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Main-chain degradable single-chain cyclized polymers as gene delivery vectors

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ABSTRACT

Single-chain technology (SCT) allows the manipulation of polymeric architectures at an individual polymer chain level, providing a new platform for the fabrication of nanoscale polymeric objects. However, it remains problematic to apply this newborn technology to the biological and medical fields, since synthesis of single-chain polymeric nanoparticles relies heavily on controlled/living radical polymerization of vinyl based monomers, yielding a persistent non-degradable carbon-carbon based backbone. Moreover, the ultrahigh dilution conditions often required for single-chain polymer nanoparticle synthesis limits large-scale applicability. A versatile approach to achieve backbone degradability in single-chain cyclized polymers was developed by combining ring-opening addition polymerization and intramolecular cyclization into a one-pot RAFT copolymerization of cyclic and mono/multi-vinyl monomers system under concentrated conditions. The in situ intramolecular cyclization of individual propagating chains was achieved by kinetic control and statistical manipulation of mono- and multi-vinyl monomer copolymerization. The cyclic allylsulfide monomer 3-methylidene-1,9-dioxa-5,12,13-trithiacyclopentadecane-2,8-dione (MDTD) was copolymerized via the ring-opening pathway to introduce disulfide groups into the vinyl-based backbone without compromising the single chain propagation nature. Backbone degradable single chain polymeric nanoparticles were obtained with molecular weights of 10 kDa and MDTD incorporation ratios of 4.7%. Chemical degradation of the nanoparticles confirmed both their single chain nature, as well as backbone degradability. The single-chain cyclized polymeric nanoparticles were evaluated for their gene transfection capabilities. The backbone degradable nanoparticles displayed high transfection efficiencies and low cytotoxicities in both 3T3 and HeLa cells.

1. Introduction

The preparation of nano-sized objects consisting of single polymeric chains, recently coined single-chain technology (SCT) [1] brings great promise to different areas in nanotechnology, including materials science, biomedicine and energy [2–8]. Interest in producing macromolecular architectures through manipulation of single polymer chains is rapidly increasing, because of the promise of smart, autonomous and tailor-designed functions at the individual polymeric chain level. Over a decade of development has resulted in single-chain polymer nanoparticles becoming new building blocks for bottom-up nanotechnology [2]. Nanomedicine may greatly benefit from their drug transport properties, multivalent character [2,9,10] and, though still crude, resemblance to natural proteins and enzymes [11,12].

Several techniques have been explored to synthesize single-chain polymeric nanomaterials. In particular, intramolecular crosslinking of individual linear polymer chains in ultra-dilute solution leads to unimolecular nanoparticles – i.e. single-chain polymeric nanoparticles (SCNPs) – have received ample attention [2,8]. Hawker and coworkers reported pioneering work in which random intramolecular dimerization of benzocyclobutene units was employed to form compact polystyrene nanoparticles [13]. This intrachain crosslinking has also been achieved afterwards by other covalent [7,14,15], dynamic covalent [16,17] and noncovalent bonding [18–20]. Interestingly, Meijer et al. have shown that directional self-assembly of supramolecular binding motifs can be used to guide the compaction of single polymer chains [21,22]. However, in order to avoid intermolecular crosslinking, these post-polymerization modification reactions need to be conducted under extremely dilute conditions (typically <0.1 mg/mL) [3],

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practically forbidding large-scale production. Promotion of intra-chain crosslinking is therefore a challenging key aspect in the advancement of the single-chain technology.

Recently, Wang and coworkers expanded the toolbox of the single-chain technology by demonstrating facile manipulation of inter- and intramolecular reactions during the polymerization of multivinyl monomers (MVMs) [23–27]. In contrast to the aforementioned post-polymerization crosslinking strategies, this approach eventually leads to single-chain cyclized/knotted polymeric nanoparticles (SCKNPs) under concentrated reaction conditions. Key to this approach is achieving kinetic control to ensure that same-chain pendant vinyl moieties have the highest probability of reacting with their own propagating active center in the initial linear chain growth stage. The benefits of this internal cyclization in the controlled polymerization of MVMs are two-fold: first, keeping chain propagation within single molecules, which is a prerequisite for the formation of single-chain cyclized polymer nanoparticles; second, consumption of most of the pendant vinyl moieties to minimize intermolecular coupling and hence delay gelation.

Reversible addition-fragmentation chain transfer polymerization (RAFT) polymerization is a particularly promising way to synthesize kinetically controlled polymers. The absence of possibly toxic metal ions in the polymerization, combined with the wide variety of compatible vinyl monomers bode well for future biomedical application. Moreover, intrachain cyclization is promoted by the inherently high deactivation rates [26] characteristic for RAFT polymerization. Typical constants of chain transfer (k_{tr}) are 10^2 to 10^3 times higher than for vinyl propagation (k_p) [28], largely preventing intermolecular crosslinking. As such, SCKNPs are readily obtained when the polymerization is quenched in time, before the primary polymer chains starts to combine. SCKNPs architecturally resemble SCNPs given the unimolecular carbon-carbon based backbone with numerous cyclized loops bridged by 'crosslinkers', whose degradation results in linear polymer chains [23,25,26,29]. However, in contrast to SCNPs, the synthesis of SCKNPs features a one-pot in situ intramolecular reaction, resulting in self-cyclization of the propagating polymer chains in concentrated solution, without the need for additional post-polymerization steps.

Synthesis of single-chain polymer nanoparticles relies heavily on controlled/living radical polymerization (CRP) techniques, owing to the high fidelity of the resulting vinyl polymers and the wide variety of easily accessible functional monomers. However, degradability of vinyl polymers remains a critical issue for their biomedical application, as the carbon-carbon backbone does not easily undergo hydrolysis or enzymatic degradation. As a result, unwanted immune responses and toxicity hamper their translation to the clinic [30]. Several different design strategies have been developed to introduce labile units into the backbone of vinyl polymers [30]. Bifunctional CRP initiators containing cleavable units such as redox-responsive [31], thermally degradable [32], acid-labile [33] and photocleavable groups [34] have been reported, from which polymers containing a central degradable junction can be grown [35]. For instance, Matyjaszewski and coworkers demonstrated the synthesis of backbone degradable polymers employing disulfide containing initiators for atom-transfer polymerization (ATRP) [31,36]. Multisegmented degradable polymers have been synthesized by combining difunctional macromonomers, which are generated for example by nucleophilic substitution of the halide endgroups of ATRP-derived polymers [37] or aminolysis of RAFT-derived polymers [38–40]. This step-growth polymerization approach generally results in broad molecular weight distributions, while the additional synthetic steps limit the applicability of this approach.

Radical ring opening polymerization of cyclic vinyl monomers provides for an elegant, broadly applicable way of incorporating degradable and responsive linkages [41,42]. The most studied cyclic monomers for this purpose are cyclic ketene acetals (CKAs), initially investigated by Bailey et al. [43], and later used to synthesize different copolymer structures [44–47]. A particularly versatile class of cyclic monomers is based on the allyl sulfide moiety [48–50]. Paulusse et al. demonstrated the facile incorporation of ester, thioester and disulfide functionalities into the vinyl polymer backbone under CRP conditions [51]. The introduction of multiple orthogonally degradable units into the vinyl backbone resulted in tunable, selective and stepwise degradation with the ability for further functionalization of the polymer backbone [51].

Combining backbone degradability with single-chain technology would enable its safe application in nanomedicine. However, the stringent requirements to achieve intramolecular chain collapse and prevent unwanted side reactions, such as intermolecular crosslinking, have hampered the development of degradable single chain polymer nanoparticles. Herein, we report the synthesis of backbone degradable single-chain cyclized polymer nanoparticles via one-pot RAFT copolymerization of *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA) with tri(ethyleneglycol) diacrylate (TEGDA) and a 15-membered cyclic allyl sulfide monomer, 3-methylidene1,9-dioxa-5,12,13-trithiacyclopentadecane-2,8-dione (MDTD). Incorporation of the cyclic monomer MDTD is monitored on the basis of copolymer composition and degradation behavior. Degradation profiles of the polymer nanoparticles are evaluated in vitro and the gene trafficking capabilities and cytotoxicity of the polymer nanoparticles are evaluated on 3T3 and HeLa cells.

2. Materials and methods

2.1. Materials

2,2′-Azobis(2-methylpropionitrile) (AIBN, 98%, Sigma-Aldrich), 2,2′-(dimethyl amino)ethyl methacrylate (DMAEMA, 98%, Sigma-Aldrich), poly(ethylene glycol) diacrylate (TEGDA, M_n = 250 g/mol, Sigma-Aldrich), toluene (>99.5%, Sigma-Aldrich), acryloyl chloride (≥97%, Sigma-Aldrich) 2-hydroxyethyl disulfide (90%, Sigma-Aldrich), triethylamine (TEA, 99%, Sigma-Aldrich), and hydrazine hydrate (reagent grade, Sigma-Aldrich) were used as received. The disulfide containing cyclic 3-methylidene1,9-dioxa-5,12,13-trithiacyclopentadecane-2,8-dione monomer was synthesized following a literature procedure [51]. The RAFT agent 2-cyano-2-propyl dithiobenzoate (CPBDT) was kindly provided by Dr. Tai, University of Bangor, United Kingdom. Solvents were purchased from Fisher Scientific and used as received.

2.2. Synthesis of disulfide diacrylate (DSDA)

THF (600 mL) was dried over sodium sulfate for 24 h. 2-Hydroxyethyl disulfide (15.38 g, 12.2 mL, 100 mmol) and TEA (40.66 g, 402 mmol) were mixed with THF (450 mL) in a 1 L round-bottom flask. The solution was flushed with argon for 10 min and kept under argon environment. Acryloyl chloride (32 mL, 35.65 g, 394 mmol) diluted in THF (50 mL) was added dropwise. The reaction was left stirring for 24 h in an ice bath. The product was filtered by vacuum filtration to remove undesired triethylammonium chloride salts. The solution was washed 5 times by extraction using a 0.2 M sodium carbonate solution, followed by 5 times distilled water washing. After drying with sodium sulfate, the solvent was removed by rotary evaporation. The obtained product (42% yield) was characterized by **1**H NMR spectroscopy in $CDCl_3$: 2.84 ppm (t, 4H), 4.45 ppm (t, 4H), 5.80 ppm (d, 2H), 6.05 ppm (q, 2H), 6.43 ppm (d, 2H).

2.3. Polymer synthesis

2.3.1. Synthesis of poly(DMAEMA-co-TEGDA) (**P1**)

DMAEMA (320 equiv.; 5.30 g), TEGDA (80 equiv.; 2.11 g), RAFT agent CPBDT (1 equiv.; 0.0239 g), AIBN (1 equiv.; 0.0174 g) and toluene (20 mL) were added into a 50 mL round bottom flask. The solution was bubbled for 15 min with argon and put into an oil bath at 60 °C. The reaction was sampled manually at desired time points. The obtained polymer mixture was purified by dialysis using a Spectra/Por® dialysis