Thermosensitive poly(N-isopropylacrylamide-co-glycidyl methacrylate) microgels for controlled drug release

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A new type of thermosensitive microgels with epoxy functional groups is designed and synthesized for drug delivery. The thermosensitive poly(N-isopropylacrylamide-co-glycidyl methacrylate) (designated as P(NIPAM-co-GMA)) microgels are prepared by an emulsifier-free emulsion polymerization method and the chemical composition of the copolymer is determined by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (1H NMR). The lower critical solution temperature (LCST) of the microgels is 32–34 °C based on the transmission changes at 500 nm monitored by UV/visible spectrophotometry. The hydrodynamic diameter and morphology of the microgel particles are examined by dynamic light scattering (DLS) and scanning electron microscopy (SEM), respectively. The drug release properties determined using 5-FU as the drug model in vitro reveal temperature dependence and low cytotoxicity. The thermosensitive microgels have large potential as targeted anti-cancer drug carriers.

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1. Introduction

Thermosensitive polymers constitute a class of stimuli-responsive materials and are attracting much attention due to their ability to give smart responses to the external environment temperature [1]. Poly(N-isopropylacrylamide) (PNIPAM) is one of the widely studied thermosensitive polymers exhibiting an abrupt volume phase transition at a lower critical solution temperature (LCST) of 32–34 °C [2]. The external temperature affects the hydrophilic and hydrophobic balance of the thermosensitive polymer chains in the aqueous system. At a temperature lower than LCST, the interaction between water and the polymer chains is dominated by hydrogen bonding between the hydrophilic segments, and the polymer absorbs water to become a swollen state. When the temperature is increased above the LCST, the polymer chains shrink and expel water molecules because of the hydrophobic segments. Because the thermal response occurs at a temperature close to the human body temperature of 37 °C, these thermosensitive polymer materials in the forms of micelles, hydrogels, films and spheres have many biomedical and biotechnological applications such as controlled drug delivery [3,4], gene delivery [5], tissue engineering [6,7], and so on.

Thermosensitive microgels are thermo-responsive 3-dimensional polymeric networks in the nano to micro size range. They have the ability to entrap and release drug molecules thus spurring research in both the fundamental mechanism and applications to drug delivery systems. With regard to targeting or interaction, modification of the microgels is sometimes necessary and this can be achieved by the introduction of other monomers with reactive groups into the copolymerization system. For instance, amine, carboxylic acid, and alcohol functional groups (–NH2, –COOH, and –OH) have been incorporated into thermosensitive drug delivery systems using monomers such as acrylic acid (AA), allylamine (AAm) and 2-hydroxyethyl methacrylate (HEMA) [8–11].

Epoxy groups are a good candidate to modify thermosensitive microgels. The advantage of epoxy groups is that they can be easily modified to become vicinal diols, amines, and aldehyde groups [12,13]. The activity ensures them to nonspecifically and moderately interact with bioligands, resulting in enhanced interactions between the microgels and biomolecules like antibodies, proteins and enzymes [14,15]. Moreover, the most common epoxy groups containing the monomer glycidyl methacrylate (GMA) is less hydrophilic than the aforementioned reactive monomers and hence, microgels with GMA disperse well in water. The synthesis and thermosensitive properties of epoxy containing thermosensitive microgels have been studied [16–18] but there have been few reports on their drug delivery behavior.
In this work, functionalized thermosensitive microgels are prepared by incorporating epoxy groups. The thermosensitive microgel particles with a spherical structure and narrow size distribution are synthesized by emulsifier-free emulsion polymerization of NIPAM and GMA. Besides investigation of the structure and morphology, the cytotoxicity of the microgels and associated drug release behavior are assessed in vitro.

2. Materials and methods

2.1. Materials

N-isopropylacrylamide (NIPAM) and glycidyl methacrylate (GMA) were purchased from International Laboratory (USA). Potassium persulfate (KPS) bought from International Laboratory (USA) was purified by recrystallization in ethanol before use. N, N'-methylene-bisacrylamide (MBA) and sodium hydrogen carbonate (NaHCO₃) were purchased from Sigma–Aldrich and 5-Fluorouracil (5-FU) from J&K Chemical Ltd. were used as received. The phosphate-buffered saline solution (PBS, 0.01 M, pH 7.4) was prepared according to standard protocols and Milli-Q ultrapure water (Department of biology and chemistry, City University of Hong Kong) was used in the cytotoxicity experiments.

2.2. Preparation of P(NIPAM-co-GMA) microgels

The thermosensitive P(NIPAM-co-GMA) microgels were prepared by emulsifier-free emulsion polymerization. The typical procedures are described in the following. NIPAM (2.100 g), GMA (0.70 g), crosslinking agent MBA (0.028 g, 1 wt%), NaHCO₃, and H₂O (100 mL) were added to a 250 mL round-bottom flask with a magnetic stirrer, condenser and nitrogen inlet. After dispersing sufficiently with a fine stream of nitrogen at 45 °C for 30 min, KPS (0.016 g, 0.6 wt%) was added to initiate polymerization. The continuous polymerization reaction proceeded for 8 h at 73 °C. After polymerization, the product was purified in a dialysis tube (molecular weight cut-off at 12 kDa, Sigma–Aldrich) with 1000 mL of deionized water for 72 h and it was exchanged at intervals of 12 h. The homopolymer microgels PNIPAM without the co-monomer GMA were obtained by the same polymerization procedures and parameters.

2.3. Structural characterization

The structures of both the PNIPAM and P(NIPAM-co-GMA) were determined by Fourier transform infrared spectroscopy (Spectrum 100, PerkinElmer, USA). The dried samples were compressed into KBr pellets and the spectra were recorded in the wavelength range of 450–4000 cm⁻¹.

The nuclear magnetic resonance (¹H NMR) spectrum of P(NIPAM-co-GMA) was acquired at a UNITY INOVA-600 MHz NMR spectrometer (Varian, USA) at 20 °C with DMSO-d₆ as the solvent. The chemical shifts were reported in ppm with tetramethylsilane as the internal standard.

2.4. Morphology

The morphology of the microgels was examined by scanning electron microscopy (JEOL JSM-820, JEOL, USA). A drop of the diluted sample was placed onto a clean silicon wafer, vacuum dried, and coated with gold before observation.

2.5. LCST measurement

The optical transmittance of the microgel aqueous solution was measured at a wavelength of 500 nm by ultra-violet visible spectrophotometry (Agilent 8453, Agilent technologies, USA) and recorded as a function of temperature from 25 to 45 °C. At least 5 min was allowed in order to reach equilibrium for each temperature and all measurements were conducted in triplicates. The lower critical solution temperature (LCST) of the microgels was defined as the temperature at which half of the total decrease in optical transmittance occurred.

2.6. Dynamic light scattering (DLS)

The hydrodynamic diameters and polydispersity index (PDI) of the microgels were measured using a dynamic light scattering particle size analyzer (Zetasizer Nano ZS, Malvern Instruments, UK) equipped with a He–Ne laser (λ = 632.8 nm). The measurements were performed from 25 to 45 °C. At each temperature, the sample was equilibrated for 5 min before the measurement. The swelling ratio (SR) of the microgels was calculated by the following equation [19]:

\[
SR = \frac{V_{\text{swollen}}}{V_{\text{shrunken}}} = \left( \frac{D_{25}}{D_{45}} \right)^3
\]

where \(V_{\text{swollen}}\) and \(V_{\text{shrunken}}\) were the volumes of microgel particles in the swollen state (25 °C) and shrunken state (45 °C), respectively. \(D_{25}\) and \(D_{45}\) were the mean hydrodiameters of the microgel particles at 25 and 45 °C, respectively.

2.7. Drug loading and release analysis

The anti-cancer drug 5-FU was chosen as for the in vitro drug release model. 50 mg of the P(NIPAM-co-GMA) microgels were added to the 5-FU PBS solution (2 mg/mL) at 20 °C and gently shaken for 24 h to reach equilibrium. The in vitro release behavior of 5-FU from the P(NIPAM-co-GMA) microgels at different temperatures was monitored by the dialysis tube diffusion technique [20]. The drug loaded microgels systems were enveloped into two different dialysis tubes (molecular weight cut-off of 12 kDa, Sigma–Aldrich). The tubes were then dialyzed against the PBS solution in a separate beaker under external stirring at 20 and 37 °C, respectively. At each fixed time intervals, 3 mL aliquots of the release medium was taken out and replaced by 3 mL of fresh PBS solution to maintain the volume of the medium. The released 5-FU was measured by UV spectrophotometry at 265 nm.

2.8. Cytotoxicity measurement

The relative cytotoxicity of the microgels was evaluated by a cell counting kit-8 (CCK-8) against osteoblasts (MC3T3-E1). The cells were cultured at 37 °C under a 5% CO₂ humidified atmosphere in Dulbecco’s modified Eagle’s medium (DMEM). 100 μL of the cell suspension (5 × 10⁴/well) were seeded on 96-well plates and incubated for 24 h. The culture medium was removed and the medium containing different concentrations of the microgel suspension was added and incubated for another 24 h with each concentration having four parallel wells. 10 μL of CCK-8 was added to each well and the plates were incubated for another 4 h. Finally, the absorbance was measured at 450 nm by using a Powerwave XS microplate reader (M3X200R, Biotech Instruments, USA). The relative cell viability (%) was calculated as \(\text{OD}_{450\text{sample}} / \text{OD}_{450\text{control}}\) × 100 [21], where \(\text{OD}_{450\text{sample}}\) was the absorbance at 450 nm of the cells in the medium containing different concentrations of the microgel suspension and \(\text{OD}_{450\text{control}}\) was the absorbance at 450 nm of the untreated cells in the blank medium used as a control (100% viability).
3. Results and discussion

3.1. Chemical structure analysis

The FTIR spectra of the polymers PNIPAM and P(NIPAM-co-GMA) are depicted in Fig. 1. A broad peak at 3200–3500 cm\(^{-1}\) corresponding to N–H stretching can be observed from both spectra. Peaks at 2978, 2932, and 2878 cm\(^{-1}\) are assigned to the absorption peaks of C–H in –CH\(_3\) and –CH\(_2\) and those at 1654 cm\(^{-1}\) and 1548 cm\(^{-1}\) arise from –C=O stretching and N–H stretching of the amide group, respectively. Compared to the FTIR spectrum of PNIPAM, the presence of the epoxy group in the GMA monomer is confirmed by the characteristic peak at 906 cm\(^{-1}\) [22]. The peaks at 1728 cm\(^{-1}\) and 1056 cm\(^{-1}\) can be assigned to the stretching vibration of –C=O and –C–O, indicating the presence of the ester groups [23]. The results corroborate the successful copolymerization reaction between the monomers NIPAM and GMA.

The \(^1\)H NMR spectrum of the copolymer P(NIPAM-co-GMA) measured in DMSO-d\(_6\) is shown in Fig. 2 and the peaks are labeled accordingly. The solvent and water peaks are at 2.50 ppm and 3.33 ppm, respectively. The protons of the amide groups (–CO–NH–, 7.25 ppm) and isopropyl groups (–CH\(_2\)(CH\(_3\))\(_2\)) at 3.83 ppm and 1.04 ppm) from NIPAM are accurately assigned. The peaks of the epoxy groups (–CH–Epox, 3.22 ppm) and –CH\(_2\) protons (4.27 ppm) linked to the ester groups from GMA are also visible in the spectrum. The molar ratio (GMA:NIPAM = 1.575) in the copolymer is calculated from the peak area ratio between the –CH– proton of –O–CH\(_2–\)CH–Epox (f, 3.22 ppm) in the GMA moiety and that of –NH–CH(CH\(_3\))\(_2\) (g, 3.83 ppm) in the NIPAM moiety.

3.2. LCST measurement

The LCST is a key parameter of the thermosensitive microgel representing the ability of the volumetric phase transition of the microgel in the aqueous dispersion. Below the LCST, the polymer chains in the microgels expand because of hydrogen bonding between the hydrophilic groups and water molecules. When the temperature exceeds the LCST, thermal breakage of the hydrogen bonds along with the hydrophobic groups leads to shrinkage of the polymer chains and exclusion of water from the microgel [24].

The LCST is measured by the optical transmittance change as a function of temperature. As shown in Fig. 3, the transmittance decreases significantly when the temperature is increased from 25 to 45 °C, and drops abruptly at 32 °C, which is determined to be the LCST in the system. The visible transition can be observed from the digital picture of the aqueous microgel dispersion at two different temperatures in figure inset. The picture is obtained from the same sample during the cooling and heating cycles and shows the reversibility of the P(NIPAM-co-GMA) microgels. The temperature-dependent phase transition of the P(NIPAM-co-GMA) microgels indicates that the thermosensitive property is not affected by the GMA component.

3.3. Hydrodynamic diameter and swelling ratio

The hydrodynamic diameters and PDI of the P(NIPAM-co-GMA) microgels measured at different temperatures by DLS are shown in Fig. 4. It is evident that the size variation of the microgel particles is similar to the transmittance variation of the microgels dispersions based on UV spectrophotometry. That is, the diameter decreases from 514 nm to 293 nm as the temperature is increased from 25 °C to 45 °C, with the most drastic decrease occurring at the LCST of 32 °C. As discussed above, the microgel particles collapse uniformly without aggregation when the temperature exceeds the LCST due to the volume phase transition. The uniform collapse behavior of the
particles results in the narrow size distribution with the PDI values being less than 0.05 at all the temperatures studied (Fig. 4).

According to the size change during the volume phase transition, we can obtain the swelling ratio of the microgel particles. This is a crucial parameter indicative of the encapsulation efficiency in the drug delivery system. The swelling ratio of the P(NIPAM-co-GMA) microgels is calculated to be 5.38.

3.4. Morphology of P(NIPAM-co-GMA) microgels

The P(NIPAM-co-GMA) microgels are characterized by SEM to provide additional morphological details and the images are shown in Fig. 5. The microgel particles have a regular spherical shape and a size of less than 300 nm. No severe aggregation even at the dehydration state is observed. The surface of the particles is relatively clean, thereby confirming the advantage of emulsifier-free emulsion polymerization to obtain clean polymer particles [25]. The narrow size distribution shown in the SEM image is in agreement with the polydispersity index (PDI) values from the DLS measurement.

3.5. Cytotoxicity of P(NIPAM-co-GMA) microgels

Cytotoxicity tests are carried out to investigate the biocompatibility of the obtained P(NIPAM-co-GMA) microgels. The effects of the concentration on the proliferation of MC3T3-E1 cells are evaluated and the relative viability is shown in Fig. 6. The cell viability
3.6. In vitro drug release analysis

In the design of thermosensitive drug delivery systems, the amount of released drug is an important factor. That is, it is desirable to incorporate drugs at a lower temperature in vitro and have the capacity to release more drugs in vivo at the body temperature. In order to investigate the release behavior of the P(NIPAM-co-GMA) microgels, the anti-cancer drug 5-FU released from the P(NIPAM-co-GMA) microgels is studied at 20 °C and 37 °C in PBS (pH 7.4) and the cumulative release curves are shown in Fig. 7. The cumulative release is about 90% and 98% at 20 °C and 37 °C, respectively. Comparing the two release profiles at different temperatures, the release amounts are 7–10% less at 20 °C than 37 °C at all the measurement time intervals, indicating larger release rates and more released amounts at a temperature higher than LCST. Hydrogen bonding is an important factor determining the drug release behavior [28]. At a temperature (37 °C) above the LCST when hydrogen bonding between the polymer chains and 5-FU molecules weakens or disappears, more drug molecules together with water are expelled from the microgels. In addition, breaking of hydrogen bonding of the polymer chains reduces cross linking locations in the microgel to some extent, thus producing more voids and channels allowing 5-FU to diffuse out.

4. Conclusion

Monodispersed and spherical thermosensitive P(NIPAM-co-GMA) microgels with epoxy functional groups are prepared via emulsifier-free emulsion polymerization. The thermosensitive properties of the microgels are determined by the LCST measurement and hydrodynamic diameter changes as a function of temperature. The microgels have low cytotoxicity and temperature dependent in vitro drug release behavior is observed. The P(NIPAM-co-GMA) microgels have large potential as intelligent drug delivery carriers.

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References