Published in final edited form as:

Bioconjug Chem. 2011 July 20; 22(7): 1416–1421. doi:10.1021/bc200141h.

Inflammation responsive logic gate nanoparticles for the delivery of proteins

Enas A. Mahmoud[†], Jagadis Sankaranarayanan[†], Jose' M. Morachis[†], Gloria Kim[†], and Adah Almutairi^{†,‡,§,*}

[†]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California at San Diego, La Jolla, California 92093-0657

[‡]Materials Science and Engineering, University of California at San Diego, La Jolla, California 92093-0657

[§]NanoEngineering, University of California at San Diego, La Jolla, California 92093-0657

Abstract

Oxidative stress and reduced pH are important stimuli targets for intracellular delivery and for delivery to diseased tissue. However, there is a dearth of materials able to deliver bioactive agents selectively under these conditions. We employed our recently developed dual response strategy to build a polymeric nanoparticle that degrades upon exposure to two stimuli in tandem. Our *poly* thioether ketal based nanoparticles undergo two chemical transformations, the first is the oxidation of the thioether groups along the polymer backbone of the nanoparticles upon exposure to reactive oxygen species (ROS). This transformation switches the polymeric backbone from hydrophobic to hydrophilic and thus allows, in mildly acidic environments, the rapid acid catalyzed degradation of the ketal groups also along the polymer backbone. Dynamic light scattering and payload release studies showed full particles degradation only in conditions that combined both oxidative stress and acidity and these conditions led to higher release of encapsulated protein within 24 hours. Nanoparticles in neutral pH and under oxidative conditions showed small molecule release and swelling of otherwise intact nanoparticles. Notably, cellular studies show absence of toxicity and efficient uptake of nanoparticles by macrophages followed by cytoplasmic release of ovalbumin. Future work will apply this system to inflammatory diseases.

Keywords

Smart nanoparticles; Multi response; Logic gate; *poly* thioether-ketal; Inflammation; Controlled protein release

INTRODUCTION

Inflammation, especially chronic, relates to various diseases including cancerous and cardiovascular. Although there are numerous reports of 'smart' nanoparticles that rapidly and selectively respond to different disease specific stimuli, there is a dearth of nanoparticles capable of specifically targeting inflammation¹⁻². Furthermore, nanoparticles capable of

*Corresponding author: aalmutairi@ucsd.edu .

ASSOCIATED CONTENT

Supporting Information. ¹HNMR of compound 3, ¹³CNMR of compound 3, HR-ESI-FT-MS (Orbit-Trap-MS) of compound 3, ¹HNMR of *poly* 5, ¹³CNMR of *poly* 5, GPC trace of Poly 5 calibrated against Polystyrene standards. This material is available free of charge via the Internet at <http://pubs.acs.org>.

cytoplasmic delivery are particularly challenging and this remains a major hurdle for effective therapeutic delivery³⁻⁴. In achieving increased cytoplasmic delivery burst-fragmenting nanoparticles hold promise as they can lead to elevated osmotic pressure within the endosome leading to endosomal escape of the nanoparticles payload⁵⁻⁷. Important biomarkers of diseased tissue that have been successfully used to trigger nanoparticle degradation include reduced extracellular pH⁸, thermal responsiveness⁹, reductive microenvironments¹⁰ and oxidative stress¹¹. These nanoparticles respond to a single disease stimulus. Furthermore, control over the degradation kinetics and thus the ON/OFF state of the system has been difficult to achieve. For example, polyketals have gained prominence as the choice of drug delivery nanoparticles owing to their rapid pH response¹²⁻¹³. Nanoparticles formulated from these polyketals degrade at acidic pH into acetone and other benign molecules, rapidly releasing the payload within to the cytoplasm¹⁴. However, a key drawback of these systems is that as hydrophobic polymeric nanoparticles their degradation rates are very slow (many days)^{12-13, 15} in acidic pH (pH 6-5) because of reduced water influx. Although a *hydrophobic* backbone is advantageous in formulating stable nanoparticles at physiological conditions, a *hydrophilic* backbone is needed to produce fast release in targeted tissue. On the other hand, a hydrophilic backbone often results in nanoparticles that are not stable in pH 7.4 for longer than a few hours¹⁶. Recently we introduced Logic Gate Nanoparticles that use dual response mechanisms to impart stability to the 'OFF' state while maintaining a rapid degradation or a sharp 'ON' state⁶. We demonstrated the ability of such particles to deliver their payload to the cytoplasm because of the rapid fragmentation of the nanoparticle¹⁴. Here we apply this strategy to physiological cues of inflammatory diseases¹⁷⁻¹⁹. Our new nanoparticles composed of a dual stimuli and response polymeric backbone are stable in healthy physiological conditions yet they are able to rapidly fragment in subcellular and diseased conditions. Rapid fragmentation is important for cytoplasmic release of the bioactive payload.

It is known that polyketals degrade via surface hydrolysis while polysulfides degrade via bulk erosion²⁸⁻²⁹. Herein, we designed a novel *poly* thioether-ketal by incorporating a thioether moiety in our polymer backbone; the thioether acts as a solubility switch that turns the polymer more hydrophilic when it becomes oxidized from a thioether into a sulfone. Sulfones are inherently more hydrophilic because of a strong dipole due to the presence of the sulfur oxygen double bond; this strong dipole leads to an increased affinity for water, resulting in an accelerated rate of hydrolysis of the ketal groups along the backbone. This allowed our polymers sharper more rapid degradation kinetics in comparison to conventional polyketals (Figure 1). Various other polysulfides have been used to successfully formulate oxidation sensitive vesicles¹ and nanoparticles²⁰, with such nanoparticles also being used to selectively target dendritic cells in vivo²¹.

The synthesized *poly* thioether-ketal nanoparticles were prepared to target the oxidative stress areas of many diseases including tumors²², atherosclerosis²³ and causes of aging due to mitochondrial damage²⁴. During these abnormalities, reactive oxygen species (ROS) are formed when oxygen undergoes a partial one-electron reduction to super oxide anion, and subsequently forms hydrogen peroxide, hypochlorite peroxynitrite, and other oxidants²⁵. Herein, the formulated nanoparticles that are triggered by both ROS and low pH (typical of inflamed tissue), function akin to an "AND" logic gate in circuits. We hope that our design will lead to nanoparticles that will better target diseased tissue for drug delivery or diagnostics.

MATERIALS AND METHODS

Materials

Potassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) were purchased from Alfa Aesar Organics (Ward Hill, MA). Dichloromethane (DCM, methylene chloride) was purchased from Fisher Scientific (Hampton, NH). Nile red, *poly* (vinyl alcohol) (PVA) (MW 30-70k) were purchased from Sigma Chemical Co. (St. Louis, MO). 2,2'-thiodiethanol was purchased Sigma Chemical Co. (St. Louis, MO). 4-nitrophenylchloroformate was purchased from Acros organics (Belgium). PLGA (Resomer RG 502H) was purchased from Boehringer Ingelheim (Germany). Ovalbumin Alexa Fluor® 594 was purchased from Invitrogen. All reagents were purchased from commercial sources and were used without further purification unless otherwise stated.

Synthesis of the polymer

As shown in Figure 2, 2,2'-thiodiethanol (compound 1, 3.66g, 0.03mol) was taken with 4-nitrophenylchloroformate (compound 2, 13.3g, 0.064mol) in a dry round bottom with 125 ml of dry dichloromethane and stirred under a nitrogen atmosphere. To this, triethylamine (30 ml, 0.21 mol) in 25 ml of dry dichloromethane was added drop-wise over 30 mins at room temperature. The reaction was subsequently stirred for 4 hrs. At the end of the reaction, the reaction mixture was rotavaped, diluted with 250ml of dichloromethane, and extracted from 2 × 100ml of 1% HCl. The organic layer was dried over $MgSO_4$ and rotavaped. The resulting crude solid was recrystallized from ethylacetate three times to yield 8.0g (0.017mol, 60% yield) of a white solid, MP (138.6-140.1°C)

1H NMR (500 MHz, CHLOROFORM-D) δ 8.28 (d, J = 7.5 Hz, 1H), 7.38 (d, J = 7.5 Hz, 1H), 4.47 (dd, J = 6.3, 5.3 Hz, 1H), 2.97 (dd, J = 6.4, 5.2 Hz, 1H).

^{13}C NMR (126 MHz, CHLOROFORM-D) δ 155.46, 152.52, 145.61, 125.48, 121.91, 67.84, 30.79.

HR-ESI-FT-MS (Orbit-Trap-MS) C18 H16 N2 O10 S Na

Mass Measured 475.0420 Theo. Mass 475.0418 Delta (ppm) 0.4

Polymer synthesis was completed by preparing diamine (compound 4, figure 2) as per the literature¹². Diamine (0.518g, 3.2mmol) and carbonate (compound 3, figure 2) (1.446g, 3.2mmol) were taken with 2 ml of dry dichloromethane in an 8 ml vial with a Teflon cap and purged with nitrogen. To this, 1 ml of triethylamine was added via syringe and the contents were stirred for 4 days at room temperature. The contents were precipitated into diethylether and the waxy yellow solid obtained was subsequently purified on a gel column packed with lipophilic sephadex (LH-20) using dichloromethane as the eluent, to obtain a pale yellow solid in 70% yield.

1H NMR (500 MHz, CHLOROFORM-D) δ 5.42 (s, 1H), 4.21 (t, J = 6.4 Hz, 2H), 3.45 (t, J = 5.2 Hz, 2H), 3.30 (d, J = 5.3 Hz, 2H), 2.77 (t, J = 6.5 Hz, 2H), 1.32 (s, 3H). ^{13}C NMR (126 MHz, CHLOROFORM-D) δ 156.42, 100.32, 64.24, 64.21, 59.93, 41.20, 31.34, 24.93, 24.90.

Molecular weight: Obtained via GPC against poly styrene standards using DMF(0.01%LiBr) as the eluent.

Mw=59800 Da, Mn=34100, PDI =1.75

Nanoparticle formulation

In o/w procedure, 25 mg of the synthesized polymer were dissolved in 2.5 ml DCM. Then 500 µg Nile red were added, for preparing Nile red containing particles. DCM was added to 50 ml of phosphate buffer (pH 8) containing 1% PVA, and the mixture was stirred at 1,000 rpm for 5 min to prepare an emulsion. Further emulsification was achieved using a high-pressure homogenizer (Microfluidic® 110PS, USA) at 23,000 Psi for three cycles. The nanoparticle suspension was stirred at 1,000 rpm using a magnetic stirrer to evaporate the DCM. Nile red-containing nanoparticles were filtered through 1 µm filter to remove the insoluble Nile red aggregate, and a concentrated mode tangential flow filtration system with 500 kDa Pellicon XL cassettes (Millipore, USA) was used to remove the PVA and any unencapsulated material. The nanoparticle suspension was concentrated to 10 ml and washed twice²⁶. Protein was encapsulated into the nanoparticles using the w/o/w emulsion method. Briefly, 2 mg Ovalbumin Alexa Fluor® 594 was dissolved in 0.2 ml of PBS buffer and subsequently emulsified with 5 ml of DCM containing 100 mg polymer using probe sonication (stabilized using 2% Span 80) at amplitude of 40% for 5 min (1/8" tip, Misonix S-4000, USA). The primary emulsion was added to 50 ml of 1% PVA in buffer (pH 8) under stirring at 1,000 rpm, and the secondary emulsion was produced. Additional emulsification was achieved using the high-pressure homogenizer at 23,000 Psi for two cycles and tangential flow filtration was accomplished as previously described. Finally, 5% Trehalose was added to the particles before lyophilization. The lyophilized particles were suspended in aqueous media at different pHs and H₂O₂ levels for further evaluation of stability or release.

The encapsulation efficiencies of the particles were determined by incubating them at acidic pH in presence of 100 mM H₂O₂ until no further particles are detected by the DLS and the detected fluorescence reaches its maximum.

Dynamic light scattering measurements of the nanoparticles

Nanoparticles were suspended in a phosphate buffer pH 7.4 or 5 in presence or absence 100 mM H₂O₂, with stability determined by monitoring their size for 24 hrs via dynamic light scattering (DLS) using a Zetasizer-ZS (fixed attenuator of 7, Malvern Worcestershire, UK).

Effect of pH and H₂O₂ on Nile red release

Nile red was encapsulated into the nanoparticles as a sensor of changes in the hydrophobic-hydrophilic character of the surrounding environment. Nile red fluoresces in the hydrophobic environment inside the nanoparticles and quenches once released into the aqueous release media; this quenching was measured as an indicator of Nile red release into an aqueous media. Nanoparticle powder was dispersed in PB pH 7.4 or 5 in presence or absence 100 mM H₂O₂. The pH was checked in the presence of H₂O₂ to confirm the pH. Samples were taken and Nile red fluorescence was determined at different time intervals. Decrease in the nanoparticles fluorescent was indication of Nile red release to the aqueous media.

Effect of pH and H₂O₂ on ovalbumin release

Nanoparticle powder was dispersed in PB pH 7.4 or 6.5 in presence or absence 100mM H₂O₂. PB pH 6.5 was chosen for ovalbumin release study to prevent its precipitation upon release into PB having pH near ovalbumin isoelectric point (pH~5)²⁷. Samples were taken at different time intervals and spun down at 20K xg for 10 min at 4°C. The supernatant was analyzed for released ovalbumin Alexa Fluor® 594 after appropriate dilution.

Degradation of the *poly thioether-ketal* by ^1H NMR

10 mg of the polymer were taken with deuterated a) 1:1 acetonitrile - water (pH=5, phosphate buffer); b) 1:1 acetonitrile - water (pH=5, phosphate buffer) with 20 μL of H_2O_2 ; the ^1H NMR was obtained at regular intervals.

Cell toxicity of nanoparticles

The cytotoxicity of nanoparticles was investigated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. RAW 264.7 macrophage cells were seeded at a density of 15,000 cells/well in a 96-well plate and incubated for 24 hrs to reach 60% confluency. Cells were treated with various amounts of nanoparticles that corresponded to the varying amounts of polymer contained in them (0–300 $\mu\text{g}/\text{mL}$) and incubated for 20 hrs. To each well was added 10 μL of MTT solution, and the wells were incubated for 3 hrs. Dimethyl sulfoxide (DMSO, 100 μL) was added to cells to dissolve the resulting formazan crystals. After 20 mins of incubation, the absorbance at 570 nm was measured using a FlexStation microplate reader (Molecular Devices, Inc., Sunnyvale, CA, USA). Cell viability was obtained by comparing the absorbance of nanoparticle-treated cells to that of control cells not treated with particles.

Cellular internalization of ovalbumin-containing nanoparticles

The uptake of ovalbumin Alexa Fluor®594-loaded nanoparticles was studied in RAW264.7 cells using a fluorescent microscope. Cells were plated on CultureWell Chambered Coverglass slides (Invitrogen Corporation, Carlsbad, CA, USA) at a density of 10,000 cells per well for 24 hrs followed by treatment with *poly thio-ketal* or PLGA nanoparticles. The nanoparticle payload contained a final concentration of approximately 10 $\mu\text{g}/\text{mL}$ ovalbumin Alexa Fluor®594. After 8 hrs at 37°C, the cells were washed with PBS before mounting and staining with DAPI.

RESULTS AND DISCUSSION

Infected tissues are frequently characterized by a decreased pH and the presence of reactive oxygen species (ROS) ¹⁷⁻¹⁹. In order to simulate these conditions, particle behavior was tested at acidic pH and in the presence of H_2O_2 , which is a prevalent reactive oxygen species. Accordingly, nanoparticles were subjected to different physiologically relevant conditions: pH 7.4 (pH of healthy tissue), pH 7.4/100 mM H_2O_2 (presence of ROS), acidic pH, and acidic pH/100 mM H_2O_2 . Their behavior was monitored via DLS and by measuring the release of Nile red and ovalbumin.

The hydrophobic nature of the polymer permitted the formulation of the nanoparticles using single- or double-emulsion techniques. This in turn allowed the encapsulation of different payloads with different hydrophobic-hydrophilic characteristics like Nile red and ovalbumin (Figure 3).

Monitoring the behavior of the nanoparticles using DLS (Figure 4) showed that only nanoparticles dispersed in pH 5 in the presence of H_2O_2 degraded within 24 hrs, while the particles dispersed in pH 5 alone remained detectable throughout this time period. Thus, the presence of both stimuli caused an accelerated degradation of the nanoparticles. This is further evidenced by the observed increase in particle size upon incubation in H_2O_2 at pH 7.4. This swelling is due to the backbone becoming oxidized, resulting in the hydration of the particles. When Nile Red is encapsulated in the particles it acts as a fluorescent probe of hydrophobicity and release. In presence of H_2O_2 , oxidation of the polymer backbone occurs, rendering the nanoparticles more hydrophilic. This causes quenching of encapsulated Nile red upon its release from the nanoparticles (Z-average= 200 nm). This hydrophilic change

occurred in both neutral and acidic conditions and depended entirely on whether the particles were incubated in hydrogen peroxide or not (Figure 5). Also, Nile red was minimally quenched at pH 5 due to the slow degradation of the particle via surface erosion. However, more quenching is observed when both conditions are operational. Finally, in order to confirm that our polymer degrades under the said conditions, we performed degradation and analyzed via ^1H NMR (Figure 6 and Figure 7). Here, as expected, we did not see a difference in polyketal hydrolysis rates because the polymer is completely dissolved without the need of oxidation. The control that we observed in the rates of particle degradation is due to the nanoparticle architecture, which is lost when the polymer is dissolved. We subsequently tested this systemic control in releasing ovalbumin as a model protein.

Figure 8 shows that ovalbumin release was initiated by oxidation of the polymer, or more accurately due to *swelling* of the nanoparticles. Meanwhile, higher release was observed at acidic pH in the presence of hydrogen peroxide due to *degradation* of the nanoparticles. Importantly, the nanoparticles showed excellent stability at neutral 7.4. Furthermore, the full degradation of our new nanoparticles system is important as small fragments are more readily excreted³⁰⁻³².

We evaluated the cytotoxicity of *poly* thio-ketal nanoparticles in cells using the MTT assay using RAW264.7 cell line. The RAW264.7 macrophage cell line is a great model system for studying immune response and can produce relatively higher levels of endogenous ROS. Additionally, these cells are able to take up cells very readily without the need of cell penetrating peptides. RAW 264.7 cells were incubated with various amounts of nanoparticles for 20 hrs. Figure 9 illustrates the comparison of cytotoxicity between cells treated with increasing concentrations of *poly* thioether-ketal polymer. There was no significant cytotoxicity observed with the cells incubated with up 300 $\mu\text{g}/\text{ml}$ of polymer ($p=0.294$).

Finally, we analyzed the cellular uptake of nanoparticles and release of cargo within cells by comparing *poly* thioether-ketal and PLGA nanoparticles containing equal amounts of fluorescently labeled ovalbumin (Figure 10). Nanoparticles were added to RAW 264.7 cells and evaluated by fluorescence microscopy. *Poly* thioether-ketal nanoparticles were able to efficiently deliver protein within RAW264.7 macrophage cells that have an H_2O_2 concentration of 2 μM ³³. Cells treated with *poly* thioether-ketal nanoparticles show thorough diffusion of ovalbumin throughout the cytoplasm and nucleus, indicating that labeled ovalbumin was released within the cell. This is in contrast with slow-degrading PLGA nanoparticles that show punctuated spots and less diffused ovalbumin throughout the cell.

CONCLUSION

We designed a polymeric nanoparticle with two stimuli responsive moieties in its backbone, and which undergoes programmed degradation when stimulated by inflammatory cues, ROS and acidic pH, respectively. This system functions akin to an 'AND' Logic Gate. Upon stimulation by ROS, the polymer becomes hydrophilic, followed by ketal hydrolysis promoted at an acidic pH. We observed release of small hydrophobic molecules, Nile red, in acidic pH and an increase in polymer hydrophilicity upon oxidation. Higher release of encapsulated Ovalbumin was observed in the presence of both hydrogen peroxide and at acidic pH. We found the nanoparticles were readily taken up by macrophage cells, and that ovalbumin release from the nanoparticles into the cytoplasm was in agreement with the degradation behavior of the nanoparticles as shown in the DLS measurements. We hypothesize that these nanoparticles can differentiate between the conditions of our target

areas from others. This should ensure the release of the payload in specific diseased regions such as inflammatory or tumor sites and allows for intracellular delivery. These studies suggest that our nanoparticles can be applied to the delivery of diagnostics and therapeutics to sites of inflammation. Thus our current work focuses on the application of these nanoparticles in targeting theranostics to vulnerable plaque versus stable plaque.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The research described in this paper was sponsored in part by the UCSD IRACDA Fellowship NIH Grant GM06852 (JM), the NIH New Innovator Award (1 DP2 OD006499-01), and the King Abdulaziz City for Science and Technology (KACST). We thank Dr. Eric Schopf for his help with TEM imaging.

References

- (1). Napoli A, Valentini M, Tirelli N, Mueller M, Hubbell JA. Oxidation-responsive polymeric vesicles. *Nature Materials*. 2004; 3:183–189.
- (2). Wilson DS, Dalmasso G, Wang LX, Sitaraman SV, Merlin D, Murthy N. Orally delivered thioketal nanoparticles loaded with TNF-alpha-siRNA target inflammation and inhibit gene expression in the intestines. *Nature Materials*. 2010; 9:923–928.
- (3). Vasir JK, Labhasetwar V. Biodegradable nanoparticles for cytosolic delivery of therapeutics. *Advanced Drug Delivery Reviews*. 2007; 59:718–728. [PubMed: 17683826]
- (4). Mescalchin A, Detzer A, Wecke M, Overhoff M, Wuensche W, Sczakiel G. Cellular uptake and intracellular release are major obstacles to the therapeutic application of siRNA: novel options by phosphorothioate-stimulated delivery. *Expert Opinion on Biological Therapy*. 2007; 7:1531–1538. [PubMed: 17916045]
- (5). Hu Y, Litwin T, Nagaraja AR, Kwong B, Katz J, Watson N, Irvine DJ. Cytosolic Delivery of Membrane-Impermeable Molecules in Dendritic Cells Using pH-Responsive Core-Shell Nanoparticles. *Nano Letters*. 2007; 7:3056–3064. [PubMed: 17887715]
- (6). Sankaranarayanan J, Mahmoud EA, Kim G, Morachis JM, Almutairi A. Multiresponse Strategies To Modulate Burst Degradation and Release from Nanoparticles. *ACS Nano*. 2010; 4:5930–5936. [PubMed: 20828178]
- (7). Sonawane ND, Szoka FC Jr, Verkman AS. Chloride Accumulation and Swelling in Endosomes Enhances DNA Transfer by Polyamine-DNA Polyplexes. *Journal of Biological Chemistry*. 2003; 278:44826–44831. [PubMed: 12944394]
- (8). Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Research*. 1989; 49:6449–65. [PubMed: 2684393]
- (9). Meyer DE, Shin BC, Kong GA, Dewhirst MW, Chilkoti A. Drug targeting using thermally responsive polymers and local hyperthermia. *Journal of Controlled Release*. 2001; 74:213–224. [PubMed: 11489497]
- (10). Saito G, Swanson JA, Lee K-D. Drug delivery strategy utilizing conjugation via reversible disulfide linkages: role and site of cellular reducing activities. *Advanced Drug Delivery Reviews*. 2003; 55:199–215. [PubMed: 12564977]
- (11). Vo CD, Kilcher G, Tirelli N. Polymers and sulfur: what are organic polysulfides good for? Preparative strategies and biological applications. *Macromolecular Rapid Communications*. 2009; 30:299–315. [PubMed: 21706606]
- (12). Paramonov SE, Bachelder EM, Beaudette TT, Standley SM, Lee CC, Dashe J, Frechet JMJ. Fully Acid-Degradable Biocompatible Polyacetal Microparticles for Drug Delivery. *Bioconjugate Chemistry*. 2008; 19:911–919. [PubMed: 18373356]
- (13). Michael, J. Heffernan; Murthy, N. Polyketal nanoparticles: a new pH-sensitive biodegradable drug delivery vehicle. *Bioconjugate Chemistry*. 2005; 16:1340–2. [PubMed: 16287226]

- (14). Broaders KE, Cohen JA, Beaudette TT, Bachelder EM, Frechet JMJ. Acetalated dextran is a chemically and biologically tunable material for particulate immunotherapy. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:5497–5502. [PubMed: 19321415]
- (15). Jain R, Standley SM, Frechet JMJ. Synthesis and degradation of pH-sensitive linear poly(amidoamine)s. *Macromolecules*. 2007; 40:452–457.
- (16). Murthy N, Thng YX, Schuck S, Xu MC, Frechet JMJ. A novel strategy for encapsulation and release of proteins: Hydrogels and microgels with acid-labile acetal cross-linkers. *Journal of the American Chemical Society*. 2002; 124:12398–12399. [PubMed: 12381166]
- (17). Punnia-Moorthy A. Evaluation of pH changes in inflammation of the subcutaneous air pouch lining in the rat, induced by carrageenan, dextran and staphylococcus aureus. *Journal of Oral Pathology & Medicine*. 1987; 16:36–44.
- (18). Ródenas J, Mitjavila MT, Carbonell T. Simultaneous generation of nitric oxide and superoxide by inflammatory cells in rats. *Free Radical Biology and Medicine*. 1995; 18:869–875. [PubMed: 7797094]
- (19). Greenwald RA. Oxygen radicals, inflammation, and arthritis: Pathophysiological considerations and implications for treatment. *Seminars in Arthritis and Rheumatism*. 1991; 20:219–240. [PubMed: 2042055]
- (20). Rehor A, Hubbell JA, Tirelli N. Oxidation-Sensitive Polymeric Nanoparticles. *Langmuir*. 2005; 21:411–417. [PubMed: 15620332]
- (21). Reddy ST, Rehor A, Schmoekel HG, Hubbell JA, Swartz MA. In vivo targeting of dendritic cells in lymph nodes with poly(propylene sulfide) nanoparticles. *Journal of Controlled Release*. 2006; 112:26–34. [PubMed: 16529839]
- (22). Giles GI. The redox regulation of thiol dependent signaling pathways in cancer. *Current Pharmaceutical Design*. 2006; 12:4427–4443. [PubMed: 17168752]
- (23). Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, Vahabzadeh K, Hama S, Hough G, Kamranpour N, Berliner JA, Lusis AJ, Fogelman AM. Thematic review series: The pathogenesis of atherosclerosis: The oxidation hypothesis of atherogenesis: The role of oxidized phospholipids and HDL. *Journal of Lipid Research*. 2004; 45:993–1007. [PubMed: 15060092]
- (24). Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:10771–8. [PubMed: 7971961]
- (25). Szatrowski TP, Nathan CF. Production of Large Amounts of Hydrogen Peroxide by Human Tumor Cells. *Cancer Res*. 1991; 51:794–798. [PubMed: 1846317]
- (26). Dalwadi G, Benson HA, Chen Y. Comparison of diafiltration and tangential flow filtration for purification of nanoparticle suspensions. *Pharm Res*. 2005; 22:2152–62. [PubMed: 16151669]
- (27). Smith ERB. THE EFFECT OF VARIATIONS IN IONIC STRENGTH ON THE APPARENT ISOELECTRIC POINT OF EGG ALBUMIN. *Journal of Biological Chemistry*. 1935; 108:187–194.
- (28). Khutoryanskiy VV, Tirelli N. Oxidation-responsiveness of nanomaterials for targeting inflammatory reactions. *Pure and Applied Chemistry*. 2008; 80:1703–1718.
- (29). Burkert, F. v.; Goepferich, AM. An approach to classify degradable polymers. *Mater. Res. Soc. Symp. Proc*. 1999; 550:17–22.
- (30). Greish K, Fang J, Inutsuka T, Nagamitsu A, Maeda H. Macromolecular therapeutics - Advantages and prospects with special emphasis on solid tumour targeting. *Clinical Pharmacokinetics*. 2003; 42:1089–1105. [PubMed: 14531722]
- (31). Kwon GS, Yokoyama M, Okano T, Sakurai Y, Kataoka K. BIODISTRIBUTION OF MICELLE-FORMING POLYMER DRUG CONJUGATES. *Pharmaceutical Research*. 1993; 10:970–974. [PubMed: 8378259]
- (32). Lu ZR, Parker DL, Goodrich KC, Wang XH, Dalle JG, Buswell HR. Extracellular biodegradable macromolecular gadolinium(III) complexes for MRI. *Magnetic Resonance in Medicine*. 2004; 51:27–34. [PubMed: 14705042]

- (33). Gong X, Li Q, Xu K, Liu X, Li H, Chen Z, Tong L, Tang B, Zhong H. A new route for simple and rapid determination of hydrogen peroxide in RAW264.7 macrophages by microchip electrophoresis. ELECTROPHORESIS. 2009; 30:1983–1990. [PubMed: 19517439]

Figure 1. Degradation mechanism of *poly* thioether-ketal. Hydrogen peroxide and acidic pH stimulate the degradation of the polymeric nanoparticles in tandem.

Figure 2.
Synthesis of *poly thioether-ketal*.

Figure 3.
A SEM image of the nanoparticles showing a particle diameter of $< 1\mu\text{m}$.

Figure 4.

Effect of pH and H₂O₂ on the Z-average of the nanoparticles. Triplicate measurements were taken using the DLS at a fixed attenuator of 7.

Figure 5. Release of Nile red from *poly* thioether-ketal nanoparticles. Decrease in nanoparticles suspension fluorescence was correlated to Nile red release in different conditions.

Figure 6.

Degradation of polymer 5 at pH 5 in 1:1 deuterated acetonitrile and water by ¹H NMR. Hydrolysis of ketal is evidenced by the appearance of acetone peak (at δ 2.6 ppm) and disappearance of the ketal peak (at δ 1.6 ppm). There is also concomitant change in the peak at δ 3.8 ppm to δ 3.9 ppm due to the formation of alcohol upon degradation of the ketal.

Figure 7. Degradation of polymer 5 at pH 5 in 1:1 deuterated acetonitrile and water with addition of 20 μ l of 30% H₂O₂ by ¹H NMR. In addition to the changes noted in the Figure 6 upon addition of H₂O₂ we see that the peak at δ 3.6 ppm shifts to δ 3.7 ppm. This is consistent with the oxidation of sulfur in the backbone to a sulfoxide. These NMR studies establish that the sulfur moiety undergoes oxidation when subjected to H₂O₂.

Figure 8.

Release of ovalbumin AlexaFluor® 488 from *poly* thio-ketal nanoparticles. Nanoparticles supernatants were tested for fluorescence to determine ovalbumin release profile over time (n=3).

Figure 9. Cytotoxicity of *poly* thioether-ketal nanoparticles at different concentrations in RAW 264.7 macrophage cells. The cells were incubated with the nanoparticles for 20hrs before performing the MTT assay.

Figure 10.

Uptake of nanoparticles loaded with fluorescent ovalbumin. Raw 264.7 macrophage cells were treated for 8hrs with *poly* thioether-ketal or PLGA nanoparticles containing ovalbumin Alexa Fluor 594 (red) and stained with DAPI (blue).