Lipobeads as Drug Delivery Systems

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ABSTRACT

A relatively new type of nanoparticles – lipobeads – a liposome-hydrogel assembly is a novel drug delivery system. Due to their bi-compartmental structure, lipobeads are of great potential for application as drug carriers with high therapeutic efficiency. It was shown that aggregation of lipobeads and its reversibility can be controlled by the hydrophobic modification of nanogels and their thermal or ionic collapsing within lipobeads. The discovered reversible and irreversible aggregation was proposed as a key step for designing two types of the so-called combined drug delivery systems. Their novelty and advantages were discussed.

Keywords: liposomes, nanogels, supramolecular assembly, lipobeads, drug delivery systems.

1 INTRODUCTION

Nowadays, when drug has to be delivered to the right site in the right concentration at the right time, progress in medical sciences, to the great extent, depends on the development of all classes of drug delivery systems: permeation, controlled release, targeting, and sensing. Many delivery systems such as polymers, nanoparticles, microspheres, micelles, liposomes have been applied to prolong the circulation time of certain molecules, to deliver them to the appropriate sites, and to protect them from degradation in the plasma [1-7].

Hydrogel-liposome assemblies, named lipobeads, can be considered as a new type of nanocapsules. An appropriate assembly of a lipid bilayer on a spherical hydrogel surface combines biocompatible surface properties of liposomes with the mechanical stability and a greater loading capacity of polymer network. This combination broadens the potential of nanocapsules for pharmaceutical applications, biomimetic sensory systems, controlled release devices, multivalent receptors and so on. Thus, a bi-compartmental structure of the lipobeads may serve as a container for loading various agents such as drugs, fluorescent dyes, or other molecules, and as a functionalization site for the attachment of various ligands depending on the desired applications.

Here we discuss two ways of lipobeads preparation: (i) synthesis of hydrogel within liposomal interior, and (ii) spontaneous formation of lipid layers around hydrogels after nanogel-liposome mixing, and their properties as drug carriers.

2 STRATEGY OF LIPOBEADS PREPARATION

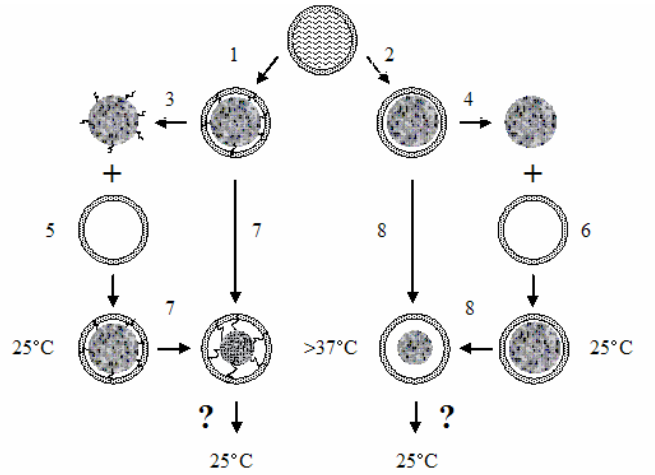

Figure 1: The strategy of liposome-hydrogel structures preparation: gelation within liposomes – formation of anchored (1) and non-anchored (2) lipobeads; nanogel preparation of anchored (3) and non-anchored (4) nanogels; spontaneous formation of lipid bilayers around hydrogel after nanogel-liposome mixing (second way of lipobeads formation); temperature induced collapse of anchored (7) and non-anchored (8) nanogels.

Figure 1 shows our strategy of liposome/hydrogel coupling in a spherical configuration. Processes (1) and (2) employ the liposomal interior as a microreactor to provide polymerization reaction inside and to form the so called lipobead. In process (1), hydrophobic anchors co-polymerize on the nanogel surface. Processes (3) and (4) illustrate preparation of hydrogel nanoparticles by removing lipid bilayer. Process (3) results in hydrophobically modified nanogels. Processes (5) and (6) show the spontaneous formation of lipid bilayers around hydrogel after nanogel-liposome mixing (the second way of lipobeads preparation). It is a well known fact that some polymer gels can swell or shrink discontinuously and reversibly in response to many different stimuli (temperature [8], pH [9], ions [10-13], electric fields [14] and light [15]) depending on the chemical composition of a gel/solvent system. Processes (7) and (8) illustrate the collapsing of a nanogel core inside a lipobead as a response to the temperature change. Our goal, pointed out by question marks, is to study how the swelling/de-swelling of

anchored and non-anchored nanogels inside lipobeads affects their properties.

3 EXPERIMENTAL SECTION

Materials. N-Isopropylacrylamide (NIPA) and 1-vinylimidazole (VI) monomers and N,N'-methylenebisacrylamide (MBA) were purchased from Aldrich (Milwaukee, WI). Chloroform solution of L-α-phosphatidylcholine from egg yolk (EPC, transition temperature Tm = –2°C, Mw = 760 g/mol) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). 2,2-Diethoxyacetophenone (DEAP) and nonionic detergent Triton X-100 (TX-100) were purchased from Fluka (Milwaukee) and Eastman Kodak (Rochester), respectively. Chemicals were used directly without additional purification. Water was purified by Milli-Q (Millipore). 50 mM Tris-HCl buffer (pH 7.5) was used in the experiments.

Preparation of lipobeads and nanogels, using liposomes as microreactors, includes several steps described elsewhere [16]. To prepare liposomes containing a pre-gel medium, a needed volume of EPC/chloroform solution with a phospholipid concentration of 20 mg/ml was poured into a round-bottom flask. To form a lipid film, the chloroform was evaporated under flushing with nitrogen. The film was held under vacuum for at least 3 more hours and then hydrated by dispersing in a mixture of monomers (5.5 wt.% of NIPA, 2 wt.% of VI), a cross-linker (0.5 wt.% of MBA), and a photoinitiator (0.1 wt.% of DEAP) in distilled water. The final concentration of EPC was chosen to be 5 mg/mL. After sonication of the multilamellar vesicles (MLV) suspension, the resultant suspension was diluted 25-fold with distilled water to prevent polymerization in the solution exterior to the liposomes. Further UV exposure of the solution initiated free radical polymerization, yielding lipobeads, i.e. hydrogel particle resides within the spherical phospholipid bilayer. The polymerization temperature (20°C) was below the lower critical volume phase transition temperature for poly(*N*-isopropylacrylamide-*co*-1-vinylimidazole (PNIPA-VI) hydrogel (T_V~37°C). To obtain bare nanogels, the phospholipid was removed with 15 mM T_{X-100}. The formed phospholipid/detergent micelles were removed by dialysis in SpectraPore 50 kDa membrane bags against water.

The experimental procedure for lipobeads and nanogels with hydrophobic anchors attached to the surface of hydrogel core was essentially the same as the preparation of unanchored nanogels except that the *N*-(n-octadecyl)acrylamide (ODAm) was dissolved in the initial phospholipid/chloroform solution with the mole ratio of phospholipid to ODAm equal to 70:1.

Instrumentation. For imaging the synthesized lipobeads and nanogels, the atomic force microscopy (AFM) was used (Multimode NanoScope IIIa, Digital Instruments, Santa Barbara, CA). The size of liposomes, lipobeads, and hydrogel nanoparticles was examined by Dynamic Light Scattering (DLS) technique using a N4 Plus particle size analyzer (Beckman-Coulter, Fullerton, CA)

4 RESULTS

4.1 Properties of Nanogels

Once extracted from liposomes, the hydrogel spherical particles, referred as nanogels, have strong compatibility with phospholipid bilayer: the phospholipids are self-assembling around the nanogels upon mixing with liposomes as detected by DLS and AFM. Although the spontaneous formation of lipid layers around hydrogels has been confirmed experimentally [17,18], the mechanism of phospholipids self-assembling around hydrogel particles and the layered structure is still a question to be answered. Nevertheless, it is not surprising that lipid bilayer tends to form a membrane on the surface of nanogels. This phenomenon reflects the tendency of the system comprising two objects of different hydrophilicity to minimize the thermodynamic potential by hiding the more hydrophobic components. This finding experimentally justifies the other method for lipobeads preparation: nanogel-liposome incubation.

As revealed by DLS [16], PNIPA-VI nanogels were temperature and pH sensitive: their volume decreased by ~8 times when temperature changed from 25°C to 40°C and increased by ~6 times as pH changes from 7 to 4.5 at the fixed temperature (25°C). Given these properties, the prepared nanogels themselves can be considered as containers for drugs and vehicles capable of targeting specific sites within the body. Herein, the trigger mechanisms of drug release will depend on chemical composition of the polymer network and its sensitivity to the environment.

Moreover, DLS analysis showed [16] that thermally collapsed nanogels preferred to aggregate. Despite aggregation, the solution remained stable and transparent, indicating that the aggregates were still colloidally stable at least in the time scale of interest. It seems reasonable to assume that the surface of the shrunken hydrogel particles is more hydrophobic than the swollen ones. Aggregation reduces the total hydrophobicity by decreasing the total surface of the particles. The fine balance of the van der Waals forces and the electrostatic forces determines the resultant size of the aggregates.

Striking ability of ionically swollen nanogels to merge and flatten on the planar surface was observed by AFM [18]. At pH 6.5 the PNIPA-VI hydrogel particles deposited on the mica surface had the shape of spherical caps of a 100-120 nm diameter. At pH 3.0, when nanogels were in swollen state, the flattening was more significant and the swollen particles exhibited the shape of spherical zone with an average diameter of 300-360 nm. What was unexpected, the swollen nanogels formed dimers which after 2 h incubation on mica finally merge into a homogeneous spots of a uniform nanometer thickness of *ca.* 15 nm. The

observed ability of swollen nanogels to form films with uniform thickness on the nanometer scale is promising for designing planar drug delivery systems like iontophoretic patches [19].

Nanogels merging in swollen state as well as aggregating in shrunken state may facilitate biochemical reactions on micro- and nanometer scales. One can imagine that if two nanoparticles loaded with two different reagents are brought into contact, a reaction between the compounds will begin. In the context of drug delivery, different drugs, which are not allowed to contact before they reach the targeted specific site within the body, can be loaded into different nanogels.

4.2 Properties of Lipobeads

Recently, it has been found [16] that the volume changes of nanogels trapped within stable liposomes could not be detected when PNIPA-VI nanogels were forced to contract at T > ~37°C or to swell/shrink in the pH range from 7 to 2.

In this work, the attention is focused on how the collapse of nanogels within lipobeads effects on the behavior of the lipobeads.

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. Interestingly, the further cooling back to 25°C restored the original unimodal size distribution of lipobeads, indicating reversibility of anchored lipobeads aggregation. Figure 2 sketches the behavior of lipobeads resulted from the anchored hydrogel collapsing.

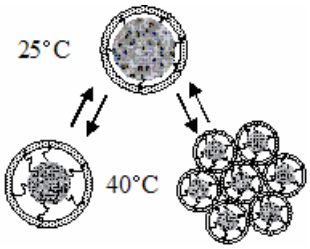

Figure 2: Schematic presentation of the anchored lipobeads and their aggregation in the course of nanogels shrinking. They reversibly disaggregate when nanogels swell back.

Two conclusions were derived from these observations. First, the collapse of nanogels even hidden within lipid bilayer results in lipobeads aggregation. Presumably, the aggregation reduces the hydrophobic/hydrophilic imbalance caused by collapsed nanogels within lipobeads. Second, the reversible dissociation of the lipobead aggregates may evidence that anchored lipobeads do not fuse. The only explanation is in that the hydrophobic chains of anchored PNIPA-VI nanogels penetrate into the lipid bilayer and stabilize the liposomal membrane against fusion. To confirm this point, a similar experiment was performed for non-anchored lipobeads.

Lipobeads with non-modified nanogels. In order to estimate the effect of hydrophobic chains on stabilization of the lipid bilayer, the lipobeads containing nanogels without hydrophobic modification of their surface were synthesized. In contrast to the hydrophobically modified lipobeads, the unimodal size distribution of unanchored lipobeads was recorded by DLS at 40°C. The single peak was significantly shifted towards a greater average diameter than that at 25°C. This observation indicated that a more pronounced aggregation occurred in this case. Furthermore, 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 confirms that the aggregation of lipobeads at elevated temperatures appeared to be irreversible because of their fusion into the "giant" lipobeads with structure (2) sketched in Figure 3.

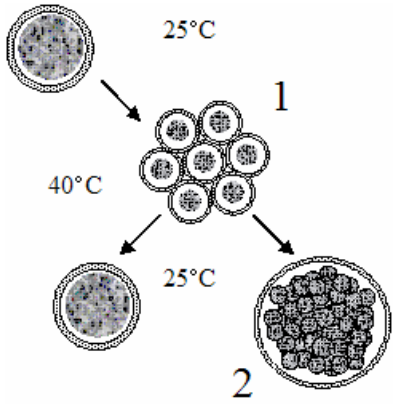

Figure 3: Schematic presentation of the non-anchored lipobeads and their aggregation (1) when nanogels shrink. In the course of the liposomal membrane fusion, the collapsed nanogels aggregate to form a giant lipobead (2). The process is irreversible.

Thus, the formation of giant lipobeads consisting of the aggregates of collapsed nanogels within the single closed lipid membrane results from two processes: (i) aggregation of lipobeads as a result of nanogel collapsing at elevated temperature, and (ii) fusion of the lipid bilayers.

5 SUMMARY AND CONCLUSIONS

Bi-compartmental structure of lipobeads makes them attractive as stable and biocompatible drug delivery containers. The novel, so-called combined drug delivery systems were proposed to design using reversible and irreversible aggregation of lipobeads. Herein, the contraction and hydrophobic modification of nanogels are the factors discovered to control those types of aggregation.

Reversible and irreversible aggregation of lipobeads could be a key step for designing two types of combined multifunctional containers: (1) different drugs entrapped in different lipobeads can be simultaneously delivered as one aggregate to the targeted organs in the body and released in desired order; (2) several nanogels loaded with different pre-drug reagents are trapped under the one lipid membrane ("giant lipobeads") to react inside without damaging surrounding organs and to be delivered to the targeted site in one "giant" container able to release final product controllably.

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