Summary: A novel comb-like poly(ethylene glycol) (CPEG), with dominant water-soluble PEG, is found to spontaneously aggregate into vesicles above a certain concentration in water. The hollow, three-dimensional structure of the vesicles is proven by TEM, SEM, and AFM. The diameters of the vesicles are from 200 to 500 nm with 50 nm walls. The spontaneously formed vesicles can be further cross-linked by the reaction between divinyl sulfone (DVS) and the hydroxy groups in the side chains of the CPEG. The spontaneously formed vesicles with dense reactive hydroxy groups will have great potential in both applications and research.

SEM image of the uncross-linked vesicles.

Spontaneous Vesicle Formation in Aqueous Solutions of Comb-Like PEGa

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Introduction

Vesicles have attracted much attention in the fields of biology, chemistry, and physics and have numerous practical applications in various branches of technology owing to their hollow and lamellar spherical structure. Of all applications, the delivery–release of various substances (e.g., drugs and active substances) could be the most potential. Vesicles are also becoming increasingly important in nanoreactors. The theoretical and experimental studies of vesicles are similarly fascinating. Recently, many theoretical studies have been devoted to developing models of their formation and physicochemical properties. In the experiment field, many scientific activities are focusing on optimising the systems to achieve particular properties in order to obtain the best fitness for the foreseen applications.

Amphiphilic copolymers can aggregate into vesicles depending on their structure (e.g., the chemical constitution and the relative length of the individual block) and the properties of the solution (e.g., concentration, pH, temperature and solvent). Many types of amphiphilic copolymers, for example, copolymers including the poly(ethylene glycol) (PEG)/poly(ethylene oxide) (PEO) block copolymers and graft copolymers composed of a hydrophilic backbone and hydrophobic side chains, have been reported to aggregate into vesicles. Usually, if the corona block in all types of aggregates is very long, the aggregates are referred to as star-like, such as micelles. Whereas if the corona block is shorter than the size of the aggregated core, they are called crew-cut aggregates, such as vesicles. Essentially, the vesicles result from a balance of the interfacial energy between the core and the outside...
solution, the stretching of the core-forming blocks, and the repulsive interactions among corona chains. If the hydrophilic block is longer than that of the hydrophobic block of the block copolymer, a question arises as to whether the block copolymers will aggregate in water to form vesicles with a special structure.

In this research, the aggregation of a hydroxy-capped comb-like poly(poly(ethylene glycol) methacrylate) (CPEG), which consists of a hydrophobic methacrylate backbone and -OCH\textsubscript{3} end-group, and dense hydrophilic PEG chains end-capped by hydroxy groups, is investigated by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM). It is very interesting to find that the CPEG with dominant water-soluble PEG actually spontaneously aggregates into vesicles above a certain concentration. The hydroxy groups on the corona of the vesicles could be further cross-linked by the reaction with divinyl sulfone (DVS), which indicates the reactivity of the hydroxy groups. To the best of our knowledge, this is the first report that a comb-like PEG with dense hydroxy end-capped PEG side chains can spontaneously aggregate into vesicles in water.

**Experimental Part**

**Materials**

The comb-like poly(ethylene glycol) (CPEG) used in this study is PEG2000-\textit{b}(PEGMA)\textsubscript{37} with $M_n = 16000$ and $M_w/M_n = 1.20$. The copolymer was synthesized by atom transfer radical polymerization (ATRP) of $\alpha$-methylacryloyl-$\omega$-hydroxy-poly(ethylene glycol) ($M_n = 365$, Aldrich) by using $\alpha$-methyl-$\omega$-2-bromoisobutyl poly(ethylene glycol) (PEG2000-Br) as initiator. A description of the detailed synthesis can be found in a previous paper and in the supporting information for this paper. A schematic structure of CPEG is shown in Figure 1.

**Preparation of the CPEG Solution**

The CPEG was first dissolved with a good solvent, tetrahydrofuran (THF). The THF solution of CPEG was filtered using a membrane with 0.22 μm apertures into a flask. After the solvent was evaporated, a certain amount of redistilled deionized water was dropped into the flask. Gentle shaking was applied to the solution in order to promote the dissolution of CPEG and homogenize the total solution.

**The Cross-Linking of CPEG Vesicles**

The CPEG solution was cross-linked by adding DVS dropwise with stirring, and the solution was left stirring for 12 h at ambient temperature. An excess amount of DVS was added into the CPEG solution because of side reactions. The target degree of cross-linking was $2 \times [\text{DVS}] / [\text{hydroxy group}] \times 100\%$.

**TEM**

A CPEG solution was dropped onto a carbon-coated grid, washed with a negative stain solution (2% uranyl acetate solution), and blotted with a filter paper. The specimens were observed with a transmission electron microscope (JEM 1230, JEOL).

**SEM**

The CPEG solution was dropped onto freshly cleaved mica. Then the sample was dried under an infrared lamp. After being coated with gold, the sample was observed using a scanning electron microscope (Sirion-100, FEI).

**Particle Size Distribution Analyzer**

The average diameter and size distribution of the vesicles were measured using a laser particle-size analyzing system (Brookhaven 90 plus, Brookhaven Instruments Corporation) over a temperature range of 10–70 °C at 5 °C intervals.

**AFM**

The CPEG solution was uniformly dip-coated onto freshly cleaved mica. The sample was then transferred into a desiccator and left for at least 24 h. The sample was scanned using the tapping mode with an atomic force microscope (SPI3800N, Seiko Instruments Inc.).

**Result and Discussion**

**The Characterization of Aggregate Morphology**

During the preparation of the CPEG solution, only a gentle shake is applied in order to homogenize the solution. A series of CPEG solutions of different concentrations are studied. It is not surprising to find the hydrophilic-PEG-dominated amphiphilic copolymer can self-aggregate into a micellar structure. The critical micelle concentration (CMC) of CPEG is $5.76 \times 10^{-3}$ g·L\textsuperscript{-1}, as determined using a fluorescence spectrometer. However, it is really interesting to find that it will spontaneously aggregate into vesicles at or above $1.13 \times 10^{-2}$ g·L\textsuperscript{-1}.  

Figure 1. The schematic structure of comb-like PEG (CPEG).
A representative TEM image for a $1.13 \times 10^{-2}$ g·L$^{-1}$ CPEG solution is presented in Figure 2(a). The hollow structure of the vesicles can be deduced from the increased blackness caused by the staining agent. The agent leads to lower transmission in the central areas of the aggregates compared with their less black periphery and outer region, which confirms that they are hollow.[24,26,29] The single, less black ring around the aggregate proves that the CPEG aggregates are lamellar, and the thickness of the lamellar wall is around 50 nm. All aggregates show a large dark center, which indicates that the diameter is indeed larger than the film thickness. In addition, the edges of the CPEG aggregates appear irregular because of shrinkage that arises as a result of evaporation of the water in the hollow structure. The TEM image shows that the radii of the aggregates are in the range of 200–500 nm.

The vesicles have been further studied by SEM, the results of which are shown in Figure 2(b). There is a remarkable contrast between the central and the edge parts of the aggregates in the SEM image, which exhibits a concave central area[30] in the aggregates. It is possible that the evaporation of the inner water of the aggregates leads to the
collapse of the central area. The diameter of the vesicles is in the range of 200–400 nm, which is very close to the TEM results. The wall is about ~100 nm, which is about twice that of the radius determined by TEM because of collapse of the aggregates to form a double wall. In the SEM image, the small spherical particles are believed to be micelles, which are determined by their radius.

An AFM topology picture of the CPEG aggregates is shown in Figure 2(c). The central areas of the aggregates appear darker than the edges, which indicates that the central area is lower than the edges. This indicates that the central area is hollow as a result of the collapse. From the height of the CPEG aggregates, it can be concluded that the aggregates are not two-dimensional plates but three-dimensional spheres. The result is consistent with those of TEM and SEM.

Based on the above results, the aggregates appear to be vesicles with hollow, lamellar, three-dimensional spheres.

Cross-Linking of Vesicles by Hydroxy Groups

In view of the foreseen application of mimicking the cell membrane, the surface functionalization of vesicles is extremely important. Here, the availability of the vesicles functionalization is proven by the cross-linking reaction between divinyl sulfone and hydroxy groups in the side chains of CPEG. Evidence for the cross-linking is provided by the S 2p peak in the XPS spectra, which is attributable to the sulfur originating from the divinyl sulfone. The TEM image of cross-linked vesicles (Figure 2(d)) provides further evidence for the cross-linking reaction as the edges have become more regular and smooth and the diameter of the vesicles has decreased after addition of the cross-linker. As seen, the cross-linker did not destroy the vesicular structure. In Figure 3(a), the cross-linker has led to the movement of the vesicles peak from 1 000 to 850 nm. In addition, in Figure 3(b) the results of diameter vs. temperature show that the cross-linker decreases the diameter and polydispersity of the aggregates. The fluctuation of the diameter of the aggregates became smaller, and from 50–70 °C the diameter tended towards a constant after the addition of cross-linker. All the results indicate that the cross-linking reaction is successful, which in turn reveals that the hydroxy groups of the outer surface of the vesicles indeed have reactivity. The cross-linking of the vesicles could realize an enhanced stability and a regulated permeability of the vesicles.

In order to mimic, on a very simple level, the complex molecular recognition and signal transition functions of a cell membrane, receptor ligands are often applied to the design of synthetic membranes. The spontaneous formation of vesicles with reactive hydroxy groups in this research could potentially be used to conjugate a bioactive receptor to the surfaces of the vesicles in order to mimic the function of a cell membrane. So the CPEG vesicles will have great potential in both application and research.

Conclusion

The aggregative behavior of a novel comb-like poly(ethylene glycol) with hydroxy groups in the CPEG side chain is investigated in water. It is very interesting to find that the dominant PEG amphiphilic copolymer CPEG actually spontaneously aggregates into vesicles above a certain concentration in water. The vesicular structure is proven by TEM, SEM, and AFM. The diameter of the vesicles is 200–500 nm with ~50 nm walls (determined by TEM). The vesicles could be further cross-linked by the reaction between divinyl sulfone (DVS) and the hydroxy groups in the side chains of CPEG, which has been confirmed by XPS, TEM, and particle size distribution. So the reactive hydroxy groups of the spontaneously formed vesicles could potentially be used to conjugate a bioactive receptor to the surfaces of the vesicles to mimic the function of a cell membrane.

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