Siloxane-crosslinked Polysaccharide Nanogels for Potential Biomedical Applications

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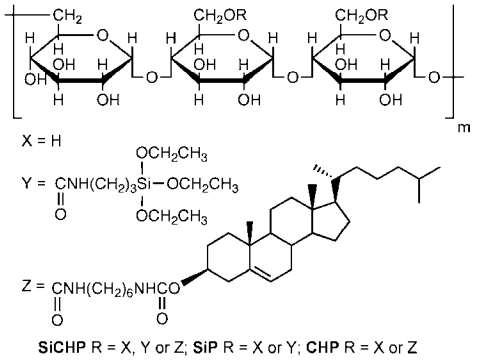
The hybrid nanogels were prepared by condensation of silanol groups grafted to cholesterol-bearing pullulan at ambient temperature and pH without any catalysts or organic solvents. This is a new method for preparation of organic–inorganic hybrid nanogels with dual network structures of both chemically crosslinked points with siloxane bonds and physically crosslinked hydrophobic association points.

Development of organic–inorganic hybrid nanomaterials is a fascinating research target in biosensing, bioimaging, and drug delivery systems (DDS)[1] since specific functionality given by the organic part and the structural stability carried by the inorganic part. Siloxane-based hybrid nanoparticles are promising candidates for use especially in DDS[2] because hydrated silica is widespread in living organisms and completely biocompatible. However, it is still difficult to control both the particle size and physicochemical stability at nano-scale.

Here a new method for preparation of polysaccharide–siloxane hybrid nanogels by condensation of silanol groups grafted to pullulan nanogels is reported. Nanogels have attracted growing interest with respect to their application in DDS.[3] We developed self-assembled nanogels. For example, hydrophobized polysaccharides such as cholesterol-bearing pullulan (CHP, Figure 1) form stable amphiphilic nanogels in water by self-association of hydrophobic groups, which form physically crosslinked points.[4] CHP nanogels trap various proteins and show molecular chaperone-like activity that is to prevent aggregation of denatured proteins and to release them as active forms.[5] CHP nanogels are useful for protein delivery such as cancer vaccine[6] and cytokine therapy.[7] On the other hand, the stability of CHP nanogels, especially in the blood stream, is not sufficient because of the physically crosslinked structure. To solve the problem, the fragile nanogel was rigidified upon covalent crosslinking with inorganic compounds which enable to provide additional platform for mineralization such as other silane coupling agents, titania, and hydroxyapatite.[8] The stable hybrid nanogels are promising candidates for controlled-release DDS.

The objective of this study is to develop siloxane-crosslinked pullulan nanogels using a silane coupling agent, triethoxy(3-isocyanatopropyl)silane (ICPTES). The isocyanate group of ICPTES reacts with hydroxy groups of pullulan and the triethoxysilyl groups act as crosslinker between polysaccharide chains. This paper reports the first attempt to synthesize hybrid polysaccharide crosslinked nanogels with siloxane linkage, though macro- or microgels of gelatin, cellulose or pullulan hybridized with the siloxane linkage have been reported.[9]

CHP, which was substituted with 1.1 cholesteryl groups per 100 glucose units of the parent pullulan ($M_w = 1.0 \times 10^5$), was synthesized as reported previously.[4] The synthetic procedure involves the synthesis of cholesteryl *N*-(6-isocyanatohexyl)carbamate and subsequent condensation with pullulan. Triethoxysilane-bearing CHP (SiCHP) and pullulan (SiP) were prepared through the reaction of hydroxy groups of the pullulan with isocyanate groups of the ICPTES in anhydrous dimethylsulfoxide in the presence of dibutyltin dilaurate as catalyst.[10,11] The conjugation of ICPTES to pullulan was confirmed by the FTIR spectrum for the products, which indicated formation of carbamate linkage (1703, 1541, and 1259 cm^{-1}) and the disappearance of the isocyanate peak (2271 cm^{-1}). The ^{1}H NMR spectra (DMSO-d_6) showed distinctive peaks: the anomeric proton of the pullulan glucopyranosyl ring at 4.69–4.71 ppm, triethoxysilylpropyl group at 0.54 ppm, 1.46 ppm, and 2.94 ppm for the propyl group, and 1.15 ppm and 3.45 ppm for ethoxy group. The degree of substitution of ICPTES coupled to the polysaccharides per 100 glucose units was quantitatively determined using the integrated areas of glucopyranosyl rings at δ 4.70 ppm and triethoxysilylpropyl groups around δ 0.54 ppm for propyl groups to be 8.8 and 7.7 for SiCHP and SiP, respectively.



SiCHP R = X, Y or Z; **SiP** R = X or Y; **CHP** R = X or Z

Figure 1. Chemical structures of cholesterol- and/or triethoxysilane-bearing pullulan.

Nanogels were prepared according to established protocol.[4] Briefly, pullulan derivatives (10 mg/mL) were dispersed in pure water and the resulting suspension was treated with a probe-type sonicator (40 W) for 15 min. The nanogel solutions were centrifuged (19000 × *g*, 30 min). The obtained nanogels were analyzed by transmission electron microscopy (TEM). Negatively stained TEM image of SiCHP clearly indicate the formation of monodispersive nanogels as shown in Figure 2a. The mean diameter of the SiCHP nanogels is 31 nm and the standard deviation is 6 nm as determined from the TEM image (inset in Figure 2a). The hydrodynamic diameter (D_{hy}) evaluated by dynamic light scattering (DLS) measurement is 87 nm with a polydispersity index (*p.i.*) of 0.17 (Table 1), which is larger than that evaluated by TEM image. This difference might be due to shrinking of the nanogel under the reduced pressure of TEM measurement.

Nanogels were also formed in the case of SiP (Figure 2b and Table 1), whereas the *p.i.* was larger than those for SiCHP. This

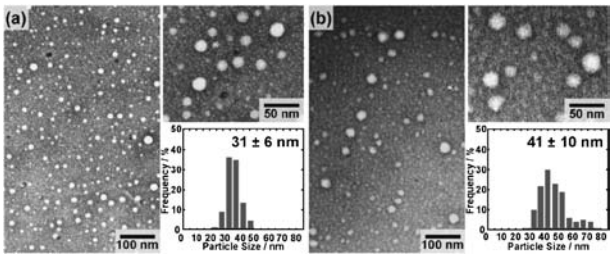


Figure 2. TEM images for SiCHP (a) and SiP (b). Inset shows the magnified TEM images and the particle size distribution determined from the TEM image.

Table 1. Hydrodynamic diameter (D_{hy}) and polydispersity index (*p.i.*) for nanogels

Sample	D_{hy}/nm (*p.i.*)	
	− methyl-β-CD	+ methyl-β-CD
SiCHP	87 (0.17)	103 (0.24)
SiP	74 (0.35)	82 (0.45)
CHP	34 (0.25)	n. d.

result suggests that ICPTES grafted on the polysaccharide chain gives siloxane-crosslinked points because the unmodified pullulan does not form any nanoparticles under the present conditions. The sol–gel processes proceed through initial hydrolysis of the silica precursor and following condensation of the silanols ($Si–OEt + H_2O \rightarrow Si–OH + Et–OH$ followed by $2Si–OH \rightarrow Si–O–Si + H_2O$). The processes come to a halt as long as a catalyst has not been introduced.[12] Generally, to facilitate the processes, an acid or alkali is added to the solution to provide a catalytic effect. However, it is worth mentioning that the hydrolysis and the condensation successfully took place without any catalyst at neutral pH in the present system. This might be attributed to the fact that catalysis is caused by a formation of hydrogen bonds between hydroxy groups in the pullulan and the products of precursor hydrolysis.[13] The hydrolysis was also confirmed by ζ potential change for SiCHP from −3.2 to −8.9 mV by altering the medium pH from 7.0 to 10, reflecting deprotonation of the silanol groups.

To obtain further information of the network structure of the nanogel, interaction with methyl-β-cyclodextrin (methyl-β-CD) was investigated. Physically crosslinked CHP nanogel was dissociated by adding methyl-β-CD owing to capping of the hydrophobic cholesteryl groups by complexation with methyl-β-CD as reported previously.[5] In fact, CHP nanogel gave dissociated CHP which did not provide enough light scattering intensity for DLS measurement upon addition of 46 mM of methyl-β-CD (Table 1). However, SiCHP nanogel swelled without dissociation in the presence of methyl-β-CD (the size of the nanogels increased). This result clearly demonstrates that the hybrids were dual network nanogels with physically crosslinked hydrophobic association points and chemically crosslinked points with siloxane bonds.

In conclusion, CHP nanogels with silane coupling agent were self-rigidified by the condensation of silanol groups. The organic–inorganic hybrid nanogels were prepared at neutral pH without any catalysts or organic solvents. They show a narrow particle-size distribution of around 90 nm in diameter, and high stability in the presence of methyl-β-CD, which dissociates conventional physical crosslinked nanogels. The hybrid nanogels open new opportunities for application as nanocarriers in DDS and as a platform of the further functional modification of inorganic scaffold in the nanogels with other silane coupling agents, titania, and hydroxyapatite.

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