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Short communication

Reversible pH-responsive aggregates based on the self-assembly of functionalized POSS and hyaluronic acid

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ABSTRACT

We report here the self-assembly of reversible pH intelligent aggregate from natural polysaccharide hyaluronic acid and functionalized polyhedral oligomeric silsesquioxane with tertiary amines and hydroxyls (POSS-AH). Various forms such as micelles, sacs and membranes were self-assembled through different procedures in this organic/inorganic hybrid system. The complex can be manipulated simply by changing pH to produce hydrogen-bond and ionic interaction. Furthermore the aggregates can form at a wide pH range by adjusting the proportion of the two constituents. These pH-responsive composites will offer potential opportunities in many fields, including cell immune barriers, drug and gene delivery systems and chemical sensors.

1. Introduction

The design and fabrication of organic/inorganic hybrid materials composed of organic polymers and inorganic nanoparticles has recently attracted much interest because of the tailored physical and chemical properties (Agrawal, Gupta, & Stamm, 2011). Polyhedral oligomeric silsesquioxane (POSS) is one of the molecules in the silsesquioxane family which has a distinctive nanocage structure consisting of an inner inorganic framework of silicon and oxygen atoms and an outer shell of organic groups (R) (Kuo & Chang, 2011). They are highly symmetric molecules with a nanoscopic size feature of approximately 1.5 nm in diameter including the R groups and can be considered as one of the perfect organic/inorganic hybrid nanomaterials (Ghanbari, Cousins, & Seifalian, 2011). The unique structure and superior biocompatibility of POSS allow it to be used in several biomedical applications including drug delivery (Kaneshiro & Lu, 2009), biosensors (Zou, Yan, Song, Zhang, & Wu, 2007), biomedical devices (Ghanbari et al., 2010), and tissue engineering (Ghanbari, de Mel, & Seifalian, 2011).

In previous research, much attention has been focused on the preparation methods and the properties of POSS-containing hybrid polymers. Using POSS as a noncovalent modifier, a variety of 3D aggregates has been obtained from various nanoparticles such as palladium (Jeon, Lim, & Kim, 2009), magnetic (Gu, Faucher, & Zhu, 2012), and gold nanoparticles (Kuo, Wu, Lu, & Chang, 2009). Atom-transfer radical polymerization (ATRP) and click chemistry have been used to prepare POSS-containing hybrid polymers with different topological structures such as star-shaped, quatrefoil-shaped and tadpole-shaped hybrid polymers (Kuo et al., 2012; Zhang et al., 2011). However, there are still many other novel aspects worthy to be studied, especially the self-assembly behaviour of these hybrid polymers with novel functions. Self-assembly is a powerful synthetic approach to obtain dynamic structures exhibiting complex properties. Especially noncovalent interactions, such as hydrogen bonds, electrostatics and supramolecular interactions to prepare self-assembly materials have given great importance in the preparation of chemically defined surfaces and ordered materials (George et al., 2012).

In this contribution, we extend the study of the self-assembly of POSS-containing hybrid polymers towards natural polyelectrolyte. Using hyaluronic acid sodium (HA) as the primary structural component, we have developed novel intelligent micelles and membranes via reversible pH-induced complexation by adding functionalized POSS with tertiary amines and hydroxyls (POSS-AH). HA, a natural negatively charged macromolecule containing a disaccharide repeat unit of *N*-acetylglucosamine and glucuronic acid, plays an important role in many biological processes, including tissue hydration, proteoglycan organization in the extracellular matrix, and tissue repair (Collins & Birkinshaw, 2013). The aggregate via self-assembly of HA and POSS-AH can provide a novel polymer system with unique biocompatibility for various biomedical applications.

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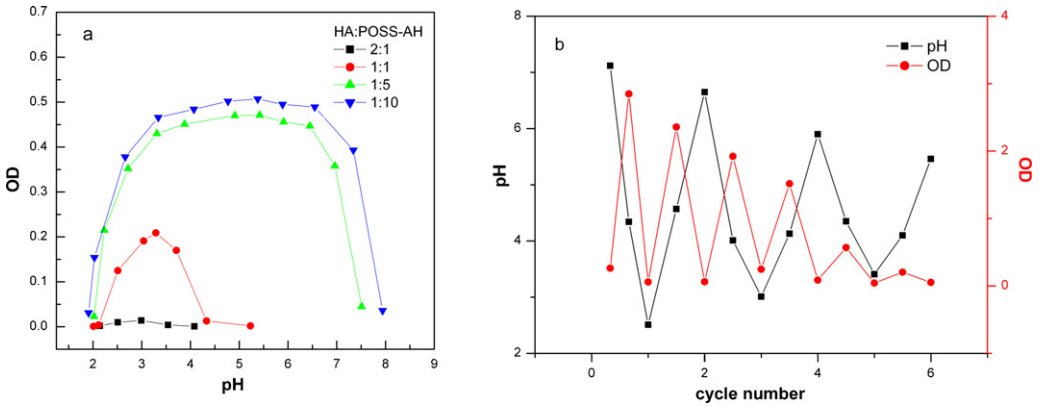

Fig. 1. (a) The optical density (OD) of the complexes under different pH with different proportion of HA and POSS-AH (the concentration of HA C_{HA} was fixed at 50 mg/L and $C_{POSS-AH}$ was different according to the proportion of the two constituents); (b) turbidity of HA/POSS-AH complexes under repeated process by adjusting pH (the concentration of the two constituents was fixed, C_{HA} = 4 g/L, $C_{POSS-AH}$ = 6 g/L, the pH is adjusted by addition of aqueous HCl (1 mol/L) and NaOH (1 mol/L) solutions).

2. Experimental

2.1. Materials

Sodium hyaluranic acid (Furuida Industries Co., Shandong province of China) was used without further purification. POSS-AH was prepared according to the procedure developed by Mori, Lanzendorfer, Muller, and Klee (2004). Briefly, the first step was the synthesis of *N*,*N*-di(2,3-dihydroxypropyl)-(aminopropyl) triethoxysilane. One mole of (aminopropyl) triethoxysilane was dropped slowly into two moles of glycidol under stirring with ice

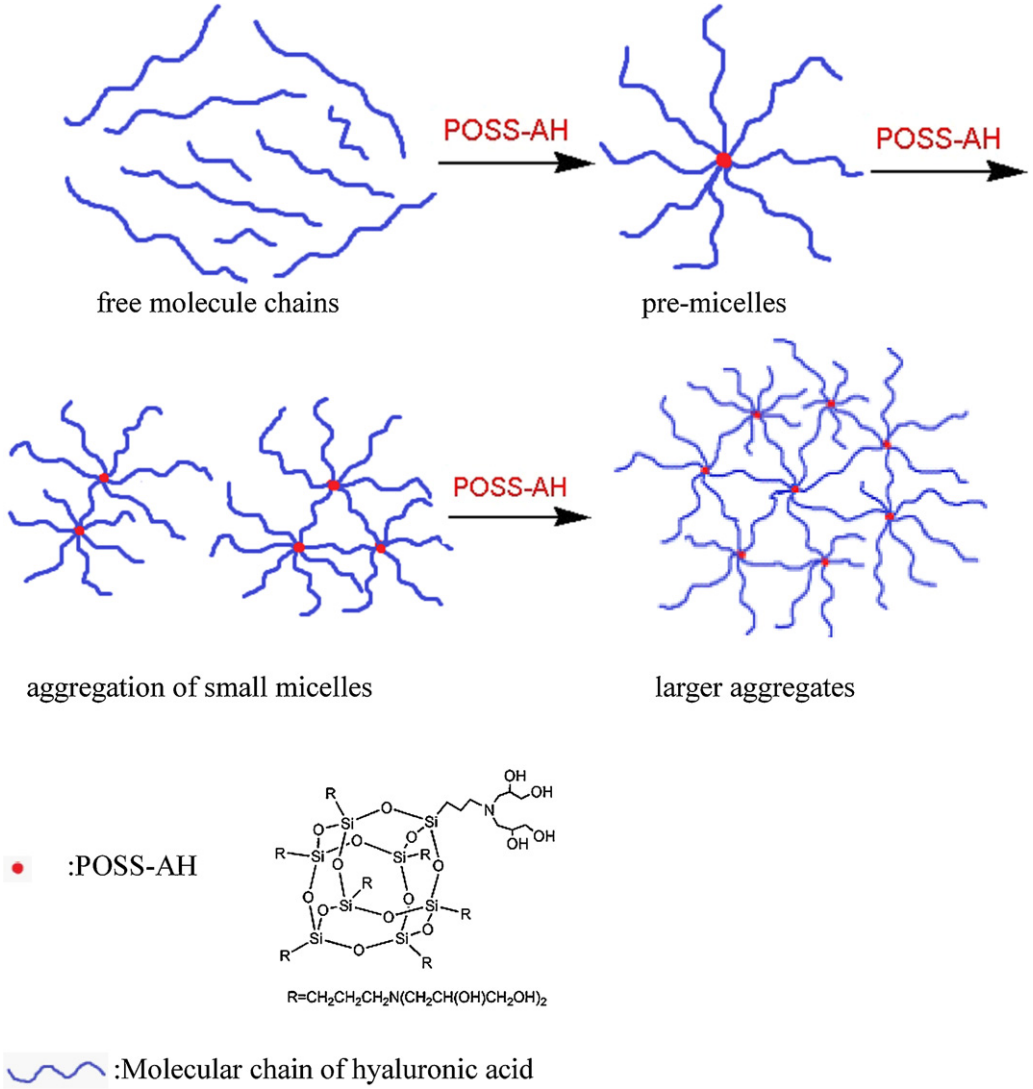

Fig. 2. Schematic illustration of the proposed model for the aggregates of HA and POSS-AH.

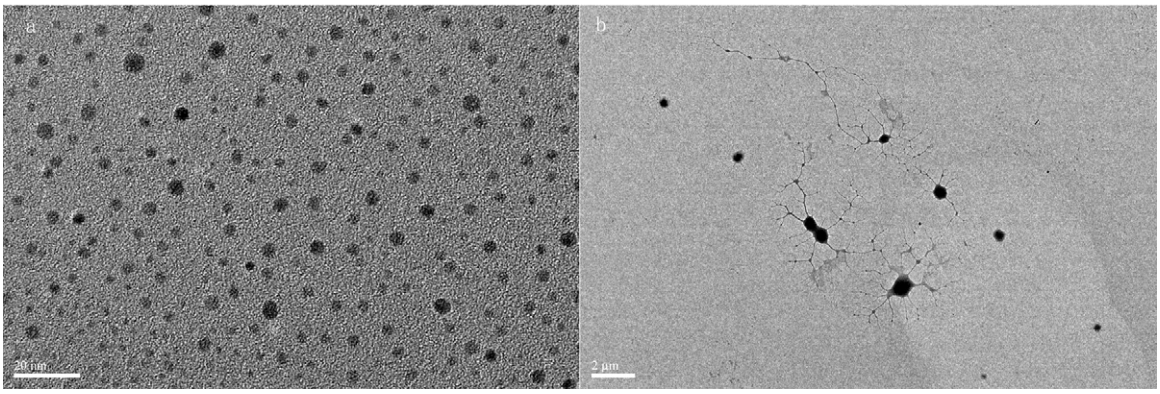

Fig. 3. TEM images of the self-assembly aggregations of HA and POSS-AH. (a) C_{HA} = 50 mg/L, $C_{POSS\text{-}AH}$ = 25 mg/L, pH 3.07; (b) C_{HA} = 50 mg/L, $C_{POSS\text{-}AH}$ = 50 mg/L, pH 3.82.

cooling and then reacted for 1 h at room temperature. The second step was the hydrolytic condensation of the product of step one in methanol with HF under stirring. After an additional reaction for 2 h under stirring at ambient temperature, the methanol, ethanol and water were removed through the vacuum drying method (40 °C, 8 mbar, about 12 h). The final product POSS-AH was obtained (yield about 80%). FT-IR spectra, ^{1}H and ^{13}C NMR spectra were recorded and the results can be found in Supporting information.

2.2. Instrumentation

The turbidity measurements of the complexation under different pH medium were carried out under 400 nm by using UV Spectrometer (Shimadzu, UV-2550, Japan). The HA and POSS-AH were dissolved in deionized water and the pH of the solutions was adjusted with HCl and NaOH aqueous solutions.

The *Z*-average size and size distribution of the complex were measured by Laser particle size analyzer (Marvin, England). Each sample was measured for 3 times.

The morphology of the aggregates was observed by transmission electron microscopy (TEM, JEOL 2100F). Samples were prepared by dripping the aggregates emulsion on the carbon-coated Cu grid, and then dried in air under infrared lamp for several minutes.

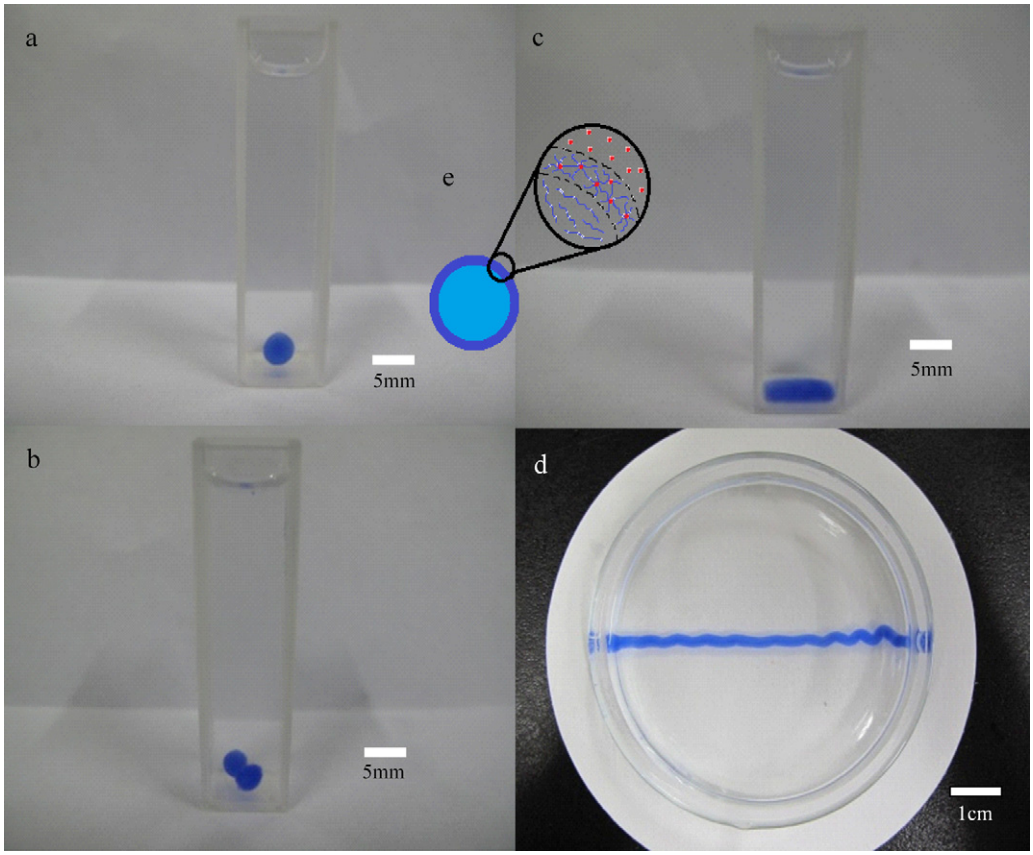

Fig. 4. (a) Closed sac formed by dropping HA solution (1.5 wt%, coloured by inert blue ink) into a POSS-AH solution (2.0 wt%, pH ≈ 4.5); (b) the closed sac cut from the middle into two parts, it can become two sacs after about 1 min of its self-sealing; (c) the dissociated sac after adding HCl (1 M) to regulate the pH value (pH≈3). (d) Continuous string formed by injecting HA solution (1.5 wt%, coloured by inert blue ink) into a POSS-AH solution (2.0 wt%, pH ≈ 4.5); (e) schematic structure of the closed sac by self-assembly.

3. Results and discussion

Both HA and POSS-AH aqueous solution are visually transparent, while the complexation leads to a white turbid dispersion under a certain pH range. Fig. 1a shows the turbidity of the complexation under different pH medium. As HA is mixed with POSS-AH in weak alkaline water the solution is transparent. By adding HCl to regulate the pH, the solution turns milky white and there are some small particles visible to naked eyes, which may be large aggregates of the micelles. Further addition of HCl to the dispersion leads to a transparent solution again. Such transformations of transparence and turbidity are observed reversibly and can be performed repeatedly by adding HCl and NaOH (Fig. 1b). After six repeated cycles the solution could not be turbid ever by further adjusting pH. This can be attributed to the increasing ionic strength in the aqueous medium.

The experimental results suggest there are at least two different equilibrium states existed during the complexation. While adding a little amount of HCl, HA is partially ionized and combined with POSS-AH mainly by hydrogen-bond. The system becomes translucent. By continuously adding HCl, the pH decreases, wherein the combination is governed mainly by the ionic interactions between the constituents and then the turbidity reaches maximum. And as the pH continues to decrease, the nitrogen atoms are protonated by HCl instead of carboxylic acid in HA, leading to the dissociation of the ionic complex and the solution becomes transparent again. Mori et al., have observed the similar self-assembly micelles formed by PAA and silica nanoparticles (Mori, Muller, & Klee, 2003), proved that the specific intermolecular interactions of hydrogen bondings and oppositely charged ionic interactions can form the intelligent colloids.

The pH range of the complexation formed by HA and POSS-AH can be controlled by varying the proportions of the two components (Fig. 1a). We find that the initial pH of the four curves is all around 2, while the values for end point are quite different, from about 3.5 to about 8. And the pH of the peak value generally increases as the concentration of POSS-AH increases. Presumably as the concentration of POSS-AH increases, the hydrogen-bonding interactions between HA and POSS-AH increase and the totally ionization of HA occur in the higher pH medium.

Laser particle size analyzer (LPSA) is used to measure the *Z*-average size and size distribution of the complex. Here we just choose the samples near the peak of the pH value in Fig. 1a to measure. With increasing of the POSS-AH concentration, the *Z*-average sizes of the four samples are 204.7 nm, 1650 nm, 687.8 nm, and 28,200 nm, respectively. In this system POSS-AH works as a multifunctional cross-linker and physically crosslink the HA chains in the aqueous solution to form micelles. As the concentration of the crosslink agent increases in the initial stage, the HA macromolecule chains are cross-linked by POSS-AH, and the size of the micelles increases. When the cross-linker concentration reaching a certain value, the continually increases cross-linker leads to more number micelles with relatively small size. The further increase in the concentration of POSS-AH will lead to the large size aggregates. The proposed mechanism for the self-assembly of HA and POSS-AH is shown in Fig. 2. The self-assembly mechanism is also confirmed by TEM as shown in Fig. 3. The aggregation structures of small micelles (Fig. 3a) and micelles aggregates (Fig. 3b) are visible obviously.

The interaction between HA and POSS-AH can also form solid membrane localized at the interface between the two liquid phase. If the denser HA solution is dropped into the POSS-AH solution, closed sacs formed instantly (Fig. 4a). Fig. 4e represents the schematic structure of the closed sac. And the sacs have the capability to self-seal a large defect instantly by triggering additional self-assembly with immersing the defect hole in the POSS-AH solution. Because the HA component was contained within the sac, application of additional POSS-AH solution created a new membrane segment with edges sufficiently sealed to the original sac membrane to prevent leakage of the fluid inside. Moreover, in the repaired region the new self sealing membrane was matching the appearance of the original sac. Even when we cut the sac into two parts, it became two smaller sacs after about 1 min for the self-sealing (Fig. 4b). We also observed that the noodle-like strings of arbitrary length could be formed by manually injecting the HA aqueous solution into POSS-AH solution (Fig. 4d). The diameter of the spheres and strings can be controlled by the size of the injection needles. In addition, the microspheres and viscoelastic strings can also sense the signal of pH and be dissociated in low or high pH area (Fig. 4c).

4. Conclusion

In conclusion, natural material hyaluronic acid and functionalized POSS-AH can form pH-induced aggregates. The nitrogen atoms provide ionic complexation and the hydroxyl provides hydrogen-bonding interactions with HA. With the pH changes, the shape, size and structure of the aggregates can be changed. And the pH range for the colloid forming can be controlled easily by the ratio of the two components. In comparison to other intelligent colloidal particles, it offers a number of crucial advantages such as good biocompatibilities, simple synthesis, easy functionalization and a possibility of tuning the particle and membrane dimensions from few nanometres to several micrometres. So the hybrid material has the potential applications in controlled release systems, gene delivery systems, chemical sensors, and so on.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.01.061.

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