Stimuli-Responsive Core Multishell Dendritic Nanocarriers

Ehsan Mohammadifar, Mohsen Adeli, Ali Nemati Kharat, Hassan Namazi, Rainer Haag*

Multidomain polymeric nanoparticles containing different blocks with different properties have gained great interest for a wide range of applications in the past two decades. In this work, core multishell (CMS) structures consisting of hyperbranched polyglycerol as core and polylactide and polycaprolactone as shell structures are presented. Number of arms and thickness of shells have been manipulated by precise control over the molar ratios of monomer to terminal functional groups in the reaction feed. The ability of CMS to load and release small guest molecules and also the loading capacities are studied using Rose Bengal as a model dye. Loading capacity of CMS depends on molecular weight, type, and thickness of shells. Due to the difference in polarity and solubility of different blocks, the CMS nanocarriers show medium-responsive behavior whereupon the encapsulated guest molecules are released in a controlled manner.

1. Introduction

Hyperbranched polymers containing hydrophobic segments have shown the ability to self-assemble in supramolecular structures.[1,2] Synthetic liposome-like polymeric structures with an internal core and multilayer shell which are covalently linked, so called core multishell (CMS) nanocarriers, are of great interest due to novel properties and applications.[3,4] As the CMSs mimic the polarity gradient of a liposome, they are benefiting from the advantages of liposomes and also overcoming the problem of their low stability due to relatively high critical micelle concentration.[5] Difference in the polarity of different domains enables CMS nanocarriers to encapsulate guest molecules with different polarities and improve their solubility in polar and nonpolar environments.[6] Guest molecules can be loaded in amphiphilic structures in two ways; encapsulation in individual unimolecular structures and/or entrapment in supramolecular or micellar aggregates.[7] Recently, varieties of amphiphilic core multishell polymers based on hyperbranched polyglycerol (hPG) have been synthesized for hyperbranched polyglycerol (hPG) have been synthesized for potential biomedical applications.[8–10] Self-assembly and solubility behavior as well as loading capacity of CMS nanocarriers can be controlled by manipulation of molecular weight and type of core and shells. For this aim, up to now various range of linear and dendritic polymers with different molecular weights have been conjugated to the hPG core to synthesize amphiphilic CMS.[11,12] One of the most important and interesting feature of CMS nanocarriers is
to response to external stimuli factors such as pH and temperature.[13] To obtain stimuli-responsive CMS, elaborate design of corresponding building blocks is required.[14]

Polylactide (PLA) is a well-known biocompatible and biodegradable polymer that has been approved by the United States Food and Drug Administration for using in drug delivery systems.[15,16] The rate of degradation of PLA can be controlled by changing its molecular weight.[17] In a previous work, Frey and co-workers[18] successfully used hPG as a macroinitiator for ring opening polymerization of lactide to synthesize multiam arm star polymer with a good control over arm length. On the other hand, polycaprolactone (PCL) is a hydrophobic and biocompatible polymer with relatively low degradation rate.[19] This polymer has been widely used in the biomedical field due to the good stability under ambient conditions, ease of synthesis, and processability.[20] As a result, combination of hPG, PLA, and PCL in one structure results in hybrid systems that are suitable for a wide range

Scheme 1. Synthetic pathway of core multishell nanoparticles: a) Lactide, Sn(Oct)$_2$, 125 °C, 4 h; b) ε-caprolactone, Sn(Oct)$_2$, 125 °C, 6 h; c) Lactide, Sn(Oct)$_2$, 125 °C, 4 h.

Figure 1. 1H NMR spectra of CMS structures containing hPG$_{8000}$ core.
Here, we report on the synthesis of CMS structures based on hPG as core and PLA and PCL as multiblock shells. The ability of the synthesized CMS nanocarriers to load small molecules and their stimuli-responsive characteristic for the controlled release of the encapsulated guest molecules have been investigated in this work.

### 2. Experimental Section

#### 2.1. Characterization

$^1$H NMR spectra were recorded in CDCl$_3$ solution, on a Bruker DRX 500 (500 MHz) apparatus with the solvent proton signal for reference. $^{13}$C NMR spectra were recorded at 125.721 MHz on the same instrument using the solvent carbon signal as a reference. All polymer NMR spectra were recorded on 250 mg mL$^{-1}$ of sample. The number-average molecular weights of the polymers were calculated from the $^1$H NMR data. The molecular weight distributions were determined by size exclusion chromatography using Agilent 1100 using PSS GRAL 100 Å column connected to a differential refractometer with refractive index (RI) and UV detector and chloroform as the mobile phase at 25 °C. Polystyrene standard samples were used for calibration. All UV–vis measurements were performed with a 100 Specord spectrophotometer and Millipore quality water was used in all the measurements. IR measurements were performed using a Nicolet 320 Fourier transform infrared spectrometer (FT-IR). Dynamic light scattering (DLS) experiments were performed on Malvern Zetasizer Nano machine (Brookhaven Instruments Corp.) at 25 °C. Millipore quality water was used in all the experiments. Transmission electron microscopy samples were prepared on copper grids (200 mesh) by blotting samples in chloroform solution and visualized using a Philips CM12 electron microscope.

### 2.2. Materials

Hyperbranched polyglycerol (degree of branching (DB) = 60%) had been synthesized according to a procedure explained in

**Table 1.** Characterizations of CMS nanocarriers containing hPG core with a molecular weight of 8000 g mol$^{-1}$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conversation [%]</th>
<th>$M_n(NMR)$</th>
<th>DP</th>
<th>$M_w/M_n$</th>
<th>Arms</th>
<th>Free OH groups</th>
</tr>
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<tbody>
<tr>
<td>hPG$<em>{8000}$-PLA$</em>{6.25}$</td>
<td>54</td>
<td>$9 \times 10^4$</td>
<td>18</td>
<td>1.77</td>
<td>65</td>
<td>44</td>
</tr>
<tr>
<td>hPG$<em>{8000}$-PLA$</em>{12.5}$</td>
<td>60</td>
<td>$15 \times 10^4$</td>
<td>30</td>
<td>1.54</td>
<td>69</td>
<td>40</td>
</tr>
<tr>
<td>hPG$<em>{8000}$-PLA$</em>{12.5}$-PCL$_{12.5}$</td>
<td>74</td>
<td>$2.6 \times 10^5$</td>
<td>15</td>
<td>1.5</td>
<td>69</td>
<td>40</td>
</tr>
<tr>
<td>hPG$<em>{8000}$-PLA$</em>{12.5}$-PCL$_{25}$</td>
<td>78</td>
<td>$4 \times 10^5$</td>
<td>40</td>
<td>1.55</td>
<td>69</td>
<td>40</td>
</tr>
<tr>
<td>hPG$<em>{8000}$-PLA$</em>{12.5}$-PCL$<em>{12.5}$-PLA$</em>{12.5}$</td>
<td>68</td>
<td></td>
<td>1.5</td>
<td></td>
<td>69</td>
<td>40</td>
</tr>
<tr>
<td>hPG$<em>{8000}$-PLA$</em>{12.5}$-PCL$<em>{25}$-PLA$</em>{12.5}$</td>
<td>70</td>
<td></td>
<td>1.6</td>
<td></td>
<td>69</td>
<td>40</td>
</tr>
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</table>

**Table 2.** Characterizations of CMS nanocarriers containing hPG core with a molecular weight of 2400 g mol$^{-1}$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conversation [%]</th>
<th>$M_n(NMR)$</th>
<th>DP</th>
<th>$M_w/M_n$</th>
<th>Arms</th>
<th>Free OH groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>hPG$<em>{2400}$-PLA$</em>{6.25}$</td>
<td>54</td>
<td>$3 \times 10^4$</td>
<td>16</td>
<td>1.61</td>
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<td>hPG$<em>{2400}$-PLA$</em>{12.5}$</td>
<td>62</td>
<td>$5 \times 10^4$</td>
<td>32</td>
<td>1.49</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>hPG$<em>{2400}$-PLA$</em>{12.5}$-PCL$_{12.5}$</td>
<td>74</td>
<td>$11 \times 10^4$</td>
<td>26</td>
<td>1.45</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>hPG$<em>{2400}$-PLA$</em>{12.5}$-PCL$_{25}$</td>
<td>86</td>
<td>$24 \times 10^4$</td>
<td>87</td>
<td>1.40</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>hPG$<em>{2400}$-PLA$</em>{12.5}$-PCL$<em>{12.5}$-PLA$</em>{12.5}$</td>
<td>77</td>
<td></td>
<td>1.44</td>
<td></td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>hPG$<em>{2400}$-PLA$</em>{12.5}$-PCL$<em>{25}$-PLA$</em>{12.5}$</td>
<td>80</td>
<td></td>
<td>1.65</td>
<td></td>
<td>20</td>
<td>13</td>
</tr>
</tbody>
</table>

**Figure 2.** GPC elution curves of the CMS structures containing hPG core 8000 g mol$^{-1}$. 

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publications\textsuperscript{[21,22]} using ring opening polymerization of glycidol monomers with a tris(hydroxymethyl)propane (TMP) core. Lactide was purchased from Aldrich and recrystallized in dry toluene before using. Stannous-2-ethylhexanoate (Sn(Oct))\textsubscript{2} and \(\varepsilon\)-caprolactone were purchased from Aldrich. Full information concerning synthesis methods and characterizations can also be found in the Supporting Information.

2.3. Preparation of the hPG-PLA

A solution of hPG with certain concentration in methanol was prepared. An exact volume of this solution was placed in polymerization ampoule and methanol was evaporated under vacuum at 80 °C for 2 h and then, a solution of Sn(Oct)\textsubscript{2} in toluene was added to this ampoule and solvent was evaporated under vacuum for 30 min at 40 °C. Then, lactide monomer was added to ampoule and mixture was placed under vacuum for 1 h. Afterward, ampoule was purged by argon three times and it was sealed under vacuum. Mixture was stirred at 125 °C for 4 h. After cooling, the polymerization ampoule contents were dissolved in chloroform and solution was filtrated off. Purified product was obtained by precipitation of polymer in cyclohexane for several times. Then, purified product was dried under vacuum at 50 °C for 1 h.

2.4. General Procedure for Preparation of CMS Polymers

For preparation of CMS, polymers with a certain number of arms (i.e., a certain number of OH functional end groups) were placed in polymerization ampoule and then purified monomer (lactide or \(\varepsilon\)-caprolactone) and Sn(Oct)\textsubscript{2} were added. Mixture was purged by argon and vacuum three times and then stirred at 125 °C for a certain time (depending on the amount and type of monomer). After cooling, the polymerization ampoule contents were dissolved in chloroform and solution was filtrated off. Purified product was obtained by precipitation in cyclohexane for several times and drying under vacuum at 50 °C for 1 h.

2.5. General Procedure for Encapsulation of Rose Bengal

A chloroform solution of CMS was prepared with certainty and stirred for 1 h, then an excess amount of Rose Bengal was added to the solution and mixture was stirred for 2 h at room temperature. Finally, solution was filtered and the filtrate was centrifuged at 11 000 rpm for 10 min and clear solution was

Figure 3. TEM images of a,b) hPG\textsubscript{2400}-PLA\textsubscript{12.5}-PCL\textsubscript{25}, c) hPG\textsubscript{8000}-PLA\textsubscript{12.5}-PCL\textsubscript{25}, e) hPG\textsubscript{2400}-PLA\textsubscript{12.5}-PCL\textsubscript{25}-PLA\textsubscript{12.5} and electron beam image of d) hPG\textsubscript{8000}-PLA\textsubscript{12.5}-PCL\textsubscript{25} and f) hPG\textsubscript{2400}-PLA\textsubscript{12.5}-PCL\textsubscript{25}-PLA\textsubscript{12.5}.

Figure 4. DLS measurements for hPG\textsubscript{8000}-PLA\textsubscript{12.5}-PCL\textsubscript{25}-PLA\textsubscript{12.5} CMS in chloroform and acetone solvents.
decanted. Loading capacity (LC) and loading efficiency (LE) for Rose Bengal were calculated according to the following formulas:

\[
LC = \left( \frac{\text{weight of loaded Rose Bengal}}{\text{weight of polymer}} \right) \times 100 \tag{1}
\]

\[
LE = \left( \frac{\text{weight of loaded Rose Bengal}}{\text{weight of Rose Bengal in feed}} \right) \times 100 \tag{2}
\]

2.6. General Procedure for Nile Red Release from Star Copolymers

2 mL of a clear solution of encapsulated polymer in chloroform was added to 10 mL of distilled water. The release of guest molecules from chloroform to water was measured at 25 °C. Water phase (3 mL) was withdrawn at regular intervals and replaced with equal volumes of distilled water. The diameter of surface of phase interface was 19 mm. The amount of released guest molecule was determined by a Specord 100 UV spectrophotometer at the absorption maximum of the free guest molecule using a 1 cm quartz cell and calibration curve obtained previously under the same conditions.

2.7. Particle Size Analysis

Dynamic light scattering experiments were performed by an equipment Zetasizer Nano from Malvern using a 4 mW He–Ne laser (633 nm wavelength) with a fixed detector angle of 173°. The measurement was performed at 25 °C and was started 10 min after the cuvette was placed in the DLS apparatus to allow the temperature to equilibrate. About 1 mL of the sample was transferred to a special dust free light scattering cell. The temperature was controlled to within ±0.02 °C.

2.8. Sample Preparation for Transmission Electron Microscopy

A solution of the CMS nanocarriers containing guest molecules in chloroform solvent was filtered using a series of ultrafilters connected together (0.4, 0.2, and 0.1 μm) to avoid dust particles. A drop of nanocarrier solution was placed on the graphite layer and solvent was evaporated at room temperature.

3. Results and Discussion

CMS structures have been synthesized via stepwise reactions using hPG as macroinitiator for ring opening polymerization of lactide and \(\varepsilon\)-caprolactone monomers and \(\text{Sn(Oct)}_2\) as catalyst.
(Scheme 1). The product in each step was purified through dissolving in a suitable solvent and precipitation in a non-solvent. Number and molecular weight of arms were controlled by choosing the ([monomer]/[OH]) molar ratio in the reaction feed.

Several types of CMS containing hPG core with two different molecular weights (2400 and 8000 g mol$^{-1}$) as well as different molar ratios of PLA and PCL have been synthesized and characterized. Molecular weight of hPG and [monomer]/[OH] molar ratio are shown as index in the nomenclature of CMS. For example, a CMS nanocarrier synthesized by hPG with 2400 g mol$^{-1}$ molecular weight and [lactide]/[OH] = 12.5 and consequently [$\varepsilon$-caprolactone]/[OH] = 25 is called hPG$_{2400}$-PLA$_{12.5}$-PCL$_{25}$.

$^1$H NMR spectra of CMS structures containing a hPG$_{8000}$ core can be seen in Figure 1 (see Figure S1 in the Supporting Information for hPG$_{2400}$). In the spectrum B, which is related to the hPG$_{8000}$-PLA$_{12.5}$, signals of protons of methylene and CH groups of PLA chains appear at 1.25–1.9 ppm and 5–5.3 ppm, respectively. Signals appearing at 2.65–2.90 ppm and 3.2–4 ppm are assigned to protons of free (unreacted) OH groups and methylene groups of hPG backbone, respectively. Signals of end methylene groups of hPG which are conjugated to PLA arms and also end CH groups of PLA chains are overlapped at 4–4.5 ppm. In spectrum D, which corresponded to the hPG$_{8000}$-PLA$_{12.5}$-PCL$_{12.5}$, signals of PLA chains, as well as those for PCL are appeared. For PCL block, signal at 2.3 ppm is assigned to the $\text{CH}_2$ adjacent to carbonyl groups and signals at 4.1–4.2 ppm are related to the $\text{OCH}_2$ groups. Also signals at 1.2–1.7 ppm corresponded to the other methylene groups of PCL chains. In the $^3$HNMR spectrum of hPG$_{8000}$-PLA$_{12.5}$-PCL$_{12.5}$ signals of hPG, PLA, and PCL chains can be observed. Clearly, the peak area of CH groups of PLA chain is increased. Two signals for CH groups of PLA part are assigned to the inner and outer PLA blocks.

Tables 1 and 2 show the characterization data of CMS nanocarriers containing hPG core with the molecular weight of 8000 and 2400 g mol$^{-1}$, respectively.

Degree of polymerization (DP), number of arms, and molecular weight of the CMS nanocarriers directly depend to the ratio of monomer to OH end functional groups of macroinitiator. Therefore, a control over DP and number of arms can be achieved by selection of the proper monomer to end group ratio. The polydispersity of CMSs are between 1.49 and 1.7.

Gel permeation chromatography (GPC) diagrams of core shell structures containing hPG$_{8000}$ and cores are shown in Figure 2 (see the Supporting Information for hPG$_{2400}$). GPC diagrams showed that molecular weight distributions are monomodal and the products are free from impurities or side products. As it can be seen in the diagrams, the molecular weight of CMS nanocarriers increased after conjugation of the additional shells. This indicates that the CMS triblock structures have been synthesized successfully.

Self-assembly of the synthesized CMS resulted in nanoparticles with the ability to encapsulate the small guest molecules. To investigate the size and structure of the CMS assemblies, transmission electron microscopy (TEM) was performed. It was found that CMSs assemble in larger nanoparticles with 200 nm diameter. TEM images showed two phases in nanocarriers; dark and crystalline part which is concentrated inside the nanoparticles is assigned to the PLA blocks and light gray part corresponded to the PCL blocks which form a shell around the dark region (Figure 3).

The CMS with a hPG core and PLA-PCL-PLA triblock shell (hPG$_{8000}$-PLA$_{12.5}$-PCL$_{25}$-PLA$_{12.5}$) showed a solvent-dependent assembly behavior. In chloroform, it can be assembled in nanosized particles with two different diameters (Figure 4).[24] However, these particles can disassemble to smaller particles with 20 nm size in acetone. This property of the CMS nanocarriers is due to the difference in the solubility of shells in chloroform and acetone. Acetone can penetrate in the aggregates and disassemble them to unimolecular, which can be efficiently used for encapsulation and controlled release of guest molecules.

In order to investigate the transport efficiency of CMS structures, Rose Bengal was loaded in nanocarriers. The
photographs of encapsulated Rose Bengal by nanocarriers in chloroform solution are displayed in Figure 5 (see the Supporting Information for hPG2400). Figure 5 shows UV spectra as well as photographs of encapsulated Rose Bengal by hPG8000-PLA12.5, hPG6000-PLA12.5-PCL25, and hPG8000-PLA12.5-PCL25-PLA12.5 nanocarriers from left to right. As it can be seen, with increasing of the shell thickness of CMS, intensity of red color of the solution is increased, indicating that the amount of encapsulated Rose Bengal and the loading capacity of nanocarriers increased. Loading capacity and loading efficiency of hPG8000-PLA12.5-PCL25-PLA12.5 for Rose Bengal was evaluated using UV–vis spectroscopy at 200–800 nm. According to these experiments, LC and LE of nanocarriers for Rose Bengal were 3% and 40%, respectively.

It was found that the loading capacity of nanocarriers depends on the thickness of the shells of CMS. For example, the amount of encapsulated Rose Bengal by assemblies of hPG6000-PLA12.5-PCL25 is higher than that by hPG8000-PLA12.5. Nanosized assemblies of CMS with thicker shell show a higher loading capacity for Rose Bengal. The type of the shell also affected the loading capacity of the CMS nanocarriers whereupon the amount of encapsulated Rose Bengal in hPG8000-PLA25 is higher than that of hPG8000-PC25 (see the Supporting Information).

In order to investigate the release of Rose Bengal, the loaded CMSs were dissolved in organic solvent and added to the water. Then, release of guest molecules from organic to the water phase was measured using UV spectroscopy. Encapsulated Rose Bengal was transferred from chloroform to water slowly (Figure 6a) (see the Supporting Information for hPG2400). However, addition of a low amount of acetone (0.1 mL) to the organic phase resulted in the fast release and transfer of the encapsulated Rose Bengal from the chloroform to the water phase (Figure 6b). This is due to the disassembly of the nanoparticles by acetone. Based on these observations, the mechanism of triggered release of guest molecules from the assemblies of CMS is depicted in Figure 7.

4. Conclusions

Different types of core–shell structures containing hPG core and PLA and PCL shells have been synthesized and characterized. Core–multishell structures were able to encapsulate small guest molecules such as Rose Bengal efficiently. The amount of encapsulated Rose Bengal depends on molecular weight of hPG core as well as the thickness and type of the shells. CMS nanocarrier self-assembled in nanosized particles in chloroform and disassembled in acetone. This feature of the CMS structures was used for the controlled release of encapsulated guest molecules.