mixing.

hydration of the lipid film by aqueous suspension of hydrogel particles. The formation of lipid bilayer around hydrogel particles can be enhanced by shaking, vortexing, pipette agitation, centrifugation, freezing-thawing, heating-cooling, or their combination. The fusion of liposomes will be more advanced at temperatures higher than the T_i of the phospholipid used. Moreover, depending on the electrostatic interaction between bilayer and hydrogel, the liposomes can adsorb on the particles surface, diffuse inside, or/and fuse on the surface with formation of lipobeads [24]. Usually, free liposomes are washed out by centrifugation or removed by ultrafiltration or dialysis. Finally, the lipobeads can be dispersed in a buffer with pH ranged from 7 to 8 or distilled water.

2.4. Modification of hydrogel core and lipid bilayer

To the great extent, the major methods for lipobeads' synthesis (polymerization within liposomal interior and liposome/hydrogel mixing) and further functionalization are analogous to those used for engineering their compartments—conventional nanogels and lipid bilayers.

2.4.1. Hydrogel core

Polymeric nanogels can be synthesized by three straightforward methods: (i) cross-linking polymer chains within already formed nanoparticles using, for example, emulsion polymerization technique [47–49], (ii) polymerization within the liposomal interior followed by the lipid bilayer removal [9], and (iii) photolithographic fabrication of submicrometer hydrogel particles using the PRINT technique [50, 51] or step and flash imprint lithography (S-FIL) [52] as an alternative nanoimprint photolithographic approach.

To engineer the stimuli-responsive nanogels (**Figure 4**), a molecule of interest can be conjugated to the polymer network through a cleavable tether, so that when the tether is cleaved, the drug is allowed to diffuse into the nearby medium. Alternatively, if different molecules are trapped within an environmentally sensitive polymer network with or without environmentally responsive cleavable linkers, the network either changes its volume (swells/shrinks) or degrades when the environmental conditions change, allowing the molecules to be released. For example [53], the doxorubicin-loaded, pH- and redox-sensitive poly(oligo(ethylene glycol) methacrylates-ss-acrylic acid) nanogels exhibited strong internalization by human hepatocellular carcinoma cells (Bel7402) under reduced opsonization and phagocytosis. Herein, the intracellular glutathione (GSH) triggered the release of doxorubicin from the nanogels into cytosol for subsequent entering the nucleus.

Figure 4. A futuristic view on a stimuli-responsive nanogel with entrapped or/and tethered molecules of interest.

2.4.2. Lipid bilayer

Specific functionality and entrapment of both hydrophilic and hydrophobic molecules into a liposomal interior are the two main objectives of the liposomal system development. Eventually, four types of liposomal systems can be distinguished (**Figure 5**), namely: classical “plain” liposomes, “stealth” liposomes, ligand conjugated liposomes, and stimuli-sensitive liposomes.

The studies on “plain” (traditional) liposomes revealed the difficulty in loading of some types of molecules and leakage of contents from the liposomal interior [54–58]. The further development of the “plain” liposome systems aimed at overcoming these obstacles. In particular, to reduce leakage from liposomes, phospholipids with a higher phase transition temperature [59] were used, and cholesterol [60] and sphingomyelin [61] were incorporated into the lipid bilayer to make it more solid at temperatures of application.

Loading and retention of molecules of interest within liposomes are the molecule dependent processes. For example, weak bases were loaded in response to pH gradients [62–66]. Some molecules, such as doxorubicin, exhibited good retention properties under conditions enhancing their precipitation inside liposomes [67–69], whereas retention of highly hydrophobic molecules, like paclitaxel, was still a challenge [70, 71] until they were converted into the weak bases [72].

Furthermore, the pharmaceutical studies revealed that (i) serum proteins effected on release of drug molecules entrapped into liposomes, (ii) liposomes were cleared very rapidly from

Figure 5. Four evolutionary steps of lipid bilayer modification and functionalization.

circulation by uptake into the cells of the mononuclear phagocyte system (MPS), predominantly in the liver and spleen [73, 74], and (iii) there existed both cellular and intracellular barriers to liposomal delivery [75]. The so-called “stealth” liposomes were developed by stabilizing liposomes with protective polymers (e.g., polyethylene glycol, PEG) [76] in order to increase their circulation time within a biologically active environment, such as blood. In addition, the clearance of liposomes from the body was found [74, 80] to be slower, if they contained neutral or slightly negative phospholipids and their size was around 100 nm. Therefore, the rate of molecules’ release should be optimized [77–79].

Ligand-conjugated liposomes were built to target specific cells, intracellular organelles, tumor microenvironment, and/or facilitating receptor-mediated endocytosis (attachment of antibodies, folate, transferrin, tyrosine kinase, vascular endothelial growth factor, introduction of fusogenic lipids, and membrane-active peptides). In particular, three ways to facilitate the intracellular drug delivery include (i) introduction of fusogenic lipids or membrane active peptides into liposomal bilayer enhances fusion or even disruption of cell/organelle membrane and thereby improves cytoplasmic delivery of drug [81–85], (ii) utilization of macrophages for natural endocytosis of drug-loaded liposomes [86], and (iii) receptor-mediated endocytosis of ligand-targeted liposomal drug carriers into the intracellular compartment (see reviews [87–90]).

The release of liposomal contents can be triggered either remotely by heat, radiation, and ultrasound or locally by pH, enzymes, and redox triggers (see review [91] and references therein). For these purposes, the lipid bilayer can be modified with stimuli-sensitive phospholipids, polymers, cleavable tethers, and linkers, as shown in **Figure 5**. Recently, the lipid bilayer consisting of molecules of the temperature-sensitive phospholipid 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (MSPC) and decorated with gold nanorods was shown to become more permeable for the pain blocking molecules (tetrodotoxin) without a tissue burn when exposed repeatedly to a low intensity near-infrared irradiance [92].

2.5. Encapsulation strategies

2.5.1. Loading during gelation within liposomal interior

Unfortunately, little work has been done so far to test loading capability of lipobeads prepared by polymerization within the lipid vesicles [11, 14, 15, 20, 30]. Typically, a hydrophobic cargo was either incorporated within lipid bilayer at the step of lipid vesicle formation or copolymerized with hydrogel core as an anchor, whereas a hydrophilic cargo was added as a component of the hydrogel-forming solution and incorporated into the intravesicular space before gelation started [9, 11, 13].

The main challenge of the scheme when a load is introduced into the aqueous phase followed by rehydration of a lipid film and further polymerization within a liposomal reactor might be the damage to the loading molecules by toxic ingredients of the hydrogel-forming solution (if any) and/or high temperature and UV radiation initiating polymerization. This approach can be especially problematic for encapsulation of proteins, because of denaturation. Nonetheless, it has been reported that antigen model (BSA) [30] or combination of protein antigen (Pfs25) and oligonucleotide sequence (CpGODN) encapsulated into pH-cross-linked PAA hydrogel core of lipobeads remain intact and active [20]. Moreover, encapsulation efficiencies of lipobeads were shown to be

by 10% higher than those for liposomal carriers. A high encapsulation efficiency of lipobeads has been demonstrated also for hemoglobin [14, 15], which withstands the conditions of free radical polymerization and UV radiation. The other example of successful coencapsulation of hydrophilic proteins (IL-2) and small hydrophobic molecules (TGF-receptor-I inhibitor, SB505124) into the biodegradable hydrogel core of lipobeads has been presented in [11]: encapsulation efficiencies were 80 and 36%, respectively, and UV polymerization did not compromise bioactivity of both immunomodulators. Recently, PAAm lipobeads with a good encapsulation efficiency (37%) of enzymes (bovine Cu, Zn-superoxide dismutase, and bovine milk lactoperoxidase) were synthesized to prove that the UV irradiation used for interior gelation did not cause any reduction in the enzymatic activity of the proteins [37].

2.5.2. Loading of lipobeads prepared by hydrogel/liposome mixing

In the case of lipobeads prepared by hydrogel/liposome mixing, hydrophilic cargos usually are introduced into the interior of hydrogel particles, whereas hydrophobic ones—into the lipid bilayer of liposomes before their mixing. Some molecules can penetrate through the lipid bilayer into the hydrogel core.

Back in 1987, the 200–600-nm agarose-gelatin nanogels were filled with colloidal gold particles prior to mixing with liposomes to form lipobeads [2]. Since colloidal gold particles are very adsorptive for proteins and peptides, their encapsulation into hydrogel core can increase the loading capacity of lipobeads. The cytokine Interleukin-2 (IL-2) plays an important role as an immunostimulator and can be relevant as a treatment by itself for cancer and HIV. However, the difficulties faced today with IL-2 are its toxicity and short half-life. To resolve these problems, this protein was bound to the lipobeads (polysaccharide hydrogel nanoparticles coated with lipid bilayer) [4, 93]. In principle, the lipobeads can be loaded with a number of entities just by their incubation in the corresponding solutions, for example, Ca^{2+} ions and drug mimicking molecules [8], adenosine triphosphate (ATP) [35], and dextran (1.5–3.0 kDa) [44]. Herein, it was found that permeability of the lipid membrane was similar to the free bilayer. To incorporate transmembrane proteins into the peripheral membrane of lipobeads, the hydrogels core particles were mixed and incubated with liposomes containing the proteins of interest within their lipid bilayer [45].

The only chemotherapeutic drug—doxorubicin—was loaded into lipobeads [6, 43]. Encapsulation was performed before lipobeads formation by soaking the dry hydrogel particles in a drug-dissolved solution. The drug diffuses inside in the course of the polymer network swelling and mesh size increase. Further mixing of hydrogel particles with liposomes encapsulates the drug into the lipobeads. As a result, the unbelievably high doxorubicin concentration of ~2 M, which is 10-fold the concentration in liposomes [94], was achieved.

A lecithin-based microemulsion method was proposed for fabrication and loading of single-core or multicore lipobeads [31]. The loading efficiency of caffeine into thus prepared sodium hyaluronate lipobeads was obtained to be 30%. A concentration of natural moisturizing factor close to the one present in corneocytes (15%) was encapsulated into the lipobeads, which acquire an enhanced water retention ability similar to corneocytes. This makes them potential for applications in cosmetics and dermatology.

Encapsulation of a protein drug into hydrogel particles before lipobeads formation can be performed either by formation of a hydrogel particle in the presence of a protein drug or by incubation of the preformed hydrogel particles in a protein solution. The first approach again could be problematic due to a danger of protein denaturation. The second approach is limited by the size-exclusion effect resulting in a lower loading concentration of proteins. However, encapsulation of proteins into microgels is a promising tool to increase the amount of drug loaded in a prelipobead (loading capacity) by using the “intelligent” properties of polymer networks (swelling/shrinking ability in response to stimuli) [95].

3. Hydrogel core swelling/collapsing and lipobeads properties

The three-dimensional polymer network within a closed lipid bilayer (liposome) can be considered as a gigantic single molecule stabilized by chemical (covalent bonds) or/and physical (ionic bonds, entanglements, crystallites, charge complexes, hydrogen bonding, van der Waals, or hydrophobic interactions) cross-links. The hydrogel core is also an open container with semi-permeable boundaries, across which water and solute molecules can move whereas charged (ionizable) groups fixed on the network chains cannot move (**Figure 4**). Herein, the network of cross-linked polymers exhibits both liquid-like and solid-like behavior [96–99]. Therefore, because of its high water content and elastic nature similar to natural tissue, hydrogel core within a liposome is solid enough to support lipid bilayers and liquid enough to keep the membrane intact and functional.

Besides the mechanical stability that hydrogel core provides to the lipid bilayer, the stimuli-sensitivity of the polymer network can be used for managing the environmental responsiveness of lipobeads.

3.1. A variety of possible hydrogel cores

Depending on the composition of a gel/solvent system, the polymer and cross-linking chemistry, nanogels swell or shrink discontinuously or continuously, reversibly or irreversibly in response to many different stimuli (temperature, pH, ion concentration, electric fields, light, reduction/oxidation, enzymatic activity, etc.) [47, 100–105]. In general, various types of hydrogels based upon either natural (e.g., hyaluronic acid, collagen, chondroitin sulfate, alginates, fibrin, and chitosan) and synthetic polymers made of neutral (e.g., 2-hydroxyethyl methacrylate, N-alkylmethacrylamides, N-alkylacrylamides, and N,N-dialkylacrylamides), acidic (e.g., acrylic acid, metacrylic acid, and 2-acrylamido-2-methyl propane sulfonic acid), or basic (e.g., N,N-dialkylaminoethyl methacrylate, 1-vinylimidazole, and methacryloyloxyethyltrialkylammonium bromide) monomers have been prepared, studied, and used in numerous applications (bioseparation, tissue engineering, sensing and molecular recognition, drug and gene delivery, controlled release, artificial muscles, and flow control).

3.1.1. Temperature-sensitive volume change

Typically, the thermoresponsive hydrogels are classified as having either positive or negative volume phase transition with a characteristic temperature (T_v) [106]. Hydrogels exhibiting

positive volume phase transition (“thermophilic” hydrogels) swell upon heating. In contrast, hydrogels exhibiting negative volume-phase transition (“thermophobic” hydrogels) collapse upon heating. The “thermophobic” hydrogels have been studied the most, and a popular example is poly(*N*-isopropylacrylamide) (PNIPA) [107]. In contrast, “thermophilic” behavior in water is not very common for synthetic polymeric materials and likely, because of that, lipobeads with a “thermophilic” hydrogel core have not been attempted yet. Nevertheless, a number of “thermophilic” hydrogels showing a positive thermosensitive volume change have been already fabricated [108–110]. Chitosan cross-linked with glutaraldehyde was found to exhibit the swelling behavior in aqueous media at physiological temperatures and pHs [111]. The graft copolymerization of mixtures of acrylamide (AAM) and acrylonitrile (AN) with *Gum ghatti* (Gg) and cross-linking with MBA resulted in a hydrogel capable of twofold swelling when temperature raised from 30 to 40°C in distilled water [112]. The interpenetrating polymer networks (IPN) composed of poly(acrylic acid) (PAAc) and poly(acrylamide(AAm)-co-butyl methacrylate (BMA), as well as the random poly(AAm-co-AAc-co-BMA) hydrogels cross-linked with MBA also increased their volume (~2.5-fold) within the 30–40°C range in water [113]. Positive thermosensitivity with a twofold swelling ability in the range from 30 to 45°C was reported for a nonionic chemically cross-linked gel made of *N*-acryloylglycinamide as a monomer and MBA as a cross-linker [109]. An abrupt increase in volume to the similar extent within the interval of 30–40°C was observed in the polyzwitterionic hydrogels consisting of *N,N'*-dimethyl(methacroylethyl)ammonium propanesulfonate or *N,N'*-dimethyl(acrylamidopropyl) ammonium propanesulfonate cross-linked with EGDM [114]. The gels with positive volume transitions at physiological temperatures, pH and in the presence of salt can be useful in biological or biomedical applications. However, in all abovementioned cases, under physiological pH and salt concentrations, the hydrogels either do not show thermophilic behavior at all or the transition temperature shifted to the nonphysiological values. Nonetheless, recently, hydrogels based on the cross-linked poly(allylurea-co-allylamine) (PAU) copolymers were prepared to unveil a fast and pronounced “thermophilic” increase in volume within the physiological ranges of temperature, pH, and concentration of salt [110].

Thus, first and foremost, a temperature range where the hydrogel shrinks or swells intrinsically depends on the chemical nature of the polymer constituting its network. Herein, the volume changes in a water-swollen hydrogel can be either continuous or discontinuous, as a function of environmental stimuli. If the system remains totally miscible at given thermodynamic conditions, one can expect continuous volume transition. On the contrary, if changes in chemical nature of the polymer network, solvent quality, or environmental stimulus “push” the system into a two-phase (unstable) region of the solubility phase diagram, one can expect that properties of the hydrogel, most notably its volume, change discontinuously. In addition, the studies [115–117] show that increased cross-linking may significantly decrease swelling ability of hydrogel, especially, below T_v but has a little effect on the value of T_v .

Figure 6 demonstrates that temperature-sensitive shrinking ability of hydrogels depends on their microstructure and method of preparation: the granular hydrogel (A) exhibits a continuous volume decrease with temperature, whereas the denser hydrogels (B and C) exhibit more abrupt changes in volume within the 35–45°C range. Interestingly, the granular structure of the PNIPA hydrogels prepared by thermal polymerization in water (A) can be broken down into separate submicroscopic domains by means of sonication (data not shown). On

Figure 6. Shrinking abilities S_v and SEM images of the microstructures of PNIPA hydrogels prepared by thermal polymerization in water (A) or DMSO (B), and by UV polymerization in water (C) (scale bar = 5 μm).

the contrary, sonication does not affect the structure of “dense” hydrogels prepared either by thermal polymerization in dimethyl sulfoxide (DMSO) (B) or by photopolymerization in water (C).

It has been shown [118] that incorporation of a small amount of ionizable groups into the nonionic gel network drives the volume phase transition from continuous volume changes toward discontinuous one (**Figure 7**). Moreover, an increase in the portion of sodium acrylate with carboxylic groups on the PNIPA network allowed one to vary the T_v from 34 to 42°C with the increasing extent of swelling ability below the transition temperature.

Figure 7. The degree of swelling of the poly(*N*-isopropylacrylamide-co-sodium acrylate) gel in water as a function of temperature. Numbers are the molar fractions of sodium acrylate in the preparations. Data were adopted from [118].

3.1.2. Ionic sensitivity of hydrogels

Figure 7 also suggests that incorporation of charged (anionic, cationic, or both) groups on the polymer network makes the volume transition temperature and degree of swelling dependent on pH and ionic strength. Indeed, the poly(N-isopropylacrylamide-co-methacrylic acid) (PNIPAA) microgel particles [119] at pH 3.4 exhibited a decrease in T_v from 33.5 to 28°C with an increase in MAA content, whereas at pH 7.5, the higher MAA content resulted in the higher T_v . In weakly charged PNIPAA hydrogels, addition of ionizable groups on the polymer network pronounced the volume changes when temperature crossed T_v [118, 120, 121]. The experimental studies [122] revealed that distribution of ionic groups in the network affects the temperature of volume change transition.

The type of ionizable groups on the polymer networks makes the maximum swelling ability of a gel strongly dependent on pH. For example, the anionic PNIPAA microgels exhibited the maximum swelling in the range of pHs from 6.5 to 10 [119], whereas for the cationic PNIPAA-VI nanogels, the maximum swelling ratio was observed in the range of pHs from 6 to 3.5 [123]. It becomes even more intriguing if the so-called polyampholyte hydrogels are designed [124] by addition of both cationic (VI) and anionic (AA) groups on the network. The polyampholyte gel was in a shrunken state near the isoelectric point ($\text{pH} \sim \text{pI}$), and it swelled at both higher and lower pHs. It is interesting that such designed polyampholyte gels can work like biomechanical systems in which the enzymatically induced pH changes control the volume of polyampholyte network or, in opposite direction, the pH sensitive volume changes control the activity of enzymes immobilized into the gel [125].

There are experimental evidences [126–130] that different monovalent (Li^+ , Na^+ , K^+ , and Cs^+) and divalent (Ca^{2+} , Mg^{2+} , Sr^{2+} , and Ba^{2+}) ions are able to promote deswelling effects in hydrogels of different chemical nature. Interestingly, at the same molar ratios of divalent to monovalent cations ($\sim 1 \text{ mM}/30 \text{ mM}$), the similar volume changes were observed in biological polyelectrolyte systems during physiological processes like nerve excitation, muscle contraction, and cell locomotion [131–137].

3.1.3. Surfactants as effectors of hydrogel volume change

The extensive theoretical and experimental [138–145] studies have shown that the addition of anionic, cationic, and nonionic surfactants to the solution containing a gel can also influence the T_v and swelling degree of hydrogels depending on their hydrophobicity and charge of the polymer network. In general, addition of anionic or cationic surfactant to the solution of nonionic hydrogel increases T_v as well as the swelling range, whereas the nonionic surfactant does not affect T_v or volume change. The surfactants with ionic head groups when bind to the nonionic polymer networks convert the neutral hydrogels to polyelectrolyte gels and elevate T_v due to introduction of additional osmotic pressure by ionization. The changes in the volume phase transition are also dependent on the length of hydrophobic tail of ionic surfactants and the critical concentration of micelle formation [142]. It was also found [144] that the amount of an ionic surfactant bound onto the swollen network of the nonionic PNIPAA hydrogel was much greater than that to the collapsed one. On the contrary, the amount of nonionic

surfactant bound onto the collapsed network of the PNIPA gel was greater than that on the swollen one. Moreover, the changes in T_v with the amount of the anionic surfactants (e.g., sodium dodecyl sulfonate) were more pronounced than for cationic ones (e.g., dodecylamine hydrochloride). Interestingly, the concentration of anionic surfactant (e.g., sodium dodecylbenzene sulfate) bound within the PNIPA hydrogel was found to be higher in the vicinity of the gel surface, whereas a central region of the gel may not contain any bound surfactant molecules [145]. Thus, peripheral layers could be in a more swollen state with a higher T_v in comparison to the central hydrogel core.

3.1.4. *Light-sensitive hydrogels*

Photosensitive hydrogels with incorporated photosensitive molecules into the gel network have been reported as well. For example, the gels with incorporated leucocyanide and leucohydroxide [146] underwent volume changes upon irradiation and removal of ultraviolet light resulted from ionization reaction and internal osmotic pressure initiated by UV light. Significant volume changes in hydrogels were also induced by visible light [147]. However, the mechanism of volume transition was different—it was due to direct heating of the polymer network by light. Nevertheless, more recent reports [148] showed that a focused laser beam was able to induce reversible shrinking in polymer gels due to radiation forces, rather than local heating, modifying the weak interactions in the gels. Herein, gel shrinkage was observed up to several tens of micrometers away from the irradiation spot. The light-induced contraction was also found in acrylamide gels, which are not temperature sensitive. In hydrogels with temperature-sensitive volume phase transitions, such as PNIPA gel, it was found that the radiation force of the laser beam not only induces the volume phase transition but also lowers the transition temperature T_v by about 10°C at an irradiation power of 1.2 W ($\lambda = 1064$ nm).

The fact that the volume change initiated by light is extremely fast seems of great importance for the development of the light-sensitive lipobeads. One could predict that the photosensitive hydrogel cores will also receive an increasing scientific and technological attention due to their capability of serving as the so-called shape-memory polymeric systems [149]. Being exposed to the light with lower wavelengths, the shape-memory materials become deformed and their temporary shape is fixed due to cross-linking. When irradiated with higher wavelengths, they recover their initial shape because of the cross-links cleavage.

3.1.5. *Electrical field-induced volume change*

Back in the 1950s, it was found that contraction, oscillation, and bending of polyelectrolyte gels can be induced electrically [150–152]. In particular, gels prepared from polymers and copolymers that contain ionizable groups exhibited remarkable contraction when placed between a pair of electrodes connected to a direct current source. Polymer gels containing no ionizable groups showed no volume change under electrical field applied. The extent and rate of volume change of the polyelectrolyte gels were shown to increase with increasing electrical field [153]. An increase in the ionic strength (e.g., an addition of NaCl) also increases the rate of gel shrinkage, whereas an addition of organic solvent (e.g., acetone or

ethanol) decreases both the extent and the rate of shrinking. In different types of hydrogels, an electrically activated volume changes were associated with the induction of the medium pH change by the electric field [154], the electrically initiated volume phase transition [155], and the so-called electrokinetic phenomena—ion transport of counter-ions in the electric field [156].

3.2. Lipobeads by hydrogel/liposome mixing

Success in formation of lipobeads by hydrogel/liposome mixing (see references from **Tables 3** and **4**) is an experimental confirmation of the main property of hydrogel and lipid bilayer—their compatibility. Indeed, the phospholipid bilayer spontaneously self-assembled around a nanogel once extracted from a lipobead (**Figure 8a**) and mixed with liposomes (**Figure 8b**) to form a secondary lipobead (**Figure 8c**) [10].

Moreover, spontaneous formation of the lipid bilayer on the surface of nonanchored microgels was shown microscopically for liposomes made of different phospholipids with or without cholesterol [36, 41]. **Figure 9** represents both laser scanning confocal (**Figure 9A, A', A''**) and scanning electron microscopy (**Figure 9B, B'**) images evidencing the fusion of liposomes on the hydrogel surface to form a lipid membrane around porous PNIPAM-co-FA microgels.

Unilamellarity, continuity, and nonleakiness of the lipid bilayer formed upon microgel/liposome mixing were proven for the micrometer-sized, hydrophobically modified, pNIPAM/p(NIPAM-co-AA) core-shell hydrogel spheres [25, 28]. It was also demonstrated by Dynamic Light Scattering (DLS) and Atomic Force Microscopy (AFM) [40] that hydrophobic modification of the nanogels is not required for spontaneous formation of the bilayer on their surface.

Interesting behavior of the lipobeads was observed when their hydrogel cores change its volume. It was shown (**Figure 10**) that a collapse of the hydrophobically modified PNIPAM microgels within the lipid bilayer caused a shape change of the lipobeads from sphere (below T_v) to a small

Figure 8. Atomic force microscopy images (amplitude data) of a PNIPAM-VI nanogel (a), EPC liposomes (b), and result of their mixture—lipobead flattened on mica surface. Scale bars = 100 nm.

Figure 9. The bright field (A) and confocal laser scanning (A', A'') microscopy images of PNIPA-co-FA hydrogel microspheres mixed with the liposomes made of HSPC with cholesterol (molar ratio 9:1): Green image (A') originated from fluorescein-o-acrylate (FA) covalently attached to the PNIP network within the core. Red image (A'') originated from rhodamine B covalently attached to the heads of PE. Scanning electron micrographs of the PNIP-co-FA microgel before (B) and after (B') mixing with liposomes (scale bars = 20 μm).

Figure 10. Fluorescence images of a giant anchored PNIP lipobead below (left) and above (right) T_v . Red images originated from 0.05 mol% RhodB-PE component within the lipid bilayer. The images were adopted from [25, 28].

central core with high curvature protrusions (above T_v), consisting of the excess lipid bilayer which still adjoin the lipid bilayer remaining bound to the hydrogel via hydrophobic anchors [25, 28]. Importantly, these changes were found to be reversible, and the bilayer remained intact and impermeable.

3.3. Lipobeads by polymerization within liposomal interior

3.3.1. Lipobeads with hydrophobically modified nanogels

As shown by DLS, the size distribution of lipobeads with hydrophobically anchored PNIPA-VI nanogels became bimodal when temperature was raised to 40°C: the position of the first peak corresponded to the initial size of lipobeads at 25°C, while the second peak was assigned to the aggregates of lipobeads. The further cooling back to 25°C restored the original unimodal size distribution of lipobeads, indicating reversibility of anchored lipobeads aggregation. **Figure 11** sketches the behavior of lipobeads resulted from the anchored hydrogel collapsing.

Presumably, the aggregation reduces the hydrophobic/hydrophilic imbalance caused by collapsed nanogels within lipobeads. The reversible dissociation of the lipobead aggregates may evidence that anchored lipobeads do not fuse. The only explanation is that the hydrophobic chains of anchored PNIPA-VI nanogels penetrate into the lipid bilayer and stabilize the liposomal membrane against fusion.

On the contrary to the hydrophobically modified giant lipobeads (**Figure 10**), the anchored PNIPA-VI nanolipobeads did not reveal a size change under temperature or pH variations, as shown by DLS [9]. Probably, on the nanometer scale, a highly curved lipid bilayer is too stiff to follow the collapse of the hydrogel core. This effect remains to be proven.

3.3.2. Lipobeads with nonmodified nanogels

In contrast to the hydrophobically modified lipobeads, the unanchored lipobeads exhibited the unimodal size distribution recorded by DLS at 40°C. The single peak was significantly shifted toward a greater average diameter than that at 25°C. This observation indicated that a more pronounced aggregation occurred in this case. After cooling back to 25°C, the bimodal size distribution of lipobeads was observed. The presence of two peaks indicated that not all aggregates of lipobeads did break up into elementary lipobeads. This pattern of the lipobeads behavior with temperature suggests that the aggregation of lipobeads at elevated temperatures can be irreversible, if lipid bilayers fuse to form a “giant” lipobead containing several nanogels as sketched in **Figure 12**. Herein, the aggregation of unfused lipobeads is reversible (see processes 1 and 2).

Figure 11. Schematic presentation of the anchored lipobeads and their aggregation in the course of hydrogel core shrinking. AFM image (amplitude data) of an aggregate is shown in the insert. Scale bar = 100 nm. The anchored lipobeads reversibly disaggregate when hydrogel swells back.

Figure 12. Schematic presentation of the nonanchored PNIPA-VI lipobeads and their aggregation when hydrogel core shrinks. In the course of the liposomal membrane fusion, the collapsed nanogels can aggregate to form a giant lipobead. The processes 1 and 2 are reversible, whereas process 3 is the irreversible one. AFM image (amplitude data) of an aggregate is shown in the insert. Scale bar = 2 μm .

The effect of temperature on shape and size of lipobeads was studied [34] using the giant (not nano-) nonanchored PNIPA lipobeads prepared by polymerization within giant vesicles. As shown microscopically, the giant lipobeads retained their spherical shape when hydrogel core collapsed at high temperature and swelled back after cooling. Their size was found to change reversibly, so that after six cycles of heating/cooling around the volume transition temperature, the lipobeads remained undamaged. The authors postulated that the membrane was coupled to the gel during the volume change, although the study of mechanisms of the gel core/lipid membrane interactions are still in demand.

4. Special applications and new perspectives

4.1. Lipobead-based drug delivery systems

To figure out which properties make lipobeads attractive for the next generation of drug delivery systems, it is worthwhile to consider first their fate in the body once being administered.

4.1.1. From injection to internalization of lipobeads into the cells

Different administration routes including intravenous, intramuscular, pulmonary, and topical could be suitable to deliver drugs by lipobeads. However, the peripheral intravenous injection seems the most reliable and reproducible route for their administration. Once entering the bloodstream after intravenous injection, lipobeads, similar to liposomal or polymeric delivery systems, should withstand a number of environmental (physiological and physicochemical) attacks on the way to targeted organs. The bloodstream is a complex environment of the serum (proteins, electrolytes, etc.) and immune system (macrophages, proteins of complement system, etc.) components, so that interaction of those components with lipobeads could result in either leakage of their content or their removal from the blood circulation as exogenous pathogens. As it was reported for liposomes, the proteins of complement system were able to produce lytic pores and enhance the release of liposomal content [157], whereas blood lipoproteins destabilized liposomes to enhance the leakage of their payload [158]. The opsonins and dysopsonins are another blood proteins, which could be responsible for recognition of

lipobeads and their enhanced uptake by the mononuclear phagocyte system (MPS) cells (neutrophils, monocytes, and macrophages) [159–162].

The effect of physicochemical factors (size, charge, hydrophobicity, surface morphology, and composition) on lipobeads' leakage in and clearance from blood is not known and should be the other target for the future study of lipobeads as a drug delivery system. Nonetheless, even just a few results available on the drug-encapsulated lipobeads (pegylated [11] or not [20]) have already demonstrated their noticeably better stability, biodistribution, nontoxicity, and therapeutic activity than those for liposomes.

Once reaching the heart, the blood with lipobeads is pumped up to organs. Undoubtedly, the mechanical stability of lipobeads in the blood flow will be higher than that of liposomes, since in this construct, a lipid bilayer is supported by hydrogel core and can be strengthened even more by anchoring. The capillaries with a diameter ranging from 2 to 10 μm constitute the first sieving constraint for the lipobead size. The particles of the size between 0.4 and 3 μm would mainly be captured by the liver macrophages. The lipobeads greater than 200 nm [75] would preferentially be filtered by the spleen. The smaller limit comes from the fact that particles less than 40 nm [162] should undergo clearance through metabolism in the liver and excretion through kidneys. Therefore, the diameter of lipobeads is supposed to be in a relatively narrow range from 50 to 180 nm for a longer retention in the bloodstream. Interestingly, it has been proven that formulations of lipobeads were the most reproducible in this range of sizes especially if prepared by polymerization within a liposomal reactor (see **Table 2**).

This range of sizes looks appropriate for lipobeads to exit systemic circulation. To reach interstitial space, lipobeads must cross a thin inner membrane of squamous endothelial cells provided by the capillaries. In normal capillaries, the endothelial cells form uninterrupted linings with typical gaps of 5–10 nm in size. In capillaries associated with pathologies such as tumor and inflammation, the gaps between endothelial cells were reported to vary from 100 to 780 nm for different types of cancer [163]. Due to rapid and imbalanced vessel formation, the tumor neovasculature is chaotic, extremely heterogeneous and “leaky” [164]. The enhanced vascular permeability of the tumor capillaries is the first factor contributing to the phenomenon referred as the enhanced permeation and retention (EPR) effect [165, 166]. The second factor of the EPR effect, an enhanced retention of lipobeads in the interstitial space, can be expected due to a poor lymphatic drainage in the tumor tissue, which results in a slower clearance of drug carriers and their accumulation in the interstitial space [167]. Biodistribution experiments have already performed in mice bearing a distant subcutaneous tumor and in mice with metastatic lung melanoma to show accumulation of drug-loaded lipobeads both in the area surrounding the tumor and within the tumor itself [11]. Therein, the payload is evident in the interstitial spaces between the tumor cells outside the vasculature.

In the interstitial space, lipobeads passively or actively target the cellular surface. Strategies of active cell targeting which has been proposed for liposomal carriers [86–88] could be applicable to lipobeads as well.

The internalization of lipobeads into the cells can proceed via several mechanisms [168, 169] sketched in **Figure 13**. Phagocytosis provides the so-called “cell eating” mechanism by which larger lipobeads can be taken into and degraded within the cells. Using pinocytosis, the cells internalize the fluid surrounding the cell simultaneously with all substances (“cell drinking” mechanism), so that if lipobeads are in the fluid phase area of invagination, they would be taken up to form pinosomes inside. Different endocytic pathways can be distinguished in accord with the specific molecular regulators (not shown in **Figure 13**), such as the clathrin-mediated endocytosis, dynamin-dependent and dynamin-independent mechanisms, as well as receptor-mediated endocytosis. In addition, the mutual fusion of cell membrane and lipid bilayer of lipobeads [170] can occur at the cell surface with internalization of just the drug-loaded nanogels. Understanding the cellular entry of lipobeads, their intracellular trafficking, drug release, and therapeutic action mechanisms are the future topics for studies on lipobeadal drug delivery systems.

4.1.2. New mechanisms of drug release

A drug release profile (the amount of drug released into the bloodstream over time) depends on the properties of the drug itself and drug carrier system. Even a few available examples of drug-encapsulated lipobeads showed that the additional element in their structure, the hydrogel core, significantly prolongs the release time for both high molecular weight (e.g., proteins) and small molecule (e.g., doxorubicin) drugs as compared to conventional liposomes and uncoated hydrogel particles. The characteristic time for release of 50% (D_{50}) of BSA (Mw 66 kDa) from 1- μ m lipobeads (~ 11 days) is 10-fold of that from 1- μ m liposomes (~ 1 day) [20]. For a lighter protein interleukin-2 (IL-2, Mw 17 kDa) [22], D_{50} equals 8, 16, and 52 h for nanogels ($\varnothing 150$ nm), liposomes ($\varnothing 100$ nm), and lipobeads ($\varnothing 120$ nm), respectively, indicating the slower release of the protein drugs from lipobeads. In comparison, the characteristic time (D_{50}) for release of doxorubicin from uncoated microgels ($\sim \varnothing 6$ μ m) was estimated [6, 43] to be about 1.5 min, whereas the release of doxorubicin from lipobeads was not detected at all within this time scale.

Figure 13. Possible mechanisms of lipobeads' internalization into the cell.

Different applications require different release profiles, and bicompartmental structure of lipobeads brings more options to change the concentration profile of a released drug from a steep rise (burst release) and a cyclic variations (pulsatile release) to a gradual increase up to the value within the therapeutic window is reached (sustained or controlled release). Of particular importance is the capability of lipobeads to provide a better-sustained release, which is the most desirable but more difficult mode to achieve and maintain.

Let us consider the novelty the hydrogel core can bring with regard to drug release mechanisms. Undoubtedly, an advanced property of polymer networks is their responsiveness to environmental stimuli. Depending on possible responses of the hydrogel core (swelling, contraction, and degradation), three mechanisms of drug release from lipobeads could be developed in the future.

In the “sponge-like” mechanism (Table 5), hydrogel core initially is in a swollen state. Nevertheless, encapsulated drug molecules release for a prolonged period as compared to conventional liposomes. When the environment changes (temperature, pH, etc.), the polymer network shrinks, so that the hydrogel core, like a squeezed sponge, releases the loaded drug into the space between gel and lipid membrane, and the drug diffuses through the membrane outside the lipobead. This mechanism provides a slow gradual drug release in response to temperature change, for example. The characteristic time of the drug diffusion through the lipid bilayer could be projected to hours.

In the “poration” mechanism, the hydrogel core initially is a shrunken state, and drug molecules are trapped more tightly within the polymer network. Their release can be even more suppressed

Mechanism	Scheme	Characteristic time
“Sponge-like” (diffusion)	<p>The diagram illustrates the 'Sponge-like' mechanism in three stages. Stage 1: A lipobead with a 'Swollen polymer network' (represented by a loose mesh of black lines) and green dots representing drug molecules. Stage 2: 'Polymer network contraction' occurs, compressing the mesh. Stage 3: 'Drug diffusion' occurs as green dots move from the contracted network, through the 'Lipid bilayer' (the outer boundary), and out of the lipobead as 'Drug molecule's.</p>	Hours
“Poration”	<p>The diagram illustrates the 'Poration' mechanism in three stages. Stage 1: A lipobead with a shrunken polymer network and green dots. Stage 2: 'Polymer network swelling' occurs, creating 'Pore formation' (gaps in the mesh). Stage 3: 'Drug release through pores' occurs as green dots exit the lipobead through the newly formed pores.</p>	Minutes
“Burst”	<p>The diagram illustrates the 'Burst' mechanism in three stages. Stage 1: A lipobead with a shrunken polymer network and green dots. Stage 2: 'Polymer network degradation' occurs, weakening the internal structure. Stage 3: 'Rupture of lipid membrane' occurs, leading to a 'Drug burst' where green dots are rapidly released from the lipobead.</p>	Seconds

Table 5. Mechanisms of drug release from lipobeads with environmentally sensitive or degradable hydrogel core.

in comparison with conventional liposomes. When the environment changes (temperature, pH, etc.), the polymer network swells so much that the volume of hydrogel core becomes greater than the space provided by the closed lipid bilayer. Therefore, a “growing” hydrogel core causes stretching of the lipid bilayer and pore formation (“poration”) resulting in the drug release through the pores. This mechanism provides a faster drug release in response to stimuli with the projected characteristic time of minutes.

The “exploding” lipobeads have been discovered [23] as a byproduct of biodegradation of microgels covered with phospholipid membrane. As schematically outlined in **Table 5** (the bottom row), if a polymer network degrades (for example, the interchain cross-links can be cleaved by hydrolysis), the swelling pressure inside increases, because the degradation products are unable to diffuse through the lipid membrane even it stretches. At some point, the internal pressure becomes sufficient to break the membrane. As a result, encapsulated drug falls out of lipobeads with the maximal release rate (“burst” release with the characteristic time of seconds).

4.1.3. Drug combination within lipobeads

In the first example [20], a combination of protein (Pfs25) and oligonucleotide (CpGODN) has been simultaneously encapsulated into lipobeads. The recombinant protein Pfs25 expressed in *Pichia pastoris* is a leading antigen of blocking stage potential and can be used as a vaccine to block malaria transmission by mosquitoes. The antigen Pfs25 has a poor immunogenicity and needs an enhancer of immunological recognition. Unmethylated CpG oligodeoxynucleotide (CpGODN) is a strong stimulator of immune response in mammalian hosts and acts as the adjuvant improving immunogenicity of coadministered protein antigen as well as reducing the amount of protein required. CpGODN stimulates the immune system through a specific receptor TLR9. The immune activity of CpG was monitored by following the levels of nonspecific and specific immunoglobulins, a variety of cytokines, gamma interferon (IFN- γ), and increased lytic activity (see [20] for references). The results of this study were impressive: (i) on the 90th day of storage at 4°C, the detected antigen leakage from lipobeads was significantly lower (5%) than from conventional liposomes (26%), (ii) like the conventional liposomes, no macroscopic sign of adverse reaction (redness, swelling, and formation of granulomas) at the site of intramuscular injection was observed for lipobeads, (iii) lipobead-encapsulated combination of Pfs25 and CpGODN showed the maximal immune response based on serum anti-Pfs25 profile of immunized mice, (iv) significantly higher levels of interferon- γ and interleukin-2 were detected in the spleen if mice immunized with lipobeads carried the drug combination.

In the second scheme [11], hydrophilic protein (IL-2, 17 kDa) and hydrophobic small molecule drug (SB505124, SB, 335 Da) have been coencapsulated into the hydrogel core of 120-nm lipobeads cross-linked by a free radical photopolymerization. The IL-2 belongs to the family of cytokines, soluble proteins that supposedly stimulate natural killer cells (NK) and enhance lytic activity against melanomas and renal cancer. However, efficiency of the IL-2 as an immunotherapeutic agent may be significantly reduced by the ability of tumor cells to secrete a number of immunosuppressive factors, such as the transforming growth factor- β (TGF- β) that decreases local immune responses. The SB is a TGF- β antagonist that inhibits TGF- β receptor. The study on coencapsulation into lipobeads and simultaneous sustained

delivery of the aforementioned drugs showed that no toxicity was observed on intravenously administrated mice. Biodistribution analysis of rhodamine-loaded lipobeads in healthy mice indicated that the lipobeads primarily accumulated in lungs, liver, and kidney, the heart and spleen were also reached though. In B16 lung metastatic animals, the highest accumulation of lipobeads and drug was found to occur in the lungs and liver. In comparison to other delivery systems including liposomal, a significantly greater reduction in both tumor growth rate and tumor mass was observed after one-week therapy of the B16/B6 mouse models of metastatic melanoma administered intravenously. It was found that the lipobead-delivered combination immunotherapy stimulated both innate and adaptive immune systems resulting in drastically increased survival.

4.1.4. Combined multifunctional drug containers

As per **Figures 11 and 12**, the nanogel core collapse at elevated temperature causes either reversible or irreversible aggregation of lipobeads depending on whether lipid bilayer fusion occurs or not. Reversible and irreversible aggregation of lipobeads is a key step for designing two types of combined multifunctional containers.

In the system made of anchored lipobeads, the initial formulation may consist of two different drugs entrapped in different lipobeads (**Figure 14**). Under switching condition 1, both drugs can be simultaneously delivered as one aggregate to the targeted organs in the body. At switching condition 2 or 3, either one or the other drug can be released in the desired order.

In the system based on irreversible aggregation of lipobeads (**Figure 15**), several nanogels loaded with different predrug reagents are trapped under the same lipid membrane ("giant lipobeads") to react inside without damaging the surrounding organs and to be delivered to the targeted site in one "giant" container able to release the final product controllably.

Figure 14. The combined drug delivery system based on reversible aggregation of lipobeads.

Figure 15. The combined drug delivery system based on irreversible aggregation of lipobeads.

4.1.5. Remarks on lipobead-encapsulated anticancer drugs

Today, cancer is one of the most dangerous illnesses on Earth, because mortality in patients with solid malignant tumors is caused mainly by tumoral metastases, the appearance of new cancerous centers in another organs or different tissues. Administration of anticancer drugs by intravenous route (chemotherapy) is the main treatment aimed at destruction of primary tumor and reduction of the probability of formation of secondary tumors due to metastases. Since single metastatic cells cannot be localized, followed, and monitored so far, a high concentration of an anticancer drug should be systematically distributed throughout the entire human body to increase the probability of the cancer cells' distraction. Being strong poisons and/or cancerogenic themselves, anticancer drugs destroy not only malignant cells but also normal ones giving rise to serious side effects of a chronic and irreversible origin and/or causing the formation of a new malignant tumor even without metastases of the primary one. Moreover, the high concentrations of anticancer drugs can induce a resistance of the malignant cells to these drugs. To reduce the toxicity of the anticancer drugs by controlling their suitable concentrations and reaching the targeted cells without healthy cells being affected, numerous polymeric nanoparticles and liposomal drug delivery systems are under development or undergo clinical trials. However, only two polymer conjugates and six liposome-encapsulated anticancer drugs were approved to market as the most clinically successful liposomal anticancer products so far (for details, see [36] and references therein). Probably, loading drugs into the environmentally responsive hydrogel core covered with the lipid layer is the right way to the chemotherapy with superior tumor response and minimal side effects even at a greater loading concentration.

4.2. Prospective applications and perspectives

4.2.1. Hydrogel/lipid bilayer assembly: Mimicking cell membrane system

Recently developed technologies of using the liposome interior as a microreactor and the concept of lipobeads itself inspire the idea of artificial membrane system with controlled properties. The bottom-up approach to design “liposomes-within-giant vesicle” structures (*vesosomes*) [171, 172] includes a series of structures mimicking cell membrane systems such as shown in **Figure 16** in the order of increase in their complexity: (a) giant vesicles with the size compared to the size of living cells (~5–200 μm), (b) large unilamellar vesicles with the

Figure 16. Liposomes-within-giant vesicle structures.

nanometer scale size (<1000 nm), (c) a number of liposomes interior to the single membrane vesicle (cell analogue), (d) a double membrane vesicle with the inner membrane surface lesser than the outer membrane surface (nucleus analogue), and (e) a double membrane vesicle with the inner membrane surface greater than the outer membrane (mitochondrion analogue).

By analogy with vesosomes, it would be intriguing to design the so-called *vesobeads*—hydrogel/membrane structures of different combinations of liposomes, giant vesicles, nanogels, microgels, lipobeads, and giant lipobeads. Some of them shown in **Figure 17** are: (a) giant vesicles with hydrogel core (“giant lipobeads”), (b) hydrogel inside liposomes with the size less than $1\ \mu\text{m}$, (c) structure that has many lipobeads inside of a giant vesicle, which can also be done by injection. By manipulating the hydrogel, one can cause the lipobeads to aggregate, as shown in structure (d). If the membranes of lipobeads fuse, the structure (e), a number of nanogels inside of a double membrane, can be engineered.

The aforementioned membrane/membrane and hydrogel/membrane structures comprise fusion/fission of LUVs and GUVs and can be served as a model system to study exo- and endocytosis, hydrogel/membrane compatibility, loading ability of the lipid bilayers, polymer networks, and interior of GUVs, and interactions between those assemblages when their surfaces are specifically modified.

Besides all the advantages of conventional lipobeads discussed in this chapter, the multicompartamental structure of vesobeads will provide additional protection against degradation and leakage in bloodstream and greater biocompatibility. Moreover, the structure of a vesobead resembles the structure of a macropinosome (**Figure 13**) and can provide simultaneous internalization of several lipobeads into the cell interior. Indeed, if an external lipid bilayer of vesobead fuses with the cellular plasma membrane, a bunch of loaded lipobeads are injected into cytoplasm.

Figure 17. Hydrogel/lipid bilayer assemblies.

4.2.2. Proteo-lipobeads

The next step of lipobead functionalization is incorporation of proteins into their lipid bilayers or/and loading of the hydrogel core with functional proteins. This type of hydrogel/membrane structures has been already named as *proteo-lipobeads*.

4.2.2.1. Proteins within lipid bilayer

The first proteo-lipobeads were prepared when transmembrane receptors were reconstituted into the lipid bilayer of lipobeads [45]. It was found that the receptors retained their native-specific binding. Recently, new proteo-lipobeads with the controlled orientation of the membrane protein and enhanced stability have been developed by modifying agarose beads with linkers, binding membrane proteins to the linkers, and surface coverage with phospholipids [173]. The lipobead incorporated cytochrome c oxidase was shown to be functional in terms of antibody binding and proton transport modulation. The proteo-lipobeads, as alternatives of living cells for monitoring properties of membrane proteins and ion transport through ionic channels and transporters, did exhibit a higher stability, capability of uniform orientation, and functional activity of the membrane proteins in comparison with proteo-liposomes and polymersomes [174]. The further interplay between lipids and the lipobead-encapsulated proteins will allow one to remodel the dynamic cell membrane systems [175] and, despite the increase in complexity of a lipobead structure, will bring about new benefits, such as tiny living cells mimicking mechanisms of drug release regulated by signaling.

If ionic channels (transmembrane proteins) incorporated into the lipid bilayer, it would be necessary to measure ionic transport through the membrane without rupturing the lipid bilayer. For these purposes, one can imagine a hemispherical configuration of the lipobead, which would allow an electrical access to the interior as shown in **Figure 18**. By the way, this device could be used as a biomimetic sensor and a cell analogue, which functional properties could be modeled and studied by changing the inner compartments of the probe.

Figure 18. Hemispherical lipobead with electrical access to the interior.

4.2.2.2. *Proteins inside hydrogel core*

As it was mentioned in Section 2.5.1, so far the only test enzymes were loaded into the lipobeads interior to show retention of their enzymatic activity after release from the hydrogel core [37].

By analogy with the enzyme-containing lipid vesicles (liposomes) [176], one can predict that in turn the near-future development of enzyme-containing lipobeads will follow the same steps and directions, including the studies on (i) methods for adsorption of a variety of enzymes onto the interior or exterior site of the lipid bilayer and for their encapsulation within hydrogel core, (ii) enzyme encapsulation efficiencies, (iii) potential applications, especially, in the biotechnology (e.g., cheese production) and biomedical fields (e.g., enzyme-replacement therapy or for immunoassays). As in the case of liposomes, the enzyme-containing lipobeads could play a twofold role: enzyme carriers and enzymatic nanoreactors. In the first case, the enzyme molecules are expected to be controllably released from the lipobeads at the target site. In the second case, enzymes trapped inside the hydrogel matrix or interspace between the hydrogel core and lipid bilayer catalyze an enzymatic reaction either upon permeation of a substrate across the bilayer or by stimuli-responsive activation of the enzyme molecules.

Obviously, a deeper understanding and modeling of the catalytic activity of the entrapped enzyme molecules will be in demand. In this context, it is worthy to highlight the key property which hydrogel core brings to the enzyme-containing lipobeads: the activity of the enzyme entrapped into the hydrogel can be affected by the 3D-polymeric network density. For example, the studies on activities of enzymes immobilized into the temperature-sensitive hydrogels explicitly indicated that their activity (e.g., urease [177], β -galactosidase [178], α -chymotrypsin [179]) decreased upon PNIPA hydrogel shrinking at elevated temperatures. Since the volume transition in the temperature-sensitive hydrogels is reversible (see Section 3.1.1 for details), the activities of the aforementioned enzymes were restored with temperature decrease beyond the T_v of PNIPA.

There may be another case, when the activity of enzyme immobilized into the hydrogel matrix increases upon a polymer network collapse. In accord with the recently proposed model of electrochemical mechanics of bacterial spores [180], all bacterial spores have a lipobead-like structure consisting of layered protein network (coat), peptidoglycan cross-linked matrix (cortex) enclosed between two lipid membranes, and the spore core containing the genetic information. Some piece of evidence indicates that the co-called cortex lytic enzymes involved into the spore cortex degradation enhance their activity in response to the collapse of the peptidoglycan cortex where these enzymes are located. Note, in this case, the cortex matrix is a substrate for the enzymes, so that its collapse is equivalent to the increase in the substrate concentration, which in turn is responsible for the rate of the degradation reaction.

5. Closing remarks

The concept of lipobeads has been proposed about 30 years ago and the time has come to explore a combination of lipid bilayer and cross-linked polymer network as a logical step of

the development of polymeric and liposomal nanoscopic systems to provide the desired level of functionality, which Nature achieves in living cells. Two general approaches to preparation of lipobeads (polymerization within lipid vesicle interior and mixing of separately prepared lipid vesicles and hydrogel particles) actually are the modified methods for fabrication of liposomes or nanogels. Besides attractive properties from both liposomal and polymeric systems, the mechanical stability of the lipid bilayer and environmental responsiveness of the total structure are the two important properties that hydrogel core brings about to the new construct. Bicompartamental structure of lipobeads predefines their numerous future applications in biotechnology and bioengineering, tissue engineering, sensing and molecular recognition, drug and gene delivery, controlled release, artificial muscles, flow control, and so on.

As a platform for drug delivery systems, lipobeads have already been loaded with chemotherapeutics (doxorubicin, a combination of immunotherapeutic agent and inhibitor of growth factor receptor), malaria vaccine (a combination of stage potential blocker and immunostimulator), and dermatological agent (natural moisturizing factor). In animal experiment, the lipobead-delivered combination chemotherapy demonstrated a drastically increased survival.

Further development of the hydrogel/lipid bilayer assemblies may include vesobeads (e.g., many dispersed lipobeads or their aggregate inside of a giant vesicle, a number of nanogels inside of a double membrane), proteo-lipobeads (lipobeads with functional proteins incorporated into lipid bilayers or/and hydrogel core), enzyme-containing lipobeads (a particular case of proteo-lipobeads with controllable enzymatic activity), and hemi-lipobeads (a cell analogue probe with electrical access to its interior).

This chapter shows that additional technological expenses on production of lipobeads will not be a high cost for the aforementioned advantages of their use in the following Era of the Bioscopic Systems.

Acknowledgements

Financial support for this work was provided by Pace University (Dyson College of Arts and Sciences, Summer Research and Scholarly Research Funds). I would like to thank the entire cohort of my collaborators and all my students participated in this project. I am also grateful to Dr Irina Gazaryan for many comments, which helped clarify my intent.

Author details

Sergey Kazakov

Address all correspondence to: skazakov@pace.edu

Department of Chemistry & Physical Sciences, Pace University, Pleasantville, NY, United States of America

References

- [1] Torchilin VP, Klibanov AL, Ivanov NN, Ringsdorf H, Schlarb B. Polymerization of liposome-encapsulated hydrophilic monomers. *Makromolekulare Chemie, Rapid Communications*. 1987;**8**:457-460
- [2] Gao K, Huang L. Solid core liposomes with encapsulated colloidal gold particles. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 1987;**897**:377-383
- [3] Samain D, Bec J-L, Cohen E, Nguyen F, Peyrot M. Particulate vector useful in particular for the transport of molecules with biological activity and process for its preparation. In: Publication Number WO1989011271; 1989
- [4] Castignolles N, Betbeder D, Ioualalen K, Merten O, Leclerc C, Samain D, Perrin P. Stabilization and enhancement of interleukin-2 in vitro bioactivity by new carriers: Supramolecular bio-vectors. *Vaccine*. 1994;**12**:1413-1418
- [5] Monshpouri M, Rudolph AS. Method of forming hydrogel particles having a controlled size using liposomes. In: US Patent 5464629; 1995
- [6] Jin T, Pennefather P, Lee PI. Lipobeads: A hydrogel anchored lipid vesicle system. *FEBS Letters*. 1996;**397**:70-74
- [7] Kiser PF, Wilson G, Needham DA. Synthetic mimic of the secretory granule for drug delivery. *Nature*. 1998;**394**:459-462
- [8] Perrott MG, Barry SE. Liposome-assisted synthesis of polymeric nanoparticles. In: US Patent 6217901; 2001
- [9] Kazakov S, Kaholek M, Teraoka I, Levon K. UV-induced gelation on nanometer scale using liposome reactor. *Macromolecules*. 2002;**35**:1911-1920
- [10] Kazakov S, Kaholek M, Kudasheva D, Teraoka I, Cowman KM, Levon K. Poly(N-isopropylacrylamide-co-1-vinylimidazole) hydrogel nanoparticles prepared and hydrophobically modified in liposome reactors: Atomic force microscopy and dynamic light scattering study. *Langmuir*. 2003;**19**:8086-8093
- [11] Park J, Wrzesinski SH, Stern E, et al. Combination delivery of TGF-inhibitor and IL-2 by nanoscale liposomal polymeric gels enhances tumour immunotherapy. *Nature Materials*. 2012;**11**:895-905
- [12] Viallat A, Dalous J, Abkarian M. Giant lipid vesicles filled with a gel: Shape instability induced by osmotic shrinkage. *Biophysical Journal*. 2004;**86**:2179-2187
- [13] Stauch O, Uhlmann T, Frohlich M, et al. Mimicking a cytoskeleton by coupling poly(N-isopropylacrylamide) to the inner leaflet of liposomal membranes: Effects of photopolymerization on vesicle shape and polymer architecture. *Biomacromolecules*. 2002;**3**:324-332
- [14] Patton JN, Palmer AF. Engineering temperature-sensitive hydrogel nanoparticles entrapping hemoglobin as a novel type of oxygen carrier. *Biomacromolecules*. 2005;**6**:2204-2212

- [15] Patton JN, Palmer AF. Photopolymerization of bovine hemoglobin entrapped nanoscale hydrogel particles within liposomal reactors for use as an artificial blood substitute. *Biomacromolecules*. 2005;**6**:414-424
- [16] Van Thienen TG, Lucas B, Flesch FM, Nostrum CF, Demeester J, De Smedt SC. On the synthesis and characterization of biodegradable dextran nanogels with tunable degradation properties. *Macromolecules*. 2005;**38**:8503-8511
- [17] Jesorka A, Markstrom M, Karlsson M, Orwar O. Controlled hydrogel formation in the internal compartment of giant unilamellar vesicles. *Journal of Physical Chemistry B*. 2005;**109**:14759-14763
- [18] Jesorka A, Markstrom M, Orwar O. Controlling the internal structure of giant unilamellar vesicles by means of reversible temperature dependent sol-gel transition of internalized poly(N-isopropyl acrylamide). *Langmuir*. 2005;**21**:1230-1237
- [19] Schillemans JP, Flesch FM, Hannink WE, van Nostrum CF. Synthesis of bilayer-coated nanogels by selective cross-linking of monomers inside liposomes. *Macromolecules*. 2006;**39**:5885-5890
- [20] Tiwari S, Goyal AK, Mishra N, et al. Development and characterization of novel carrier gel core liposomes based transmission blocking malaria vaccine. *Journal of Controlled Release*. 2009;**140**:157-165
- [21] Hong JS, Stavis SM, DePaoli Lacerda SH, Locascio LE, Raghavan SR, Gaitan M. Microfluidic directed self-assembly of liposome-hydrogel hybrid nanoparticles. *Langmuir*. 2010;**26**:11581-11588
- [22] Pépin-Donat B, Campillo C, Quemeneur F, et al. Lipidic composite vesicles based on poly(NIPAM), chitosan or hyaluronan: Behaviour under stresses. *International Journal of Nano Dimension*. 2011;**2**:17-23
- [23] De Geest BG, Stubbe BG, Jonas AM, Van Thienen T, Hinrichs WLJ, Demeester J, De Smedt SC. Self-exploding lipid-coated microgels. *Biomacromolecules*. 2006;**7**:373-379
- [24] Helwa Y, Dave N, Liu J. Electrostatically directed liposome adsorption, internalization and fusion on hydrogel microparticles. *Soft Matter*. 2013;**9**:6151-6158
- [25] MacKinnon N, Guerin G, Liu B, Gradinaru CC, Rubinstein JL, Macdonald PM. Triggered instability of liposomes bound to hydrophobically modified core-shell PNIPAM hydrogel beads. *Langmuir*. 2010;**26**:1081-1089
- [26] Kazakov S, Kaholek M, Levon K. Lipobeads and their production. In: US Patent 7618565 B2; 2009
- [27] Kazakov S, Kaholek M, Levon K. Lipobeads and their production. In: US Patent 7883648 B2; 2011
- [28] Saleem Q, Liu B, Gradinaru CC, Macdonald PM. Lipogels: Single-lipid-bilayer-enclosed hydrogel spheres. *Biomacromolecules*. 2011;**12**:2364-2374
- [29] Kiser PF, Wilson G, Needham D. Lipid-coated microgels for the triggered release of doxorubicin. *Journal of Controlled Release*. 2000;**68**:9-22

- [30] Tiwari S, Goyal A, Khatri K, Mishra N, Vyas SP. Gel core liposomes: An advanced carrier for improved vaccine deliver. *Journal of Microencapsulation*. 2009;**26**:75-82
- [31] An E, Jeong CB, Cha C, Kim DH, Lee H, Kong H, Kim J, Kim JW. Fabrication of microgel-in-liposome particles with improved water retention. *Langmuir*. 2012;**28**:4095-4101
- [32] Campbell A, Taylor P, Cayre OJ, Paunov VN. Preparation of aqueous gel beads coated by lipid bilayers. *Chemical Communications*. 2004;**21**:2378-2379
- [33] Campillo CC, Schroder AP, Marques CM, Pépin-Donat B. Composite gel-filled giant vesicles: Membrane homogeneity and mechanical properties. *Materials Science and Engineering: C*. 2009;**29**:393-397
- [34] Faivre M, Campillo C, Viallat A, Pepin-Donat B. Responsive giant vesicles filled with poly(*N*-isopropylacrylamide) sols or gels. *Progress in Colloid & Polymer Science*. 2006;**133**:41-44
- [35] Buck S, Pennefather PS, Xue HY, Grant J, Cheng Y-L, Allen CJ. Engineering lipobeads: Properties of the hydrogel core and the lipid bilayer shell. *Biomacromolecules*. 2004;**5**: 2230-2237
- [36] Kazakov S. Liposome-nanogel structures for future pharmaceutical applications: An updated review. *Current Pharmaceutical Design*. 2016;**10**:1391-1413
- [37] Bobone S, Miele E, Cerroni B, et al. Liposome-templated hydrogel nanoparticles as vehicles for enzyme-based therapies. *Langmuir*. 2015;**31**:7572-7580
- [38] Gasperini L, Mano JF, Reis RL. Natural polymers for the microencapsulation of cells. *Journal of the Royal Society Interface*. 2014;**11**:20140817
- [39] Peyrot M, Sautereau AM, Rabanel JM, Nguyen F, Tocanne JF, Samain D. Supramolecular biovectors (SMBV): A new family of nanoparticulate drug delivery systems. Synthesis and structural characterization. *International Journal of Pharmaceutics*. 1994;**102**:25-33
- [40] Kazakov S, Kaholek M, Levon K. Hydrogel nanoparticles compatible with phospholipid bilayer. *Polymer Preprints*. 2002;**43**:381-382
- [41] Rahni S, Kazakov S. Hydrogel micro-/nanosphere coated by a lipid bilayer: Preparation and microscopic probing. *Gels*. 2017;**3**:00007
- [42] Major M, Prieur E, Tocanne JF, Betbeder D, Sautereau AM. Characterization and phase behavior of phospholipids bilayers adsorbed on spherical polysaccharidic nanoparticles. *Biochimica et Biophysica Acta*. 1997;**1327**:32-40
- [43] Ng CC, Cheng Y-L, Pennefather PS. One-step synthesis of a fluorescent phospholipid-hydrogel conjugate for driving self-assembly of supported lipid membranes. *Macromolecules*. 2001;**34**:5759-5765
- [44] Ng CC, Cheng Y-L, Pennefather PS. Properties of a self-assembled phospholipid membrane supported on lipobeads. *Biophysical Journal*. 2004;**87**:323-331

- [45] Park PS-H, Ng CC, Buck S, Wells JW, Cheng Y-L, Pennefather PS. Characterization of radio-ligand binding to a transmembrane receptor reconstituted into Lipobeads. *FEBS Letters*. 2004;**567**:344-348
- [46] Umamaheshwari RB, Jain NK. Receptor-mediated targeting of lipobeads bearing aceto-hydroxamic acid for eradication of *Helicobacter pylori*. *Journal of Controlled Release*. 2004;**99**:27-40
- [47] Shidhaye S, et al. Nanogel engineered polymeric micelles for drug delivery. *Current Drug Therapy*. 2008;**3**:209-217
- [48] JK O, Siegwart DJ, Matyjaszewski K. Synthesis and biodegradation of nanogels as delivery carriers for carbohydrate drugs. *Biomacromolecules*. 2007;**8**:3326-3331
- [49] Shah PP, et al. Skin permeating nanogel for the cutaneous co-delivery of two anti-inflammatory drugs. *Biomaterials*. 2012;**33**:1607-1617
- [50] Perry JL, et al. PEGylated PRINT nanoparticles: The impact of PEG density on protein binding, macrophage association, biodistribution, and pharmacokinetics. *Nano Letters*. 2012;**12**:5304-5310
- [51] Perry JL, et al. PRINT: A novel platform toward shape and size specific nanoparticle theranostics. *Accounts of Chemical Research*. 2011;**44**:990-998
- [52] Glangchai LC, Caldorera-Moore M, Shi L, Roy K. Nanoimprint lithography based fabrication of shape-specific, enzymatically-triggered smart nanoparticles. *Journal of Controlled Release*. 2008;**125**:263-272
- [53] Yang H, Wang Q, Chen W, et al. Hydrophilicity/hydrophobicity reversible and redox-sensitive nanogels for anticancer drug delivery. *Molecular Pharmaceutics*. 2015;**12**:1636-1647
- [54] Gregoriadis G, Ryman BE. Liposomes as carriers of enzymes or drugs: A new approach to the treatment of storage diseases. *Biochemical Journal*. 1971;**124**:58P
- [55] Gregoriadis G. Drug entrapment in liposomes. *FEBS Letters*. 1973;**36**:292-296
- [56] Juliano RL, Stamp D. Pharmacokinetics of liposome-entrapped anti-tumor drugs. *Biochemistry & Pharmacology*. 1978;**27**:21-27
- [57] Mayhew E, Papahadjopoulos D, Rustum YM, Dave C. Use of liposomes for the enhancement of the cytotoxic effects of cytosine arabinoside. *Annals of the New York Academy of Sciences*. 1978;**308**:371-386
- [58] Allen TM, Cleland LG. Serum-induced leakage of liposome contents. *Biochimica et Biophysica Acta*. 1980;**597**:418-426
- [59] Storm G, Roerdink FH, Steerenberg PA, de Jong WH, Crommelin DJ. Influence of lipid composition on the antitumor activity exerted by doxorubicin-containing liposomes in a rat solid tumor model. *Cancer Research*. 1987;**47**:3366-3372
- [60] McIntosh TJ. The effect of cholesterol on the structure of phosphatidylcholine bilayers. *Biochimica et Biophysica Acta*. 1978;**513**:43-58

- [61] Cullis PR, Hope MJ. The bilayer stabilizing role of sphingomyelin in the presence of cholesterol: A ³¹P NMR study. *Biochimica et Biophysica Acta*. 1980;**597**:533-542
- [62] Mayer LD, Tai LC, Bally MB, et al. Characterization of liposomal systems containing doxorubicin entrapped in response to pH gradients. *Biochimica et Biophysica Acta*. 1990;**1025**:143-151
- [63] Deamer DW, Prince RC, Crofts AR. The response of fluorescent amines to pH gradients across liposome membranes. *Biochimica et Biophysica Acta*. 1972;**274**:323-335
- [64] Mayer LD, Bally MB, Cullis PR. Uptake of adriamycin into large unilamellar vesicles in response to a pH gradient. *Biochimica et Biophysica Acta*. 1986;**857**:123-126
- [65] Madden TD, Harrigan PR, Tai LC, et al. The accumulation of drugs within large unilamellar vesicles exhibiting a proton gradient: A survey. *Chemistry and Physics of Lipids*. 1990;**53**:37-46
- [66] Bolotin EM, Cohen R, Bar LK, Emanuel SN, Lasic DD, Barenholz Y. Ammonium sulphate gradients for efficient and stable remote loading of amphipathic weak bases into liposomes and ligandosomes. *Journal of Liposome Research*. 1994;**4**:455-479
- [67] Drummond DC, Noble CO, Guo Z, Hong K, Park JW, Kirpotin DB. Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilization strategy. *Cancer Research*. 2006;**66**:3271-3277
- [68] Maurer N, Wong KF, Hope MJ, Cullis PR. Anomalous solubility behavior of the antibiotic ciprofloxacin encapsulated in liposomes: A ¹H-NMR study. *Biochimica et Biophysica Acta*. 1998;**1374**:9-20
- [69] Johnston MJ, Edwards K, Karlsson G, Cullis PR. Influence of drug-to-lipid ratio on drug release properties and liposome integrity in liposomal doxorubicin formulations. *Journal of Liposome Research*. 2008;**18**:145-157
- [70] Bartoli MH, Boitard M, Fessi H, et al. In vitro and in vivo antitumoral activity of free and encapsulated taxol. *Journal of Microencapsulation*. 1990;**7**:191-197
- [71] Cabanes A, Briggs KE, Gokhale PC, Treat JA, Rahman A. Comparative in vivo studies with paclitaxel and liposome-encapsulated paclitaxel. *International Journal of Oncology*. 1998;**12**:1035-1040
- [72] Zhigaltsev IV, Winters G, Srinivasulu M, et al. Development of a weak-base docetaxel derivative that can be loaded into lipid nanoparticles. *Journal of Controlled Release*. 2010;**144**:332-340
- [73] Gregoriadis G, Neerunjun D. Control of the rate of hepatic uptake and catabolism of liposome-entrapped proteins injected into rats. Possible therapeutic applications. *European Journal of Biochemistry*. 1974;**47**:179-185
- [74] Juliano RL, Stamp D. The effect of particle size and charge on the clearance rates of liposomes and liposome encapsulated drugs. *Biochemical and Biophysical Research Communications*. 1975;**63**:651-658

- [75] Mahli S, Dixit K, Sohi H, Shegokar R. Expedition of liposomes to intracellular targets in solid tumors after intravenous administration. *Journal of Pharmaceutical Investigation*. 2013;**43**:75-87
- [76] Gabizon A, Catane R, Uziely B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene glycol coated liposomes. *Cancer Research*. 1994;**54**:987-992
- [77] Laginha KM, Verwoert S, Charrois GJ, Allen TM. Determination of doxorubicin levels in whole tumor and tumor nuclei in murine breast cancer tumors. *Clinical Cancer Research*. 2005;**11**:6944-6949
- [78] Johnston MJ, Semple SC, Klimuk SK, et al. Therapeutically optimized rates of drug release can be achieved by varying the drug-to-lipid ratio in liposomal vincristine formulations. *Biochimica et Biophysica Acta*. 1758;**2006**:55-64
- [79] Charrois GJR, Allen TM. Drug release rate influences the pharmacokinetics, biodistribution, therapeutic activity, and toxicity of pegylated liposomal doxorubicin formulations in murine breast cancer. *Biochimica et Biophysica Acta*. 1663;**2004**:167-177
- [80] Poste G, Bucana C, Raz A, Bugelski P, Kirsh R, Fidler IJ. Analysis of the fate of systemically administered liposomes and implications for their use in drug delivery. *Cancer Research*. 1982;**42**:1412-1422
- [81] Weissmann G, Cohen C, Hoffstein S. Introduction of missing enzymes into the cytoplasm of cultured mammalian cells by means of fusion-prone liposomes. *Transactions of the Association of American Physicians*. 1976;**89**:171-183
- [82] Ozawa M, Asano A. The preparation of cell fusion-inducing proteoliposomes from purified glycoproteins of HVJ (Sendai virus) and chemically defined lipids. *Journal of Biological Chemistry*. 1981;**256**:5954-5956
- [83] Parente RA, Nir S, Szoka FCJ. pH-dependent fusion of phosphatidylcholine small vesicles. Induction by a synthetic amphipathic peptide. *Journal of Biological Chemistry*. 1988;**263**:4724-4730
- [84] Bailey A, Monck MA, Cullis PR. pH-induced destabilization of lipid bilayers by a lipopeptide derived from influenza hemagglutinin. *Biochimica et Biophysica Acta*. 1997;**1324**:232-244
- [85] Torchilin VP, Rammohan R, Weissig V, Levchenko TS. TAT peptide on the surface of liposomes affords their efficient intracellular delivery even at low temperature and in the presence of metabolic inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**:8786-8791
- [86] Kelly C, Jefferies C, Cryan SA. Targeted liposomal drug delivery to monocytes and macrophages. *Journal of Drug Delivery*. 2011;**2011**:727241
- [87] Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*. 2005;**4**:145-160

- [88] Zhao G, Rodriguez BL. Molecular targeting of liposomal nanoparticles to tumor micro-environment. *International Journal of Nanomedicine*. 2013;**8**:61-71
- [89] Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews*. 2013;**65**:36-48
- [90] Allen TM. Ligand-targeted therapeutics in anticancer therapy. *Nature Reviews Cancer*. 2002;**2**:750-763
- [91] Bibi S, Lattmann E, Mohammed AR, Perrie Y. Trigger release liposome systems: Local and remote controlled delivery? *Journal of Microencapsulation*. 2012;**29**:262-276
- [92] Zhan C, Wang W, Santamaria C, Wang B, Rwei A, Timko BP, Kohane DS. Ultrasensitive phototriggered local anesthesia. *Nano Letters*. 2017;**17**:660-665
- [93] Von Hoegen P. Synthesis biomimetic supra molecular Biovector™ (SMBV™) particles for nasal vaccinatedelivery. *Advanced Drug Delivery Reviews*. 2001;**51**:113-125 and references therein
- [94] Lasic DD, Frederic PM, Stuart MCA, Barenholz Y, McCintosh TJ. Gelation of liposome interior. A novel method for drug encapsulation. *FEBS Letters*. 1992;**312**:255-258
- [95] Zhang Y, Zhu E, Wang B, Ding J. A novel microgel and associated post-fabrication encapsulation technique of proteins. *Journal of Controlled Release*. 2005;**105**:260-268
- [96] Harland RS, Prud'homme RK, editors. *Polyelectrolyte Gels: Properties, Preparation, and Applications*. Washington, DC: American Chemical Society; 1992
- [97] Peppas NA. *Hydrogels in Medicine and Pharmacy*. Boca Raton, FL: CRC Press; 1987
- [98] Galaev IY, Mattiasson B. Smart polymers and what they could do in biotechnology and medicine. *Trends in Biotechnology*. 1999;**17**:335-340
- [99] Hennink WE, van Nostrum CF. Novel cross-linking methods to design hydrogels. *Advanced Drug Delivery Reviews*. 2002;**54**:13-36
- [100] Shinoda A et al. Dual cross-linked hydrogel nanoparticles by nanogel bottom-up method for sustained-release delivery. *Colloids and Surfaces, B: Biointerfaces*. 2012;**99**:18-44
- [101] Morimoto N, et al. Hybrid nanogels with physical and chemical cross-linking structures as nanocarriers. *Macromolecular Bioscience*. 2005;**5**:710-716
- [102] Lee ES, et al. A virus-mimetic nanogel vehicle. *Angewandte Chemie, International Edition*. 2008;**47**:2418-2421
- [103] Kopecek J. Polymer chemistry—Swell gells. *Nature*. 2002;**417**:388-391
- [104] Kettel MJ, et al. Aqueous nanogels modified with cyclodextrin. *Polymer*. 2011;**52**:1917-1924
- [105] Shen W, et al. Thermosensitive, biocompatible and antifouling nanogels prepared via aqueous raft dispersion polymerization for targeted drug delivery. *Journal of Controlled Release*. 2011;**152**:e75-e76

- [106] Mah E, Ghosh R. Thermo-responsive hydrogels for stimuli-responsive membranes. *Processes*. 2013;**1**:238-262
- [107] Schild HG. Poly(N-isopropylacrylamide): Experiment, theory and application. *Progress in Polymer Science*. 1992;**17**:163-249
- [108] Yang M, Liu C, Li Z, Gao G, Liu F. Temperature-responsive properties of poly(acrylic acid-co-acrylamide) hydrophobic association hydrogels with high mechanical strength. *Macromolecules*. 2010;**43**:10645-10651
- [109] Liu F, Seuring J, Agarwal S. A non-ionic thermophilic hydrogel with positive thermo-sensitivity in water and electrolyte solution. *Macromolecular Chemistry and Physics*. 2014;**215**:1466-1472
- [110] Shimada N, Kidoaki S, Maruyama A. Smart hydrogels exhibiting UCST-type volume changes under physiologically relevant conditions. *RSC Advances*. 2014;**4**:52346
- [111] Rohindra DR, Nand AV, Khurma JR. Swelling properties of chitosan hydrogels. *The South Pacific Journal of Natural and Applied Sciences*. 2004;**21**:32-35
- [112] Kaith BS, Jindal R, Mittal H. Superabsorbent hydrogels from poly(acrylamide-co-acrylonitrile) grafted *Gum ghatti* with salt, pH and temperature responsive properties. *Der Chemica Sinica*. 2010;**1**:92-103
- [113] Katono H, et al. Thermo-responsive swelling and drug release switching of interpenetrating polymer networks composed of poly (acrylamide-co-butyl methacrylate) and poly (acrylic acid). *Journal of Controlled Release*. 1991;**16**:215-227
- [114] Georgiev GS, Mincheva ZP, Geordieva VT. Temperature-sensitive polyzwitterionic gels. *Macromolecular Symposia*. 2001;**164**:301-312
- [115] Gehrke S. Synthesis, equilibrium swelling, kinetics, permeability and applications of environmentally responsive gels. *Advances in Polymer Science*. 1993;**110**:81-144
- [116] Harsh D, Gehrke SJ. Controlling the swelling characteristics of temperature-sensitive cellulose ether hydrogels. *Journal of Controlled Release*. 1991;**17**:175-186
- [117] McPhee W, Tam KC, Pelton RJ. Poly(N-isopropylacrylamide) latexes prepared with SDS. *Journal of Colloid and Interface Science*. 1993;**156**:24-30
- [118] Hirotsu S, Hirokawa Y, Tanaka T. Volume-phase transition of ionized N-isopropylacrylamide. *Journal of Chemical Physics*. 1987;**87**:1392-1395
- [119] Zhou S, Chu B. Synthesis and volume phase transition of poly(methacrylic acid-co-N-isopropylacrylamide) microgel particles in water. *Journal of Physical Chemistry*. 1998;**102**:1364-1371
- [120] Shibayama M, Mizutani S, Nomura S. Thermal properties of copolymer gels containing N-isopropylacrylamide. *Macromolecules*. 1996;**29**:2019-2024
- [121] Hirose T, Amiya T, Hirokawa Y, Tanaka T. Phase transition of submicron gel beads. *Macromolecules*. 1987;**20**:1342-1344

- [122] Kokufuta E, Wang B, Yoshida R, Khokhlov A, Hirata M. Volume phase transition of polyelectrolyte gels with different charge distributions. *Macromolecules*. 1998;**3**:6878-6884
- [123] Kazakov S, Kaholek M, Gazaryan I, Krasnikov B, Miller K, Levon K. Ion concentration of external solution as a characteristic of micro- and nanogel ionic reservoirs. *Journal of Physical Chemistry*. 2006;**110**:15107-15116
- [124] Ogawa Y, Ogawa K, Wang B, Kokufuta EA. Biochemo-mechanical system consisting of polyampholyte gels with coimmobilized glucose oxidase and urease. *Langmuir*. 2001;**17**:2670-2674
- [125] Ereemeev NL, Kukhtin AV, Kazanskaya NF. Enzyme-dependent responses of stimulus-sensitive systems. *Bio Systems*. 1998;**45**:141-149
- [126] Horkay F, Tasaki I, Basser PJ. Effect of monovalent-divalent cation exchange on the swelling of polyacrylate hydrogels in physiological salt solutions. *Biomacromolecules*. 2001;**2**:195-199
- [127] Horkay F, Tasaki I, Basser PJ. Osmotic pressure of polyacrylate hydrogels in physiological salt solutions. *Biomacromolecules*. 2000;**1**:84-90
- [128] Eichenbaum GM, Kiser PF, Shah D, Meuer WP, Needham D, Simon SA. Alkali earth metal binding properties of ionic microgels. *Macromolecules*. 2000;**33**:4087-4093
- [129] Amiya T, Tanaka T. Phase transitions in cross-linked gels of natural polymers. *Macromolecules*. 1987;**20**:1162-1164
- [130] Horkay F, Tasaki I, Basser PJ. Ion-induced volume transition in synthetic and biopolymer gels. *Polymer Preprints*. 2001;**42**:267-268
- [131] Iwasa K, Tasaki I. Mechanical changes in squid giant axons associated with production of action potentials. *Biochemical and Biophysical Research Communications*. 1980;**95**:1328-1331
- [132] Tasaki I, Iwasa K. Rapid pressure changes and surface displacements in the squid giant axon associated with production of action potentials. *Japanese Journal of Physiology*. 1982;**32**:69-81
- [133] Tasaki I, Nakaye T, Byrne PM. Rapid swelling of neurons during synaptic transmission in the bullfrog sympathetic ganglion. *Brain Research*. 1982;**331**:363-365
- [134] Tasaki I, Byrne PM. Large mechanical changes in the bullfrog olfactory bulb evoked by afferent fiber stimulation. *Brain Research*. 1988;**475**:173-176
- [135] Tasaki I, Byrne PM. Discontinuous volume transitions in ionic gels and their possible involvement in the nerve excitation process. *Biopolymers*. 1992;**32**:1019-1023
- [136] Tasaki I, Byrne PM. Discontinuous volume transitions induced by calcium-sodium ion exchange in anionic gels and their neurobiological implications. *Biopolymers*. 1994;**34**:209-215
- [137] Tasaki I. Rapid structural changes in nerve fibers and cells associated with their excitation processes. *Japanese Journal of Physiology*. 1999;**49**:125-138

- [138] Kokufuta E, Zhang YQ, Tanaka T, Mamada A. Effects of surfactants on the phase transition of poly(N-isopropylacrylamide) gel. *Macromolecules*. 1993;**26**:1053-1059
- [139] Khokhlov AR, Kramarenko EY, Makhaeva EE, Starodubtsev SG. Collapse of polyelectrolyte networks induced by their interaction with oppositely charged surfactants. *Macromolecules*. 1992;**25**:4779-4783
- [140] Gong JP, Osada Y. Theoretical analysis of the cross-linking effect on the polyelectrolyte-surfactant interaction. *Journal of Physical Chemistry*. 1995;**99**:10971-10975
- [141] Safran A, Yoshida M, Omichi H, Katakai R. Effect of surfactants on the volume phase transition of cross-linked poly(acryloyl-L-proline alkyl esters). *Langmuir*. 1994;**10**:2955-2959
- [142] Yoshida M, Asano M, Omichi H, Kamimura W, Kumakura M, Katakai R. Dependence of volume phase transition temperature of poly(acryloyl-L-proline methyl ester) gel on hydrophobic tail length of anionic surfactants. *Macromolecules*. 1997;**30**:2795-2796
- [143] Okuzaki H, Osada Y. Effects of hydrophobic interaction on the cooperative binding of a surfactant to a polymer network. *Macromolecules*. 1994;**27**:502-506
- [144] Murase Y, Onda T, Tsujii K, Tanaka T. Discontinuous binding of surfactants to polymer gel resulting from a volume phase transition. *Macromolecules*. 1999;**32**:8589-8594
- [145] Ogawa K, Ogawa Y, Kokufuta E. Effect of charge inhomogeneity of polyelectrolyte gels on their swelling behavior. *Colloids and Surfaces*. 2002;**209**:267-279
- [146] Mamada A, Tanaka T, Kungwatchakun D, Irie M. Photoinduced phase transition of gels. *Macromolecules*. 1990;**23**:1517-1519
- [147] Suzuki A, Tanaka T. Phase transitions in polymer gels induced by visible light. *Nature*. 1990;**346**:345-347
- [148] Juodkazis S, Mukai N, Wakaki R, Yamaguchi A, Matsuo S, Misawa H. Reversible phase transitions in polymer gels induced by radiation forces. *Nature*. 2000;**408**:178-181
- [149] Lendlein A, Jiang H, Junger O, Langer R. Light-induced shape-memory polymers. *Nature*. 2005;**434**:879-882
- [150] Kuhn W, Hargitay B, Katchalsky A, Eisenberg H. Reversible dilation and contraction by changing the state of ionization of high-polymer acid networks. *Nature*. 1950;**165**:514-516
- [151] Steiberg IZ, Oplatka H, Katchalsky A. Mechanochemical engines. *Nature*. 1966;**210**:568-571
- [152] Sussman MV, Katchalsky A. Mechanochemical turbine: A new power cycle. *Science*. 1970;**167**:45-47
- [153] Osada Y, Hasebe M. Electrically activated mechanochemical devices using polyelectrolyte gels. *Chemistry Letters*. 1985;**14**:1285-1288
- [154] DeRossi DE, Chiarelli P, Buzzigoli G, Domenci C, Lazzeri L. Contractile behaviour of electrically activated mechanochemical polymer actuators. *Transactions—American Society for Artificial Internal Organs*. 1986;**32**:157-162

- [155] Tanaka T, Nishio I, Sun ST, Nishio SU. Collapse of gels in an electric field. *Science*. 1982;**218**:467-469
- [156] Kishi R, Osada Y. Reversible volume change of microparticles in an electric field. *Journal of the Chemical Society, Faraday Transactions 1*. 1989;**85**:655-662
- [157] Ricklin D, Hajishengallis G, Yang K, et al. Complement: A key system for immune surveillance and homeostasis. *Nature Immunology*. 2010;**11**:785-797
- [158] Allen TM. A study of phospholipid interactions between high density lipoproteins and small unilamellar vesicles. *Biochimica et Biophysica Acta*. 1981;**640**:385-397
- [159] Frank MM. The reticuloendothelial system and bloodstream clearance. *Journal of Laboratory and Clinical Medicine*. 1993;**122**:487-488
- [160] Scherphof GL, Kamps JA. The role of hepatocytes in the clearance of liposomes from the blood circulation. *Progress in Lipid Research*. 2001;**40**:149-166
- [161] Ishida T, Harashima H, Kiwada H. Liposome clearance. *Bioscience Reports*. 2002;**22**:197-224
- [162] Immordino ML, Dosio F, Cattel L. Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential. *International Journal of Nanomedicine*. 2006;**1**:297-315
- [163] Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. *Urologic Oncology*. 2008;**26**:57-64
- [164] Nagy JA, Chang S-H, Dvorak AM, Dvorak HF. Why are tumour blood vessels abnormal and why is it important to know? *British Journal of Cancer*. 2009;**100**:865-869
- [165] Iwai K, Maeda H, Konno T. Use of oily contrast medium for selective drug targeting to tumor: Enhanced therapeutic effect and X-ray image. *Cancer Research*. 1984;**44**:2115-2121
- [166] Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent SMANCS. *Cancer Research*. 1986;**46**:6387-6392
- [167] Noguchi Y, Wu J, Duncun R, et al. Early phase tumor accumulation of macromolecules: A great difference in clearance rate between tumor and normal tissues. *Japanese Journal of Cancer Research*. 1998;**89**:307-314
- [168] Sahay G, Alakhova DY, Kabanov AV. Endocytosis of nanomedicines. *Journal of Controlled Release*. 2010;**145**:182-195
- [169] Iversen TG, Skotland T, Sandvig K. Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. *Nano Today*. 2011;**6**:176-185
- [170] Düzgüneş N, Faneca H, Pedroso de Lima MC. Methods to monitor liposome fusion, permeability, and interaction with cells. *Methods in Molecular Biology*. 2010;**606**:209-232

- [171] Kisak ET, Coldren B, Evans CA, Boyer C, Zasadzinski JA. The vesosomes—A multi-compartment drug delivery vesicle. *Current Medicinal Chemistry*. 2004;**11**:199-219
- [172] Paleos CM, Tsiourvas D, Sideratou Z, Pantos A. Formation of artificial multicompart-ment vesosome and dendrosome as prospected drug and gene delivery carriers. *Journal of Controlled Release*. 2013;**170**:141-152
- [173] Frank P, Siebenhofer B, Hanzer T, et al. Proteo-lipobeads for the oriented encapsulation of membrane proteins. *Soft Matter*. 2015;**11**:2906-2908
- [174] Geiss AF, Khandelwal R, Baurecht D, Bliem C, Reiner-Rozman C, Boersch M, Ullmann GM, Loew LM, Naumann RLC. pH and potential transients of the bc1 complex co-reconstituted in proteo-lipobeads with the reaction center from Rb. *Sphaeroides*. *Journal of Physical Chemistry B*. 2017;**121**:43-152
- [175] MacMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. *Nature*. 2005;**438**:590-596
- [176] Walde P, Ichikawa S. Enzymes inside lipid vesicle: Preparation, reactivity and applica-tions. *Biomolecular Engineering*. 2001;**18**:143-177
- [177] Kukhtina AV, Ereemeev NI, Belyaeva EA, Kazanskaya NF. Relationship between state of a termosensitive matrix and the activity of urease immobilized in it. *Biokhimiya*. 1997;**62**:437-443
- [178] Park TG. Stabilization of enzyme immobilized in temperature sensitive hydrogels. *Biotechnology Letters*. 1993;**15**:57-60
- [179] Ereemeev NL, Kazanskaya NF. Relationship between the hydration degree of poly-N-isopropylacrylamide gel and activity of immobilized α -chymotrypsin. *Russian Chemical Bulletin*. 2001;**50**:1891-1895
- [180] Kazakov S, Hosein H. Electrochemical mechanics of nanometer-scaled structural layers of bacterial spores. *NSTI-Nanotech*. 2010;**3**:486-489

IntechOpen