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SHORT COMMUNICATION

Polyglycol-templated synthesis of poly(*N*-isopropyl acrylamide) microgels with improved biocompatibility

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Abstract We report on the synthesis and characterization of thermally responsive poly(*N*-isopropyl acrylamide) (PNIPAM) nanoparticle hydrogels (i.e., microgels). Microgels with narrow size distributions were synthesized after optimizing the concentrations of monomer, surfactant, and initiator. Polyglycol block copolymers (trade name Pluronic) and sodium dodecylsulfate (SDS) surfactants were compared. In all cases, the particles' size decreased with increasing surfactant concentration, and comparable sizes could be produced with any of the surfactants. The choice of surfactant, however, had a significant influence on the biocompatibility of the PNIPAM microgels. The copolymer-stabilized microgels were less cytotoxic than those stabilized by SDS, as measured using 3(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assays. Even after dialysis (for 3 days) to remove most surfactant, the SDS-based microgels remained more cytotoxic than particles prepared with Pluronics. After exposing cells to polyglycol surfactant solutions, it was found that the polyglycol with highest fraction of polyethylene oxide (Pluronic F127) showed the lowest level of cytotoxicity over the studied range of concentrations. Similarly, PNIPAM microgels synthesized with this surfactant had the lowest level of cytotoxicity. Finally, drug loading and release studies were performed using doxorubicin as a model drug.

Keywords *N*-isopropylacrylamide · Cytotoxicity · Temperature-sensitive nanoparticles · Pluronic · Drug delivery

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Introduction

Nanoscale particles consisting of cross-linked poly(*N*-isopropyl acrylamide) (PNIPAM) hydrogels have been extensively investigated due to their potential for use in many applications, including drug delivery systems [1–7]. Lyon et al. have recently provided a tutorial review describing PNIPAM-based microgels [8]. When dispersed in water, PNIPAM microgels can undergo rapid reversible transitions from a swollen hydrated state to a collapsed state when subjected to small changes in temperature. This feature of PNIPAM microgels allows for the loading and release of substances, resulting from swelling and collapse, to be directed by environmental stimuli, thereby making them attractive for use in controlled drug delivery. The temperature-induced transition in PNIPAM microgels

results from weakening of the hydrogen bonds between the amide functional groups and the water molecules at elevated temperature. The transition for pure PNIPAM occurs at a lower critical solution temperature (LCST) of approximately 32 °C, which is between room temperature and physiological temperature. It has been found that the LCST can be adjusted through copolymerization with various monomers [9–11]. For example, Gao and Frisken recently evaluated the impact of various comonomers and found that hydrophobic comonomers produced smaller collapsed particles with lower LCST, whereas hydrophilic comonomers yielded larger swollen particles with higher and broader LCST [12]. In addition, Chen et al. used vinyl pyrrolidone as a comonomer and found it sharpened the LCST and when also using an acrylamide comonomer, they could obtain abrupt LCST at and above body temperature [5].

Several studies have specifically examined the use of PNIPAM microgels for drug delivery. One of the main findings is that the chemical change from hydrophobic to hydrophilic, above and below the LCST, can be used to make the drug-loaded microgels accumulate in targeted regions. Chen et al. loaded PNIPAM copolymer microgels (LCST >38 °C) with a near-infrared (IR) dye, injected them into a mouse, and found that the particles accumulated in locally heated regions of the body (i.e., hyperthermia treatment) [13]. Tailfer et al. prepared drug-loaded pH-sensitive PNIPAM copolymer micelles and found that these micelles were incorporated into model tumor cells at a higher level than surfactant micelles [14]. In addition, the chemical interactions between the drug and microgel have been found to result in delayed release, especially for hydrophobic drugs. Lopez et al. synthesized PNIPAM microgels in the presence of representative drugs, and found that the drugs were incorporated into the center of the microgels. The release rate was dominated by the drugs' hydrophobicity, with hydrophobic drugs released at a delayed rate [4]. Hsiue et al. loaded PNIPAM particles with glaucoma treating drugs and found that they were released over a longer period than conventional ophthalmic formulations [15]. Chen et al. evaluated microgels and drugs with varying hydrophobicity to optimize the loading and release rates of the representative drug molecules [5]. Another aspect of using microgels for drug delivery is to prevent degradation of the drugs. Leobandung et al. loaded insulin into poly(ethylene glycol)-PNIPAM microgels and found the insulin was not degraded after being subjected to high temperature and high shear stress [16]. Recently, in this laboratory, we explored the synthesis of poly(*N*-isopropylacrylamide-co-acrylamide-co-allylamine) to increase the LCST and provide pH-sensitive amine functional groups. Microgels were prepared with LCST of 39 °C and 40 °C. After loading these microgels with the drug doxorubicin, it was found that the drug was released rapidly at 41 °C whereas the drug release significantly slowed at 37 °C [17]. These results demonstrate that the LCST can be used to control drug release, by abruptly transitioning to a hydrophobic state. In addition, the expulsion of water, upon microgel collapse, is expected to sweep drug out, as was outlined by Hsiue et al. [15]. Another study in our laboratory embedded PNIPAM microgels within a poly (ethylene glycol) matrix, and it was found that bovine serum albumin release from the composite hydrogel was accelerated at temperatures above to the PNIPAM LCST [2].

PNIPAM particles (100 nm to a few micrometers) were first synthesized by Pelton et al. without any surfactant, where the particles were stabilized by the low level of surface charge provided by the initiator [18]. They later modified the synthesis to include sodium dodecyl sulfate (SDS) as a surfactant to stabilize the microgels in aqueous solution and control their size, which allowed for smaller and more uniform size particles to be made [19, 20]. Several other studies have examined details of PNIPAM microgel synthesis and their properties [2, 21–26]. Syntheses of PNIPAM microgels typically use SDS, an anionic surfactant. Anionic surfactants, however, are known to exhibit toxic symptoms. Specifically, they have been found to modify protein structures, resulting in malfunctioning of enzymes and phospholipid membranes [27, 28]. The undesirable properties of SDS have been, in part, addressed by removing most of the SDS through dialysis. On the other hand, Konak et al. recently evaluated the use of various surfactants to disperse uncrosslinked PNIPAM homopolymers above the LCST, finding that a wide variety of surfactants were successful [29]. They noted that given the success of various other surfactants, it would be reasonable to select more biocompatible ones.

The polyglycol copolymer surfactants used in this work (trade name Pluronic, also referred to as poloxomers) are commonly used biocompatible surfactants. They are a class of nonionic surfactants that are approved excipients by the US Food and Drug Administration. Pluronics consist of hydrophilic poly(ethylene oxide) chains covalently bonded to the ends of a relatively hydrophobic poly(propylene oxide) chain, thus forming a linear triblock copolymer (often denoted as ethylene oxide $(EO)_nPO_mEO_n$, where n and m represent the degree of polymerization for each block). In dilute aqueous solutions, these polymers self assemble into micelles, having critical micelle temperatures from 25 °C to body temperature 37 °C [30]. Due to these characteristics, these polyglycols have been used in drug delivery applications [31–34]. For example, Kabanov et al. have evaluated specific uses of Pluronic surfactants in drug delivery, including cancer treatments. They found that Pluronic L61 hypersensitized cancer cells, improving the effect of an anti-cancer drug by two orders of magnitude [35]. Kabanov et al. have noted that Pluronic-formulated

Table 1 Reagents and their respective amounts for synthesizing various PNIPAM microgels

Surfactant type	Surfactant (g)	NIPAM (g)	KPS (g)	Water (mL)	Microgel diameter (nm, DLS[a], poly[b])	Microgel diameter (nm, TEM[c])
SDS	0.021	1.0	0.040	66.7	293, 0.053	179±12
L64	0.125	1.0	0.065	63.9	421, 0.032	168±15
P65	0.118	1.0	0.073	60.1	398, 0.155	169±13
P85	0.118	1.0	0.069	61.3	374, 0.050	172±7
F127	0.184	1.0	0.069	61.3	216, 0.232	184±11

All samples contained 0.024% by mass of BIS

[a] Particle size measured by DLS (Brookhaven 200SM)

[b] Second order polydispersity indexes, μ_2/Γ^2, resulting from Cumulants method fitting

[c] Particle diameter±standard deviation

drugs have met the efficiency criteria in all models by the National Cancer Institute for tumor inhibition and increased lifespan [32, 33, 36]. In another work, an example of using polyglycols as dispersants in nanoparticle synthesis was described by Vandervoot et al. [37]. They have recently used Pluronic copolymers to synthesize polylactic acid nanoparticles, thereby improving their biocompatibility.

In this work, we conduct dispersion polymerizations of PNIPAM microgels using polyglycol copolymers as steric stabilizers. The microgels are compared with those synthesized with SDS in terms of their ability to produce stable particles with narrow size distributions. We also study the impact of surfactant type on the LCST transition of the microgels. Furthermore, cytotoxicity measurements using fibroblast cells are carried out to evaluate the effect of surfactant type. In addition, the impact of dialyzing the microgels is examined. Finally, we examine the loading and release of doxorubicin from dialyzed PNIPAM microgels as influenced by surfactant.

Experimental methods[1]

Materials

N-isopropyl acrylamide (NIPAM, 97% purity, Sigma-Aldrich), sodium dodecyl sulfate (SDS, 99% purity, Sigma-Aldrich), *N,N′*-methylenebisacrylamide (BIS, Sigma-Aldrich), and potassium persulfate (KPS, 99%+ purity, Sigma-Aldrich) were purchased and used without further purification. Unpurified NIPAM was only used after comparing the obtained materials to those produced from NIPAM that had been recrystallized, and observing no difference. Polyglycol block copolymers, nominally $EO_{13}PO_{31}EO_{13}$ (Pluronic L64, M_n of 2,900 g/mol, 40% ethylene oxide (EO) by mass), $EO_{19}PO_{30}EO_{19}$ (Pluronic P65, M_n of 3,400 g/mol, 50% EO by mass), $EO_{26}PO_{41}EO_{26}$ (Pluronic P85, M_n of 4,600 g/mol, 50% EO by mass), and $EO_{100}PO_{67}EO_{100}$ (Pluronic F127, M_n of 12,600 g/mol, 70% EO by mass) were purchased from BASF and used as received. Doxorubicin hydrochloride (DOX) was purchased from Sigma-Aldrich and used as received. Deionized water (Millipore, specific resistance=18 MΩ·cm) was used for all experiments.

Synthesis of PNIPAM microgels

PNIPAM microgels were synthesized using either SDS or a polyglycol surfactant. The microgels were produced by a free radical dispersion polymerization described elsewhere [2, 20]. As a brief example, when producing SDS-stabilized PNIPAM microgels (PNIPAM-SDS), NIPAM (1.5 g) was dissolved in deionized water (90 mL). Under an argon atmosphere, BIS (26.1 mg) and SDS (30.8 mg) were added to the reaction flask, and stirred for 30 min at room temperature. The solution was heated to 70 °C, and the KPS initiator (6 mg/mL, 10 mL) was added. After 4 h, the nanoparticle product solution was cooled and dialyzed (molecular mass cutoff of 6,000 to 8,000 Da) for 3 days to remove free surfactants and unreacted monomers. The dialyzed microgel solution was lyophilized, and solid PNIPAM-SDS nanoparticles were collected. PNIPAM microgels using Pluronic surfactants L64 (PNIPAM-L64), P65 (PNIPAM-P65), P85 (PNIPAM-P85), and F127 (PNIPAM-F127) were synthesized according to the concentrations specified in Table 1. The reactions were conducted multiple times in different labs to ensure reproducibility.

Characterization of PNIPAM microgels

Transmission electron microscopy (TEM; JEOL 1200 EX Model, JEOL) was used to determine the size of the synthesized PNIPAM microgels. Samples were prepared by

[1] Equipment and instruments or materials are identified in the paper in order to adequately specify the experimental details. Such identification does not imply recommendation by NIST, nor does it imply the materials are necessarily the best available for the purpose.

drop casting an aqueous dispersion of microgels onto a carbon coated copper grid. The nanoparticles were positively stained with 1% mass fraction of uranyl acetate before observation, following previous reports [38]. Measurements of microgel size and size distributions were also performed using dynamic light scattering (DLS) instruments (Nanotrac 150, Microtrac Inc. and a Brookhaven BI-200SM). The LCST of PNIPAM microgels was measured using differential scanning calorimetry (DSC; Perkin-Elmer 7 series, DSC 7). The PNIPAM microgel solution was placed in hermetically sealed aluminum pans and heated at a rate of 1 °C/min. DSC described the value and breadth of the LCST. In addition, the LCST was directly observed by noting the associated abrupt change in turbidity upon passing through the LCST. Visual measurements were facilitated by comparing each heated sample solution with an identical unheated one.

Effect of free surfactants on cell viability and morphology

To evaluate the effect of free surfactants on fibroblast cell survival, fibroblast cells were cultured in complete medium consisting of Dulbecco's Modified Eagle Medium supplemented with 10% (by volume) fetal bovine serum and 1% (by mass) penicillin-streptomycin (Invitrogen) and seeded in a 48-well plate for 2 days to allow cells to adhere and grow prior to experiments. All surfactants (SDS, L64, P65, P85, and F127) were sterilized under ultraviolet light for 30 min and dissolved in cell complete media to obtain final concentrations of 0.54, 1.62, 4.87, 14.63, and 43.9 mg/mL. Cells were incubated with surfactant solutions for 6 h. Cells cultured in media without surfactants were used as a control. Cell survival was determined using colorimetric 3 (4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2H-tetrazolium, inner salt (MTS) assays (CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay, Promega), following the manufacturer's instructions.

To visually observe the effect of the free SDS surfactants on cell adhesion and morphology, fibroblast cells were incubated with SDS solutions with 0.54 mg/mL and 14.63 mg/mL concentrations. After 6 h of incubation media from the wells were removed and replaced by a fresh media. Cells then were observed and photographed under an inverted microscope (Axiovert 40 CFL, ZEISS).

Effect of non-dialyzed and/or dialyzed PNIPAM microgels on cell viability

To evaluate the impact of particle dialysis, undialyzed and dialyzed PNIPAM-SDS microgels (3 days of dialysis) were studied. Microgels were sterilized under UV light for 30 min and dissolved in cell complete media to obtain final concentrations of 5 and 20 mg/mL. The microgel solutions were added to the wells containing cells. Cells were incubated for 6 h and cell survival was determined using MTS assays. The cells incubated with microgel-free complete media served as controls.

Upon analyzing the results of the effect of dialysis study, all types of PNIPAM microgels (synthesized using both SDS and polyglycol surfactants) were dialyzed for 3 days to remove free surfactants and unreacted monomers. A dose dependent cytotoxicity study was performed to evaluate the effect of nanoparticles on cell survival. Microgels were sterilized as described above, and were dissolved in cell complete media to obtain final concentrations of 1 and 5 mg/mL. Cells were incubated in microgel solutions for 6 h and cell survival was determined using MTS assays.

In addition, statistical analysis was performed on the data related to the effect of free surfactants and nanoparticles on cell viability, and was performed using ANOVA post-hoc comparisons and t tests with $p<0.05$ (StatView 5.0 software, SAS Institute). For each study, the sample size was four ($n=4$) and all the results are presented as mean±standard deviation. Percentage cell survival was determined by dividing the absorbance reading of a cell sample by the representative control.

To evaluate the impact of dialysis time, microgels were synthesized and dialyzed for 0 to 5 days. The microgels were sterilized and dissolved in cell complete media at a concentration of 5 mg/mL. The microgels were then removed from the solution via filtration (0.2 μm filter) and centrifugation (37 °C, 10 min, 2,000 rpm). Absence of microgels was observed by solution clarity above the LCST. The filtrate was used to conduct cell viability measurements, with the results describing the toxicity of the materials free in solution, but not dialyzed out. That is, it was a means of evaluating how successful the dialysis period was at removing non-microgel materials from the solution, and to what extent these non-microgel materials contribute to cytotoxicity.

Drug loading and release study

DOX, used as a model drug, was loaded into the PNIPAM microgels. Doxorubicin and PNIPAM nanoparticles were dissolved in phosphate buffer solution (PBS), and the solution was incubated at 4 °C on a shaker for 3 days to allow DOX to be incorporated into the microgel network. The solution was transferred into dialysis bag (10 kDa relative molecular mass cut-off) and dialyzed against PBS for 3 h (this dialysis time was predetermined, based on the optimal separation of the unencapsulated drug) to separate free doxorubicin from the solution. The dialysate was analyzed using an Infinite M200 plate reader (Tecan) to quantify the amount of DOX in the dialysate (λ_{ex} 470 nm and λ_{em} 585 nm). The loading efficiency was taken as the difference between the total amount of DOX used and the

amount present in the dialysate, divided by the total amount of DOX used (i.e., loading efficiency $=([\text{DOX}]_{\text{initialtotal}} - [\text{DOX}]_{\text{dialysate}}) / [\text{DOX}]_{\text{initialtotal}} \times 100\%$).

To study the doxorubicin release over time, doxorubicin loaded PNIPAM microgels were dialyzed against PBS at 37 °C. At predetermined time intervals, dialysate (1 mL) was removed and stored at −20 °C for later analysis. Fresh PBS (1 mL) was added to reconstitute the dialysate volume. After a minimum 86 h of release, the dialysate samples were read at an excitation wavelength of 470 nm and emission wavelength of 585 nm using a spectrofluorometer (Infinite M200, Tecan). The concentration of doxorubicin in the dialysate samples were calculated against the doxorubicin standard samples. The drug release rates (R) were calculated using

$$R = \frac{D_2 - D_1}{t_2 - t_1}$$

where R is drug release rate between two time points (microgram per hour) whereas D_1 and D_2 (microgram) are amount of drug release at time t_1 and t_2, respectively.

Results and discussion

PNIPAM microgel synthesis

PNIPAM microgels were synthesized using various polyglycol copolymers as dispersants. Table 1 lists example recipes that successfully produced particles with narrow size distributions. The size distribution was characterized both with DLS and TEM. TEM micrographs are shown in Fig. 1. The particles ranged in size from 200 to 420 nm, diameter, in the hydrated state, as determined by DLS measurements of solutions below the LCST, whereas the particles were much smaller in their collapsed dried state, as measured by TEM. TEM images also showed that the microgels were spherical in shape. In all cases, the microgels were observed to remain dispersed in aqueous solution for long periods of time. That is, no precipitation or sedimentation was observed after 6 weeks. DLS measurements, however, are quite sensitive to observing small levels of aggregation and in the case of the microgels stabilized with Pluronic F127 and L64, DLS identified some 1 μm aggregates after a few days of storage at room temperature. It was observed that proper dispersion of microgels by polyglycol copolymers, as opposed to SDS, was more sensitive to the reaction composition. That is, a higher number of reactions involving polyglycols were found to result in particle aggregation or precipitation. This is expected, due to the lack of electrostatic repulsion associated with the non-ionic polyglycol surfactants. On the other hand, many reactions did yield well-dispersed microgels with narrow size distribution. Several example reaction compositions yielding narrow size distributions are listed in the Table S1. It was observed that the particle size consistently decreased with increased surfactant loading, and with decreased initiator loading. Thus, a wide range of microgel sizes, beyond the range presented in this work, can be synthesized.

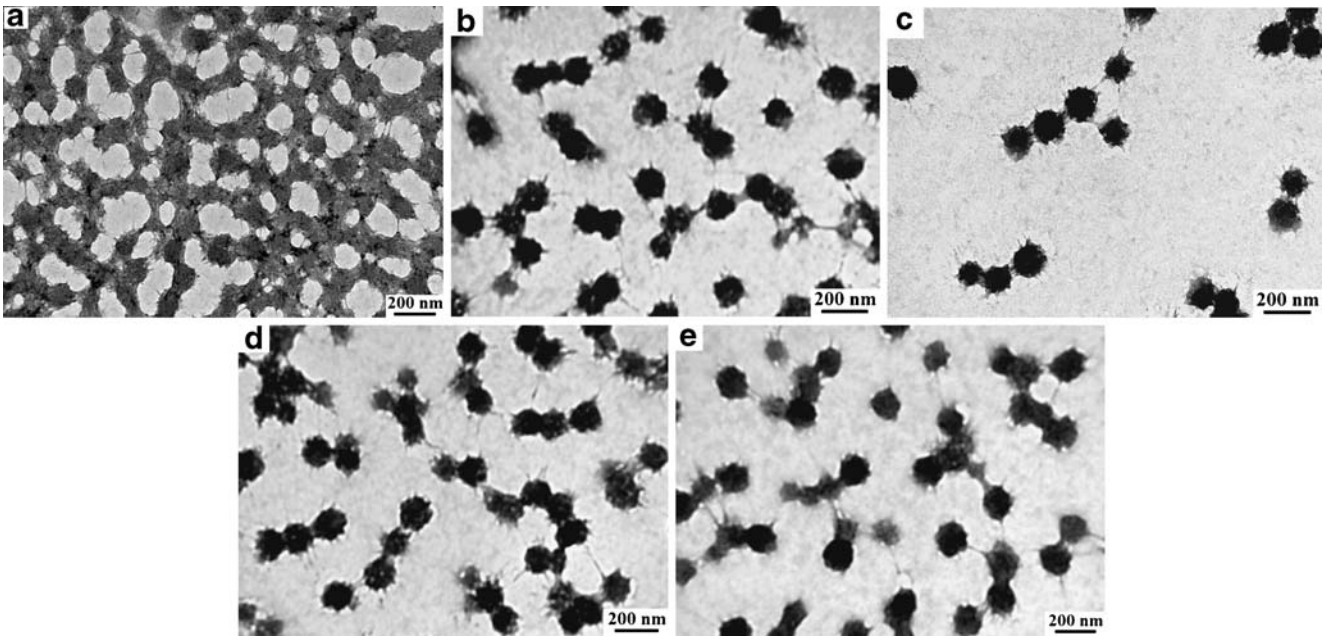

Fig. 1 TEM images of samples described in Table 1, **a** PNIPAM-SDS, **b** PNIPAM-L64, **c** PNIPAM-P65, **d** PNIPAM-P85, and **e** PNIPAM-F127 show these microgels possess a narrow size distribution. All of the samples were stained, resulting in a black color. Excess stain is present in (**a**), partially obscuring outline of the microgels

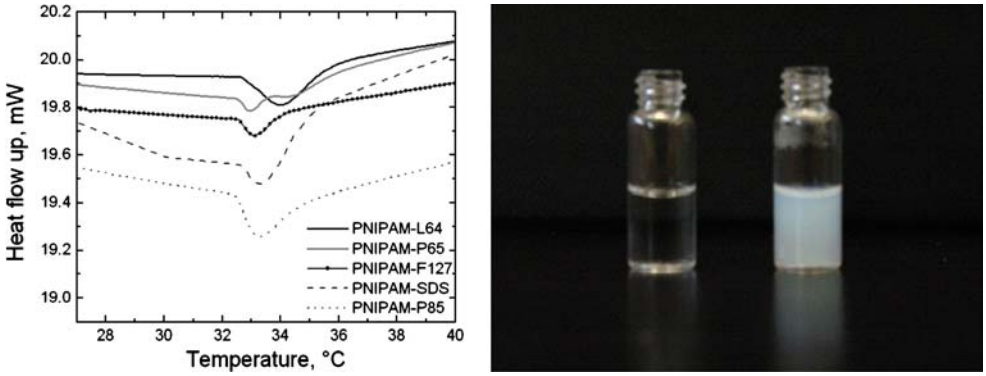

Fig. 2 The LCST of PNIPAM microgels was determined by DSC (*left*) and in all samples, was 33.5±1.5 °C. These measurements were made on samples described in Table 1. An example of a microgel sample (*right*) demonstrates the change in turbidity associated with LCST, allowing the LCST to be visually monitored

DSC data are shown in Fig. 2. In each sample there is a detectable endothermal peak, with a minimum located at 33.5±1.5 °C, corresponding to the LCST. In all cases, the onset of the LCST, upon heating, occurs at 33 °C, and spans <3 °C. The LCST were also observed visually by heating the PNIPAM solutions through the LCST while comparing to an unheated solution. The LCST were observable through an abrupt change in turbidity, which is caused by the increase in refractive index contrast between the microgels and the solvent after they collapse and expel water. Thus, the polyglycol-based microgel solutions have LCST values (33 °C) and temperature spans (<3 °C) that are in good agreement with SDS microgel samples examined in this work and reported in literature [25]. Thus, switching to polyglycol surfactants does not noticeably modify the thermal properties of PNIPAM microgels.

Effect of free surfactants on cell viability and morphology

Cells were incubated with various concentrations of free surfactants to evaluate their biocompatibility, in terms of cell survival. An MTS assay was used to quantify the number of viable cells after incubation. The MTS assay contains a salt that the mitochondrial dehydrogenase enzyme of viable cells reduces to a colored formazan product, whose concentration can be determined by absorption measurements at λ=492 nm. Thus, the absorption measurements quantify the number of cells that remained viable after culturing. The biocompatibility of free surfactants is described in Fig. 3. SDS had higher toxicity than the polyglycol surfactants at all concentrations. Pluronic L64 and P65 were found to be the least biocompatible among the polyglycol surfactants and exhibited cytotoxicity at low concentrations (1.62 and 4.87 mg/mL). Pluronic P85 and F127 were more biocompatible than L64 and P65 at high concentrations. In addition, F127 showed the highest percentage cell survival among all surfactants and exhibited little toxicity even at the highest concentration (43.9 mg/mL). It is noted that the biocompatibility of the polyglycol surfactants was dictated by the percentage of poly(ethylene oxide) in the polymer, as is shown in Fig. 3b. The level of cell survival increased as the percentage of poly(ethylene oxide) in the polymer increased. F127 contains the highest mass fraction of poly

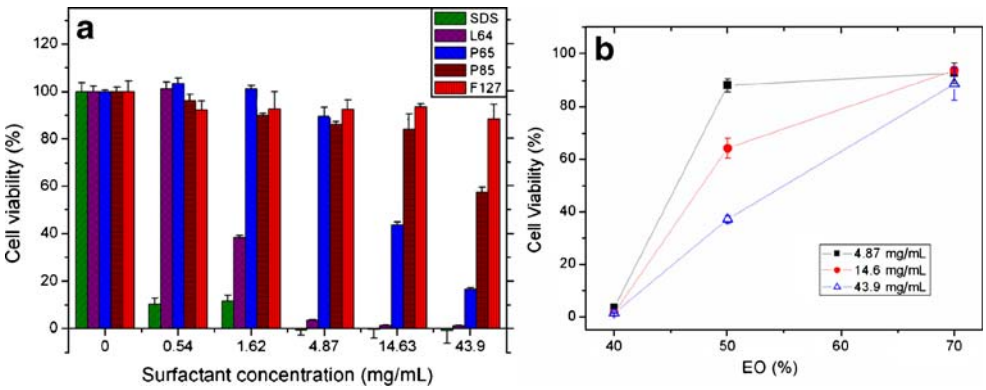

Fig. 3 Effect on cell viability of **a** free surfactants at various concentrations. **b** Cell viability as a function of the percent, by mass, of ethylene oxide in the copolymer surfactant. Standard uncertainties are reported

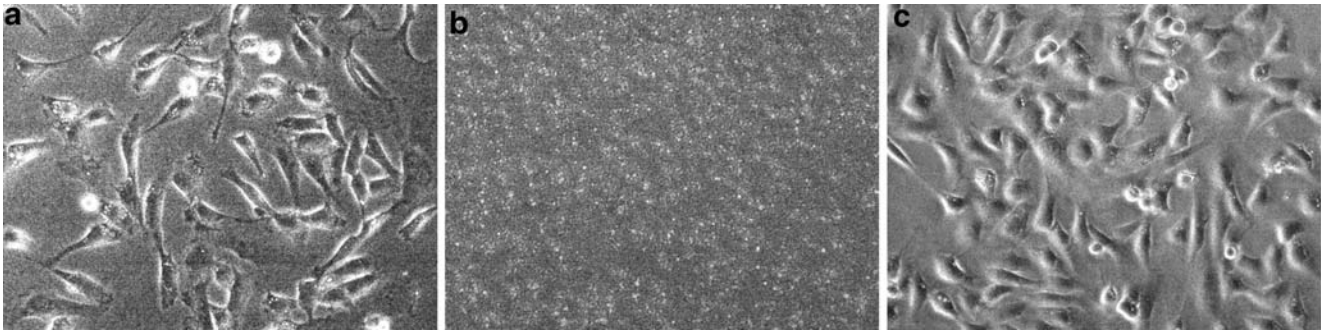

Fig. 4 Phase micrographs of fibroblast cells to show the effect of cell adhesion and morphology for **a** control, **b** 0.54-mg/mL free SDS surfactant addition, and for (c) 14.63-mg/mL free F127 surfactant addition after 6 h of exposure. All images were taken at magnification of ×20 (with a camera ×4 zoom)

(ethylene oxide), 70% by mass, and also was the most biocompatible. Pluronics P65 and P85 had the same mass fraction of poly(ethylene oxide), 50% by mass, but P85 was consistently more biocompatible. This difference may be attributable to the higher molecular mass of P85.

In addition to quantifying biocompatibility with the MTS assay, cells were imaged with optical microscopy, as is shown in Fig. 4. After 6 h of exposure to the surfactant solutions, the level of cell adhesion and the morphology of the cells were observed. The control cells did not show any apparent differences in cell morphology compared with those exposed to polyglycol surfactants (Fig. 4a and c). However, cells exposed to SDS (0.54 mg/mL) had an abnormal spherical morphology, and very few cells were observed as a result of decreased cell survival (Fig. 4b). No surviving cells were observed after exposing them to a higher concentration of SDS (14.63 mg/mL; image not shown). The lack of any cells on the surface, in this case, is likely due to cell detachment from the surface after dying. On the other hand, cells exposed to F127 at

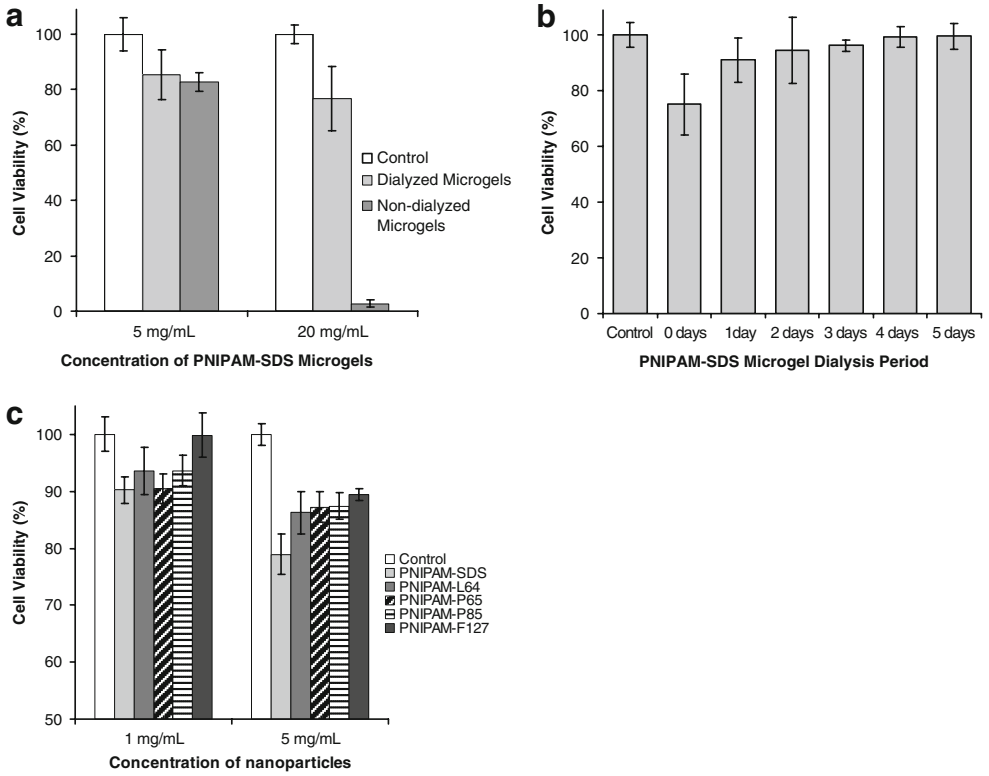

Fig. 5 Evaluation of cell viability. **a** Undialyzed PNIPAM-SDS microgels decrease cell viability, especially at elevated concentration. **b** The impact of dialysis duration on the cytotoxicity of the solution (after the microgels were removed). **c** The impact of various dialyzed microgel solutions on cell viability is compared. Standard uncertainties are reported in all cases

Table 2 Characterization of doxorubicin loading and released

Sample name	Loading efficiency (%)	Overall release (%)[a]	Burst release (μg/h)[b]	Plateau release (μg/h)[c]
PNIPAM-SDS	70.6	70	10.1	0.14
PNIPAM-L64	74.4	84[d]	10.7	0.57
PNIPAM-P65	70.5	70[d]	8.6	0.42
PNIPAM-P85	73.0	77	10.8	0.39
PNIPAM-F127	72.1	86	11.6	0.49

[a] Based on the first 103 h of measurement

[b] Measured from 0 to 24 h

[c] Measured from 24 to103 h (86 h)

[d] Value after 86 h

14.63 mg/mL show normal morphology similar to those of the control sample (Fig. 4c). The corollary of this is that polyglycol surfactants affected neither cell viability nor detachment.

Biocompatibility studies of various PNIPAM microgels

Pluronics in solution were found to be more biocompatible than SDS. Additional measurements were made to evaluate the biocompatibility of PNIPAM microgels synthesized using these surfactants, as is shown in Fig. 5. PNIPAM particles synthesized with SDS (denoted PNIPAM-SDS) were evaluated before and after dialysis. At a high concentration (20 mg/mL), undialyzed PNIPAM-SDS was quite toxic, with only 3% of cells surviving. Dialysis of PNIPAM-SDS greatly improved the biocompatibility, with 77% of cells surviving at 6 h exposure. For consistency, 3 days of dialysis was carried out in all the samples. However, it has been noted that cytotoxicity can arise from both the inherent structure of the microgels and from free SDS not removed by dialysis. In the case of free SDS, cytotoxicity could, in principle, be eliminated through longer dialysis times. To evaluate this issue, microgels were removed from solutions subjected to varying dialysis durations (0 to 5 days). The cytotoxicity of the microgel-free solutions is shown in Fig. 5b. Extending dialysis from 3 days to 5 days does slightly increase the biocompatibility of the filtrate from 96±2% to 99.5±4.5%. However, when comparing to the corresponding sample that does contain microgels (Fig. 5c, 5 mg/mL, PNIPAM-SDS), the cell viability is much lower (79±4.5% vs. 96±2%). Thus, the cytotoxicity observed is, at least in part, attributable to the inherent structure of the microgels, and cannot be erased with a longer dialysis time. The biocompatibility of a series of dialyzed microgel solutions, evaluating SDS and Pluronic surfactants, was also examined, as is shown in Fig. 5c. Despite dialysis, over the course of 3 days, the PNIPAM-SDS microgels were consistently the least biocompatible. The statistical significance of cell survival measurements was evaluated. As shown in Fig. 5c, all four polyglycol surfactants yielded materials that had statistically significant higher biocompatibility (5 mg/mL microgel loading, 95% significance level or higher). Among the polyglycol-based microgels, there appears to be relatively minor differences in biocompatibility compared to SDS. In addition, the results are consistent with examinations made directly on the polyglycol copolymers (Fig. 3), where the biocompatibility was found to be proportional to the relative amount of poly(ethylene oxide) with respect to poly(propylene oxide) in the material. For example, Pluronic F127, which has the highest poly(ethylene oxide) content (70% by mass), exhibited the least cytotoxicity in free form, and also produced microgels that appeared to be slightly more biocompatible than the other materials. It should be noted that since some of the free polyglycol surfactants did not exhibit cytotoxicity at the relevant concentrations (Fig. 4), it may be possible to avoid dialysis, thereby reducing the processing time. On the other hand, use of SDS requires dialysis to avoid reducing the biocompatibility of the microgel materials.

Drug loading and release

Using doxorubicin as a model cancer drug, the loading efficiency for microgels prepared by SDS and polyglycol surfactants was examined. It was found that all of the microgel samples could be loaded with a relatively high level of drug, ranging from 71% to 74% (Table 2). The doxorubicin was loaded at 4°C, well below the LCST (LCST=31°C to 34°C) where the microgels are in a swollen state. When free drug is removed and the microgels suspended in PBS, negligible release of doxorubicin was observed. However, when heated to 37°C, which is above

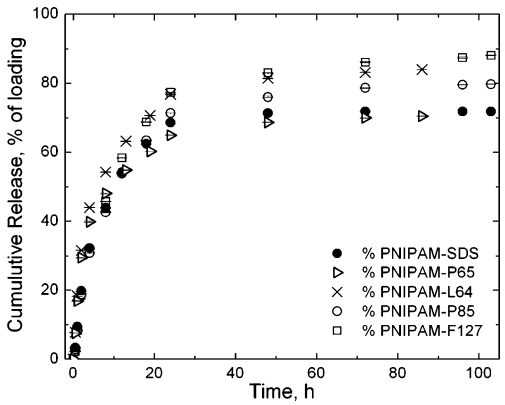

Fig. 6 Doxorubicin release profiles at 37°C. The error bars indicate the standard deviation

the LCST, there was significant release. Release above the LCST is expected to be triggered by the change of the microgels to a hydrophobic character, and by the hydrodynamic motion of the water leaving the collapsing microgel. Figure 6 and Table 2 describe the release of doxorubicin from the microgels at 37°C, where the initial time is taken as the moment when the solution was heated to 37°C. All of the microgels released significant levels of doxorubicin ranging from 70% to 86% after 103 h. The drug release was also evaluated with a biphasic release profile, as described in the experimental section and represented in Table 2. The burst rates (calculated over the first 24 h) were comparable for all microgel samples, whereas the plateau release for the PNIPAM-SDS sample was noticeably lower than the Pluronic based microgels. It is noted that PNIPAM-F127 microgels, which were identified as being the most biocompatible, exhibited high levels of drug loading and release, suggesting they are the optimal surfactant for producing PNIPAM microgels for drug delivery.

These results, as well as those in our previous report [17]. Represent promising demonstrations relevant to the development of temperature-based targeted drug delivery systems. The ability to selectively release a drug at elevated temperature is expected to find applications through hyperthermia treatment or in selectively delivering drug to the intrinsically elevated temperature of tumors and tissue sites. It is also known that loading and release properties strongly depend on the chemical nature of the drug and microgel (e.g., the level of hydrophilicity and whether it is charged). Thus, there remains an ongoing challenge of understanding how to formulate optimized drug-microgel interactions, [4, 5] which warrants further studies. It should be noted, however, that the use of polyglycol surfactants, while notably increasing biocompatibility, did not lower the loading and release of the representative doxorubicin drug. Finally, ongoing work in this laboratory is directed at understanding the implications of switching from an anionic to nonionic surfactant when using other types of drug molecules.

Conclusion

This work has presented a modified synthesis of PNIPAM microgels that uses poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) copolymers (i.e., Pluronics) as surfactants. Substituting these polyglycol copolymers for the typical SDS surfactant removed a cytotoxic ingredient, notably improving the biocompatibility of the microgel solutions. Even after dialysis, over 3 days, the polyglycol templated microgels were observably more biocompatible. It should be noted that a key driver for PNIPAM microgel research is the development of drug delivery systems, thus there is a need to minimize the cytotoxic properties of these materials. PNIPAM microgels are promising materials for drug delivery systems because of their unique thermally responsive properties. It was found, in this work, that the changes made to improve biocompatibility did not significantly affect any of the microgels' key characteristics. Stable dispersions of microgels were prepared with narrow size distributions. However, after replacing the strong electrostatic repulsions of SDS with weaker steric repulsions of the polyglycol copolymers, the reagent composition needed to be formulated more carefully to create stable dispersions. With respect to the thermal properties, there was little observed impact on the LCST value and its breadth. On the other hand, there was some improvement in the drug loading and release properties. An example of drug loading and release was presented using doxorubicin. There were higher levels of both loading and release of this drug, with best results obtained for PNIPAM microgels made with Pluronic F127. This demonstrates the potential for developing these materials into useful temperature-responsive targeted drug delivery systems.

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References

1. Kim IS, Jeong YI, Cho CS, Kim SH (2000) Int J Pharm 205:165–172
2. Ramanan RMK, Chellamuthu P, Tang LP, Nguyen KT (2006) Biotechnol Progr 22:118–125
3. Tasdelen B, Kayaman-Apohan N, Guven O, Baysal BM (2004) Polym Adv Technol 15:528–532
4. Lopez VC, Raghavan SL, Snowden MJ (2004) React Funct Polym 58:175–185
5. Chen HY, Gu YQ, Hu YZ (2008) J Mater Sci - Mater Med 19:651–658
6. Barratt G (2003) Cell Mol Life Sci 60:21–37
7. Rzaev ZMO, Dincer S, Piskin E (2007) Prog Polym Sci 32:534–595
8. Lyon LA, Meng ZY, Singh N, Sorrell CD, John AS (2009) Chem Soc Rev 38:865–874
9. Zha L, Hu J, Wang C, Fu S, Elaissari A, Zhang Y (2002) Colloid Polym Sci 280:1–6
10. Kratz K, Hellweg T, Eimer W (2000) Colloids Surf A 170:137–149
11. Hoare T, Pelton R (2004) Macromolecules 37:2544–2550
12. Gao J, Frisken BJ (2005) Langmuir 21:545–551
13. Chen HY, Zhang J, Qian ZY, Liu F, Chen XY, Hu YZ, Gu YQ (2008) Nanotechnology 19:185707
14. Taillefer J, Jones MC, Brasseur N, Van Lier JE, Leroux JC (2000) J Pharm Sci 89:52–62
15. Hsiue GH, Chang RW, Wang CH, Lee SH (2003) Biomaterials 24:2423–2430

16. Leobandung W, Ichikawa H, Fukumori Y, Peppas NA (2002) J Control Release 80:357–363
17. Rahimi M, Kilaru S, Sleiman GEH, Saleh A, Rudkevich D, Nguyen KT (2008) J Biomed Nanotech 4:482–490
18. Pelton RH, Chibante P (1986) Colloids Surf 20:247–256
19. Wu X, Pelton RH, Hamielec AE, Woods DR, McPhee W (1994) Colloid Polym Sci 272:467–477
20. McPhee W, Tam KC, Pelton R (1993) J Colloid Interface Sci 156:24–30
21. Pelton R (2004) Macromol Symp 207:57–65
22. Gao J, Frisken BJ (2003) Langmuir 19:5217–5222
23. Andersson M, Maunu SL (2006) J Polym Sci, Part B: Polym Phys 44:3305–3314
24. Tan BH, Tam KC (2008) Adv Colloid Interface Sci 136:25–44
25. Pelton R (2000) Adv Colloid Interface Sci 85:1–33
26. Keerl M, Pedersen JS, Richtering W (2009) J Am Chem Soc 131:3093–3097
27. Cserhati T, Forgacs E, Oros G (2002) Environ Int 28:337–348
28. Hrabák A, Antoni F, Szabó MT (1982) Bull Environ Contam Toxicol 28:504–511
29. Konak C, Panek J, Hruby M (2007) Colloid Polym Sci 285:1433–1439
30. Alexandridis P, Hatton TA (1995) Colloids Surf A 96:1–46
31. Nagarajan R (1999) Colloids Surf B: Biointerfaces 16:55–72
32. Kabanov AV, Batrakova EV, Alakhov VY (2002) Adv Drug Deliv Rev 54:759–779
33. Kabanov AV, Batrakova EV, Alakhov VY (2002) J Control Release 82:189–212
34. Allen C, Maysinger D, Eisenberg A (1999) Colloids Surf B: Biointerfaces 16:3–27
35. Venne A, Li S, Mandeville R, Kabanov A, Alakhov V (1996) Cancer Res 56:3626–3629
36. Oh KT, Bronich TK, Kabanov AV (2004) J Control Release 94:411–422
37. Vandervoort J, Ludwig A (2002) Int J Pharm 238:77–92
38. Hoare T, Pelton R (2006) J Colloid Interface Sci 303:109–116