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Published on Web 04/18/2007

Biodegradable Nanogels Prepared by Atom Transfer Radical Polymerization as Potential Drug Delivery Carriers: Synthesis, Biodegradation, in Vitro Release, and Bioconjugation

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Abstract: Stable biodegradable nanogels cross-linked with disulfide linkages were prepared by inverse miniemulsion atom transfer radical polymerization (ATRP). These nanogels could be used for targeted drug delivery scaffolds for biomedical applications. The nanogels had a uniformly cross-linked network, which can improve control over the release of encapsulated agents, and the nanogels biodegraded into water-soluble polymers in the presence of a biocompatible glutathione tripeptide, which is commonly found in cells. The biodegradation of nanogels can trigger the release of encapsulated molecules including rhodamine 6G, a fluorescent dye, and Doxorubicin (Dox), an anticancer drug, as well as facilitate the removal of empty vehicles. Results obtained from optical fluorescence microscope images and live/dead cytotoxicity assays of HeLa cancer cells suggested that the released Dox molecules penetrated cell membranes and therefore could suppress the growth of cancer cells. Further, OH-functionalized nanogels were prepared to demonstrate facile applicability toward bioconjugation with biotin. The number of biotin molecules in each nanogel was determined to be 142 000, and the formation of bioconjugates of nanogels with avidin was confirmed using optical fluorescence microscopy.

Introduction

Polymer-based drug delivery systems (Polymer-DDS) for targeted biomedical applications and controllable release have attracted significant attention in polymer chemistry, pharmaceutics, and biomaterials science. Polymer-DDS have the potential to treat numerous diseases, including cancer.[1] In addition, Polymer-DDS carriers conjugated with ligand biomolecules that can recognize specific cell receptors may reduce the side effects of drugs. There are several types of polymer-DDS that may be exploited for these purposes, including polymer−protein conjugates,[2] polymer−drug conjugates,[3,4] micelles,[5−10] and vesicles[11−13] based on amphiphilic and doubly hydrophilic block copolymers, dendrimers,[14] and submicrometer-sized particulates.[15,16]

Polymer-DDS may be designed as microgels. Microgels, cross-linked polymeric particles, are a class of hydrogels that may have a tunable chemical composition and three-dimensional physical structure enabling control over water content, mechanical properties, and biocompatibility.[17,18] Moreover, microgel particles offer significant opportunities for targeted applications as a consequence of their tunable size (from nanometers to several micrometers), interior network enabling incorporation

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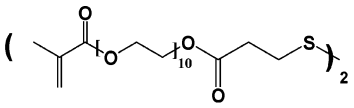
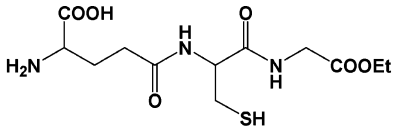
Scheme 1. Chemical Structures of the DMA Cross-Linker and Glutathione Ethyl Ester



Dithiopropionyl poly(ethylene glycol) dimethacrylate (DMA)



Glutathione ethyl ester, reduced form gamma-Glu-Cys-Gly-OEt

of biorelated molecules such as drugs, proteins, and DNA, and a large surface area for multivalent bioconjugation.[19,20]

Biodegradability of microgels can be constructed to respond to external stimuli such as enzymes and pH change, which offer a number of benefits as selective drug delivery carriers. Responsiveness to enzymes or pH could facilitate controllable release of encapsulated molecules from the interior of microgels and ensure the removal of empty vehicles after the drug release. A significant amount of research has been directed toward the development of cross-linkers functionalized with degradable linkages to control biodegradability. Cross-linking molecules include peptides,[21,22] anhydrides,[23] and oligo(lactate) esters.[24−27] The resulting cross-linked hydrogels may be subsequently degraded to water-soluble polymers. Disulfides present another class of biodegradable groups, which can be cleaved to the corresponding thiols in the presence of reducing agents such as tributyl phosphine (Bu_3P), tris(2-carboxyethyl)phosphine (TCEP), and dithiothreitol (DTT).[28−31] Disulfide-functionalized dimethacrylate cross-linkers have been developed for biodegradable bulk gels.[30,32] Furthermore, the degradation of disulfides has been utilized for the preparation of stimulus-responsive gelators,[33] reversible core-cross-linked polyplex[34−37] and shell-cross-linked[38] micelles, miktoarm star copolymers,[39] and polymer capsules.[40]

Microgels are generally prepared by a heterogeneous polymerization of hydrophilic water-soluble monomers in the presence of either difunctional or multifunctional cross-linkers.[41−43] Inverse (mini)emulsion polymerization is a heterogeneous water-in-oil (W/O) polymerization process where aqueous droplets (including water-soluble monomers) are dispersed stably with the aid of oil-soluble surfactants in a continuous organic medium.[44] Polymerization occurs within the aqueous droplets, producing stable colloidal particles upon the addition of radical initiators. Recent reports have demonstrated the synthesis of hydrophilic nanometer-sized polymeric particles[45,46] and microgels.[47,48] Frechet and co-workers reported inverse emulsion polymerization utilizing the conventional free radical polymerization process to prepare degradable microgels based on poly(*N*-isopropylacrylamide) and poly(acrylamide) for protein, plasmid DNA, and antigen delivery.[49−51]

Recently, controlled/living radical polymerization (CRP) techniques[52−54] have been explored for the synthesis of either cross-linked particles or gels of well-controlled polymers in the presence of cross-linkers.[30,55−57] We recently reported atom transfer radical polymerization (ATRP)[58−60] in inverse mini-emulsion for the synthesis and functionalization of stable (bio)-degradable cross-linked nanoparticles (called nanogels) of well-controlled water-soluble polymers in the presence of a disulfide-functionalized dimethacrylate (DMA).[31] Application of ATRP allowed for the preparation of materials with many useful features. First, the resulting particles preserve a high degree of halide end-functionality to enable further chain extension to form functional block copolymers and functionalization with biorelated molecules, such as by utilizing click reactions.[61−66] Second,

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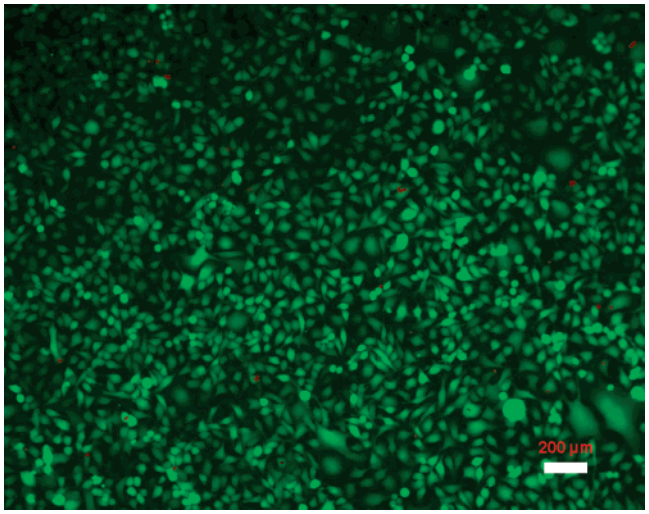

Figure 1. Fluorescent image of live (green) and dead (red) HeLa cells after 24 h incubation with 10 mg/mL nanogels. The gels were purified by extensive dialysis in water for 7 days. Scale bar = 200 μm.

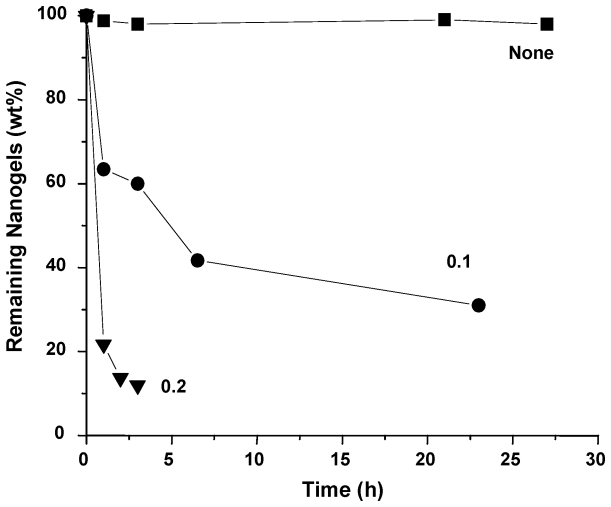

Figure 2. Evolution of weight fraction of remaining nanogels with time in the presence of different amounts of glutathione, expressed as wt ratio of glutathione/nanogels.

they are degradable in a reducing environment to individual polymeric chains with a relatively narrow molecular weight distribution (M_w/M_n < 1.5), indicating the formation of a uniformly cross-linked network in the individual particles. This uniform structure is anticipated to improve control over the release of encapsulated agents. Third, properties including swelling ratio, degradation behavior, and colloidal stability of particles prepared by ATRP are superior to those prepared by conventional free radical inverse miniemulsion polymerization. Fourth, nanogels will enhance circulation time in the blood, because they can consist of poly(oligo(ethylene oxide) monomethyl ether methacrylate) (POEOMA), an analogue of linear poly(ethylene oxide) (PEO) that can prevent nanoparticle uptake by reticuloendothelial system (RES).[67] These unique properties suggest that the well-defined functional nanogels prepared by this newly developed method hold great potential as useful biomaterials for biological and biomedical applications.

As an initial step toward demonstrating utility in biological and biomedical applications, we report our results on nanogels as carriers for controlled drug delivery scaffolds to target specific cells. We prepared stable nanogels using inverse miniemulsion ATRP and assessed biocompatibility using HeLa human cervix epithelial cells. Further, we studied biodegradation of nanogels cross-linked with degradable disulfide bonds in the presence of the tripeptide glutathione as a biocompatible reducing agent. In addition, release of a fluorescent dye and Doxorubicin (Dox) from nanogels was determined in vitro to demonstrate that the degradation triggers controllable release of encapsulated molecules. Last, bioavailability of nanogels was demonstrated by conjugation with biotin, which in turn binds to avidin resulting in the formation of avidin−nanogel bioconjugates.

Results and Discussion

Synthesis of Cross-Linked Particles. Our approach to synthesizing biodegradable, cross-linked POEOMA nanoparticles involves an ATRP of OEOMA in inverse miniemulsion in the presence of a disulfide-functionalized dimethacrylate (DMA) cross-linker at ambient temperature (30 °C) (Scheme 1).[31] In the absence of DMA, well-controlled POEOMA with narrow molecular weight distribution (M_w/M_n < 1.3) was prepared in uncross-linked colloidal particles of 151 ± 8 nm in diameter with narrow, monodisperse size distribution.[68] In the presence of DMA, expressed as $[DMA]_0/[initiator]_0$ = 4/1, the resulting particles were not soluble in any solvents, including THF and water, indicating that the particles were cross-linked during the polymerization. The diameter of particles dispersed in cyclohexane was 225 ± 24 nm. However, a small amount of larger particles (less than 0.5 wt % of total solids) was detected, suggesting the formation of some aggregates during polymerization in the presence of the cross-linker.

Cytotoxicity of Nanogels. The live/dead viability/cytotoxicity assay was used to examine the cytotoxicity of nanogels. An aliquot of nanogels was further purified using dialysis for 7 days and then evaluated for cytotoxicity. HeLa cells were cultured for 24 h to permit cell attachment. After this time, 10 mg of the nanogel suspended in phosphate buffer was added. After 24 h incubation of HeLa cells with nanogels (48 total h), differential interference contrast (DIC) and fluorescence microscopy were used to visualize live and dead cells. Live cells were viewed with green fluorescence using the FITC filter, while dead cells were viewed with red fluorescence using the Rhod filter. The combined images are shown in Figure 1. Careful counting of live and dead cells indicated 95% viability of HeLa cells in the presence of nanogels, as compared to 96% viability of HeLa cells in the absence of nanogels as a control experiment. These results suggest that nanogels of POEOMA prepared by inverse miniemulsion ATRP are nontoxic to cells and biocompatible.

To measure the cytotoxicity of the degradation products, 10 mg of nanogels was suspended in 1 mL of complete Dulbecco's

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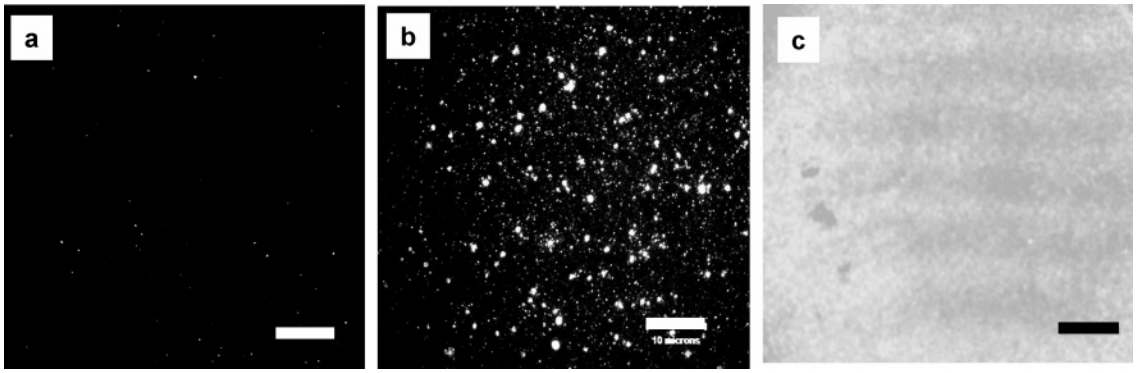

Figure 3. Optical fluorescence microscopy images of nanogels loaded (a) without, and with (b) R6G fluorescent dyes before and (c) after degradation in the presence of glutathione in water. Thin films were spun-cast on a cleaned glass plate and dried at room temperature for 10 min. All scale bars = 10 μm.

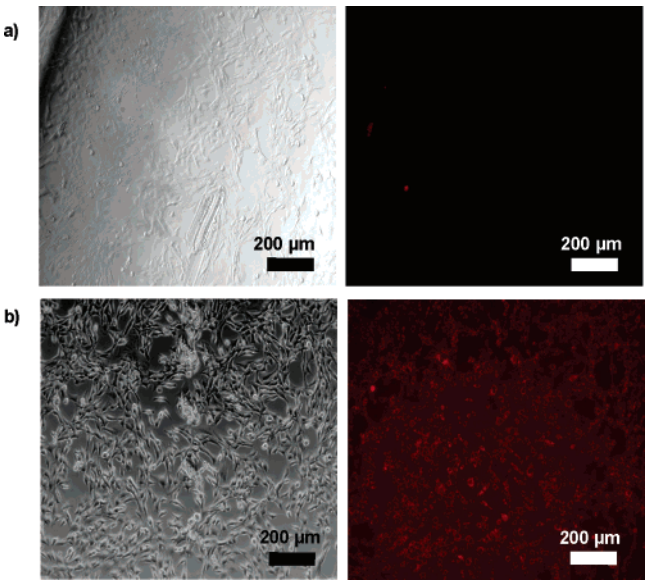

Figure 4. Differential interference contrast (DIC) (left) and fluorescent (right) images of C2C12 cells, after 24 h incubation with 5 mg of R6G-loaded nanogels (a), and after another 24 h incubation with R6G-loaded nanogels in the presence of glutathione. The red fluorescence originates from R6G loaded (right in a) to and released (right in b) from nanogels. All scale bars = 200 μm.

Modified Eagle Medium (DMEM) and placed into the incubator at 37 °C. 50 wt % of glutathione, dissolved in sterile phosphate buffer solution (PBS), was added to degrade the nanogels. The flask was maintained at 37 °C for 2 days to permit degradation. After this time, aliquots were added to culturing HeLa cells to measure the cytotoxicity of the degradation products. Using the live/dead cell assay, 94% viability was observed in the presence of 2 mg/mL degraded nanogels after 24 h, as compared to 96% viability of HeLa cells in the absence of nanogels as a control experiment. These results suggest that the degradation products of POEOMA nanogels are also nontoxic to cells and biocompatible.

Biodegradation of Nanogels in the Presence of Glutathione. In our previous publication, we reported that nanogels cross-linked with disulfide linkages were degradable in the presence of reducing agents such as Bu_3P. The degraded polymers were soluble in THF and were characterized using gel permeation chromatography (GPC) measurements. However, because Bu_3P is hydrophobic and toxic to cells, it cannot be used as a reducing agent to degrade nanogels in vitro.

Glutathione is a tripeptide found within cells at millimolar concentrations.[33,69−71] A few reports describe the use of glutathione as a water-soluble reducing agent that can degrade disulfide-containing polymers to the corresponding thiols.[33,35] Furthermore, our preliminary results obtained from cell culture experiments demonstrated that the viability of C2C12 mouse myoblast cells in the presence of 10 mM glutathione was over 91% for 2 days, suggesting that glutathione exhibits insignificant cytotoxicity at this concentration. In the experiments, the extent of degradation of nanogel was determined by the weight loss of swollen nanogels in the presence of different amounts of glutathione in water over time.

Figure 2 shows the extent of degradation (weight fraction of remaining nanogels) in the presence of different amounts of glutathione expressed as the weight ratio of glutathione/nanogels. In the absence of glutathione, the weight fraction of nanogels was almost 100%, indicating no significant degradation. However, in the presence of glutathione, the weight fraction of nanogels decreased as a function of time, indicating the occurrence of degradation. The degradation rate of nanogels increased with the amount of glutathione. For example, in the presence of ca. 20 wt % of glutathione, over 85% nanogels was degraded within 1 h. The GPC measurements of the clear supernatant were further used to analyze polymers degraded from nanogels in the presence of glutathione. The solution containing degraded polymers could be filtered through a 0.2-μm-filter without strong press, prior to its injection to GPC, suggesting the formation of degraded linear polymers. In addition, the GPC trace indicated that degraded polymers had M_n = 43 000 and M_w/M_n = 1.6.

For comparison, nanogels prepared by free-radical polymerization (FRP) in inverse miniemulsion under similar conditions were also examined for biodegradation in the presence of glutathione. However, these gels did not degrade to any significant degree, which is similar to the results obtained in the presence of Bu_3P in THF.[31]

In Vitro Release of Fluorescent Dyes from Nanogels upon Degradation in Cellular Media. The above results indicate that nanogels cross-linked with disulfide linkages prepared by inverse miniemulsion ATRP can be degraded in the presence

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Table 1. Loading Level and Efficiency of Dox into Nanogels

Dox/nanogels (wt ratio)	loading level (wt %)	loading efficiency (wt %)
0.07/1	5.4	70
0.35/1	16.4	51

of glutathione and further suggest that such degradation of the nanogels can trigger controllable release of encapsulated molecules, including drugs. To demonstrate this idea, a water-soluble fluorescent dye, Rhodamine 6G (R6G), was selected as a hydrophilic model drug. Figure 3 shows a typical optical fluorescence microscopy (OFM) image of the R6G-loaded nanogels. As compared to the OFM image of the controlled nanogels without fluorescent dyes (Figure 3a), the distinct bright spots on the dark background indicate that dyes were localized in the nanogel particles (Figure 3b). In contrast, a diffuse fluorescent signal in the background points out the release of dyes from the nanogels (Figure 3c). Upon addition of glutathione in water, the nanogels degraded into individual polymeric chains, resulting in the release of the R6G dyes. These results demonstrate that degradation of the nanogels could indeed trigger the release of encapsulated drugs in a reducing environment, through disulfide-thiol chemistry.

Next, in vitro release of R6G fluorescent dyes from R6G-loaded nanogels was demonstrated in cellular environments. Figure 4 shows the DIC and fluorescent images of C2C12 cells incubated with 5 mg of R6G-loaded nanogels before and after degradation in the presence of glutathione. Before the addition of glutathione into a mixture of C2C12 cells incubated with R6G-loaded nanogels, red fluorescence was only localized in large R6G-loaded nanogels. None of the cells were stained by free or released R6G dyes, which can be confirmed by comparison to the corresponding DIC images showing the location of cells. When glutathione was added, nanogels were degraded to release R6G dyes, which entered and stained the cells, as shown in Figure 4b. Furthermore, most cells in the presence of glutathione survived, because glutathione is biocompatible.

Loading and in Vitro Release of Doxorubicin. Doxorubicin (Dox, called adriamycin or hydroxyldaunorubicin) is a DNA-interacting drug widely used in chemotherapy. Dox is amphiphilic and has both amino and hydroxyl functional groups, which have often been used as sites for conjugation to macromolecules, resulting in the formation of polymer prodrugs and polymer micelles useful as drug delivery carriers.[5] Dox has also been physically entrapped in hydrogels.[48,72] The detailed procedures and methods to determine the amount of Dox loaded into nanogels were described in the experimental section (Supporting Information). Table 1 presents the detailed results. The loading level of Dox into nanogels (amount of Dox in nanogels) increased from 5.4 to 16.4 wt % when the initial ratio of Dox/nanogel in mixture increased from 0.07/1 to 0.35/1. The loading efficiency (amount of Dox loaded in nanogels versus amount added to mixture) was as high as 50−70%; however, the efficiency decreased as the amount of Dox added to mixtures increased. Similar results were observed for a somewhat different system with nanogels based on Pluronic F127 containing hydrophobic poly(propylene glycol).[48] In that system, the

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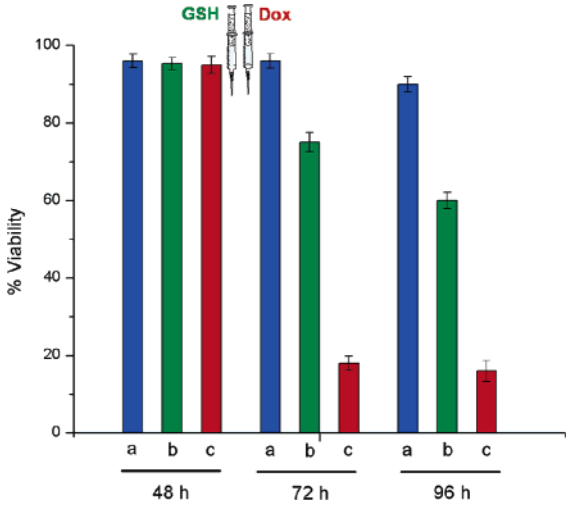


Figure 5. Viability of HeLa cells after incubation for 48, 72, and 96 h in different systems: (a) basic control without nanogels; (b) Dox-loaded nanogels (0.4 mg/mL), where glutathione (0.08 mg/mL) was added after 48 h incubation to release Dox from Dox-loaded nanogels; (c) control, where free Dox (0.064 mg/mL) was added after 48 h incubation.

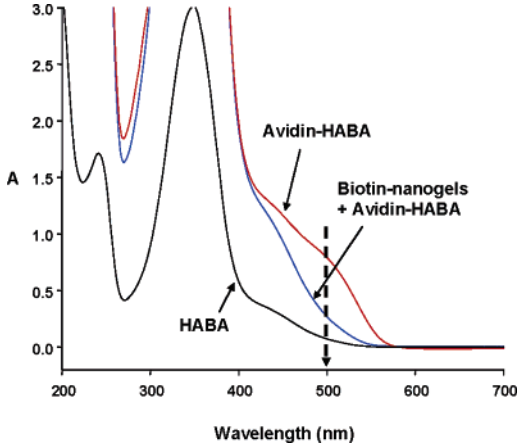
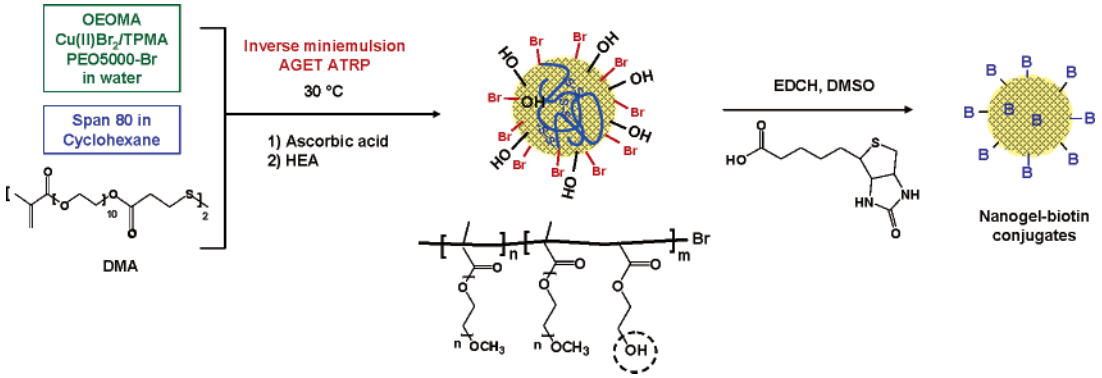


Figure 6. UV spectra of HABA and avidin−HABA complex before and after addition of biotin-nanogels in PBS buffer.

amount of Dox loaded into nanogels increased from 2.7 to 8.9 wt % when the initial ratio of Dox/nanogel in mixtures increased from 0.1/1 to 0.2/1. This type of increase in adsorption may be due to an increase in the extent of both hydrophobic and hydrophilic interactions between Dox and OEG segments, because they have dual characteristics.

It can be anticipated that Dox-loaded nanogels will be degraded upon the addition of glutathione in cellular media, and, in turn, the released Dox molecules will kill cancer cells. To demonstrate this idea, HeLa cancer cells were incubated with and without 16.4% Dox-loaded nanogels. 5×10^4 HeLa cells were added to each well of a 24-well plate and cultured for 24 h to permit cell attachment. After 24 h, a sterilized suspension of nanogels in phosphate buffer was added such that 0.4 mg of nanogels was added to each well. The cell viability was measured at the 48-h time point using live/dead staining to estimate the cytotoxicity of the Dox-loaded nanogel before addition of the reducing agent. After this analysis was performed, 20 wt % glutathione (as compared to the amount of nanogels, corresponding to 0.26 mmol/L) was added to the wells containing the nanogels, and 16 wt % free Dox (as compared to the amount of nanogels, corresponding to 0.12 mmol/L) was

Scheme 2



added to some control wells without nanogels. The resulting mixtures were maintained for another 48 h, and live/dead assays were performed at the 72 and 96 h time points. Figure 5 shows the results of cell viability. Before addition of glutathione, it was interesting to observe that the viability of HeLa cells in the presence of Dox-loaded nanogels was similar to the control. This suggests that most Dox remained in the nanogels with no significant nonspecific leaching of Dox from the nanogels. This minimization of nonspecific release of drugs during circulation in the blood will be important for in vivo application of the nanogels as drug delivery carriers. Upon the addition of glutathione, the nanogels were degraded and released Dox to kill HeLa cells. In addition, control wells without nanogels, but containing 20 wt % glutathione, also showed high viability (greater than 90%) throughout the experiment (not shown). The addition of free Dox to control wells after 48 h quickly decreased the cell viability.

From an analysis based on live/dead cytotoxicity assays after Dox release, the cell viability was ca. 60% after 96 total h (48 h after adding glutathione) in the presence of Dox-loaded nanogels, which is lower than that for the control (90%) but around 3.3 times higher than that (18%) in the presence of free Dox. This can be explained through the visual observance of some non-degraded nanogel fragments seen in the DIC images. The addition of 20 wt % glutathione did not fully degrade the nanogels in the time period allowed, leading to partial release of Dox from the nanogels. A progressive decrease in viability was observed, indicating that the Dox was released gradually, and not instantaneously. As shown in Figure 2, the addition of 20 wt % glutathione resulted in 85% degradation within 1 h. However, the method for determining degradation involved rapid mixing, as well as removal of soluble species in the supernatant after centrifugation. The degradation rate is overestimated using this protocol because large partially degraded but soluble pieces could be removed following this procedure. These pieces may still contain a tight network and may be unable to release Dox. For the experiment described in Figure 5, the glutathione must diffuse from the media into the gel without agitation. Furthermore, partial degradation may not effectively release the Dox molecules. The slower release in vitro can therefore be attributed to these factors.

This experiment demonstrates that the Dox-loaded nanogels are essentially nontoxic before addition of the reducing agent (48 h mark in the graph), but after the reducing agent is added, the drug is released, and the cell growth is significantly inhibited due to the presence of released Dox (96 h mark). In another experiment where Dox-loaded nanogel (1 mg/mL) and 100 wt % of glutathione were added, the viability decreased to 50% after only 24 h incubation. This suggests that the presence of more glutathione increases the rate of degradation in vitro.

Bioconjugation of Nanogels. To explore applicability toward bioconjugation, OH-functionalized nanogels were prepared by introducing 2-hydroxyethyl acrylate (HEA, 10 mol % of OEOMA) at certain conversion (ca. 30%) during inverse miniemulsion ATRP of OEOMA (Scheme 2). In this way, the nanogels consist of cross-linked block copolymers of POEOMA-*b*-P(OEOMA-*co*-HEA) with pendent OH groups. The resulting P(OEOMA-*co*-HEA) is a gradient copolymer with HEA units located mostly at the end of the gradient block copolymers because methacrylates are more reactive than the corresponding acrylates. Dynamic light scattering (DLS) measurement shows that the average particle size was 225 ± 24 nm for the homopolymeric nanogels and 236 ± 29 nm for OH-containing nanogels, indicating an increase in the average size of OH-nanogels by 10 nm after the addition of HEA.

After purification, the facile functionalization of the OH-nanogels was demonstrated by reaction with biotin (Vitamin H) using a carbodiimide coupling reaction, where the pendent OH groups on the nanogels were reacted with the carboxylic acid groups of biotins (Scheme 2). Excess biotins were removed by extensive dialysis in aqueous $NaHCO_3$ solution, and the biotin-functionalized nanogels were lyophilized.

The bioavailablity of biotin present in the OH-functionalized nanogels to its protein receptor was evaluated by the avidin/2-(4-hydroxyphenylazo)benzoic acid (HABA) binding assay and optical fluorescence microscopy. The avidin/HABA assay allows for determination of the amount of biotin in the functionalized nanogels. Figure 6 shows the UV−vis spectra of avidin−HABA complex before and after addition of biotin-functionalized nanogels. Upon the addition of biotin-functionalized nanogels, absorbance at 500 nm sharply decreased from 0.79 to 0.27, indicating that the biotin molecules in the nanogels competitively bind to avidin, by replacing HABA molecules. Using the calibration plot of free biotin in water, reported by Wooley and cowokers,[73] the difference of absorbance allows determination of the amount of biotin in nanogels to be 16.7 nmol/mg polymer, which corresponds to 142 000 biotin molecules available in each nanogel particle, based on the size of nanogels in water, 310 nm in diameter.

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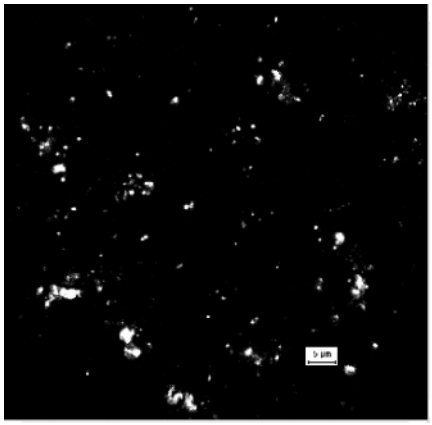

Figure 7. Optical fluorescence microscopy image of FITC−avidin−biotin-nanogel conjugates. Scale bar = 5 μm.

The bioavailability of biotins in nanogels was further demonstrated by mixing the biotin-functionalized nanogels with fluorescein isothiocyanate-labeled avidin (FITC−avidin) in PBS buffer. The formation of aggregates of avidin−biotin-nanogels was observed by optical fluorescence microscopy. As shown in Figure 7, the distinct bright spots on the dark background indicate the formation of a complex of biotin-nanogels with FITC−avidin, contained 2−4 mol of fluorescein isothiocyanate per avidin molecule). The large fluorescent spots may indicate the formation of large aggregates because each avidin has four binding pockets to biotin and each nanogel is conjugated with 140,000 biotin molecules. Such formation of aggregates of avidin and biotin-functionalized polymers was also found in the literature.[73−75]

Conclusions

Stable biodegradable nanogels cross-linked with disulfide linkages were prepared using ATRP in inverse miniemulsion. These nanogels consist of POEOMA with pendent oligo(ethylene glycol), an analogue of linear PEO, which is expected to prevent protein adsorption to the nanogels. The live/dead cytotoxicity assays revealed that the viability of HeLa cells was 95%, indicating that the resulting nanogels were biocompatible and nontoxic to cells.

These nanogels were biodegraded into soluble polymers in the presence of reducing agents such as a water-soluble biocompatible glutathione tripeptide. The results suggested that over 90% of the nanogels degraded within 3 h in the presence of 20% glutathione in water.

Water-soluble R6G was loaded into nanogels. The resulting R6G-loaded nanogels were degraded in the presence of water-soluble reducing agent, and the release of R6G molecules from the nanogels was monitored using optical fluorescence microscopy. The controlled degradation of nanogels was demonstrated in vitro where addition of glutathione to R6G-loaded nanogels incubated with C2C12 cells released R6G molecules, which penetrated cell membranes, staining the cells.

The loading of Dox as a model anticancer drug into nanogels was explored. It was found that the loading level increased from 5.4% to 16.7%, while the loading efficiency decreased from 70% to 50%, when the initial ratio of Dox/nanogels increased from 0.07/1 to 0.35/1. An aliquot of Dox-loaded nanogels (16.4% loading level) was incubated with HeLa cells. After 48 h, glutathione (0.26 mmol/L) was added to degrade Dox-loaded nanogels, resulting in the release of Dox to the cells. The viability of HeLa cells was determined to be ca. 60%, as compared to 90% for control (no nanogel added). With a 5-fold higher glutathione ratio, the viability of HeLa cells with Dox-loaded nanogels dropped to 50% after only 24 h.

In addition, HEA was introduced into nanogels to form OH-functionalized nanogels. This accomplishment underscored the feasibility of bioconjugation of the nanogels by reacting with biotin through the carbodiimide coupling reaction in water. The bioavailability of biotin-conjugated nanogels was determined using an avidin−HABA binding assay to be 16.7 nmol/mg polymer, corresponding to 142 000 biotin molecules in each nanogel particle. Biotin-conjugated nanogels were mixed with FITC-labeled avidin, and the formation of bioconjugates of nanogels with avidin was confirmed using optical fluorescence microscopy.

These overall results from the work reported suggest well-defined functional nanogels may have potential as carriers for controlled drug delivery scaffolds to target specific cells. This outcome would be attainable as a consequence of conjugating the nanogels with biomolecules such as proteins and antibodies that would be cell receptor or ligands. Subsequent to receptor binding, through receptor-mediated endocytosis, the internalized nanogel bioconjugates would be degraded within the cell glutathione. As a consequence of glutathione-induced intracellular biodegradation, the contents of the nanogel would be released. This concept is especially compelling for targeting oncologic cells to induce apoptosis. Moreover, the conjugated nanogel may contain a bioactive molecule that would enhance normal cellular function, such as glucose or protein metabolism. Conjugated nanogels, therefore, may offer considerable versatility for several biomedical applications.

Acknowledgment. Support from the NSF (DMR 05-49353) is gratefully acknowledged. We thank K. Min for the synthesis of TPMA ligand, W. Tang for HPLC measurements of PEOMA526, and Dr. J. Spanswick and Dr. A. Srinivasan for helpful discussions. J.K.O. thanks the Natural Sciences and Engineering Research Council (NSERC) Canada for a post-doctoral fellowship. D.J.S. acknowledges the NSF and Japan Society for the Promotion of Science (JSPS) for the East Asia and Pacific Summer Institutes (EAPSI) fellowship. Partial support for this work was provided by the NIH R01 DE15392 (J.O.H.).

Supporting Information Available: Experimental details including UV−vis spectra of aqueous Dox solution at different concentrations and a linear plot of absorbance versus concentration of Dox. This material is available free of charge via the Internet at http://pubs.acs.org.

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