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Fabrication of Nanoparticles Via Polymerization Induced Self-Assembly for Drug Delivery and Diagnosis

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Abstract
Overcoming biological barriers and achieving effective treatment for cancer therapy still require new strategies and designs in the drug delivery area. Nanoparticles have emerged as versatile and effective research tools at last decades for these purposes. Size, shape and surface chemistry are determinative characteristics of nanoparticles for their pharmacokinetic and biodistribution profiles. At that point, Polymerization Induced Self-Assembly (PISA) is an eligible technique to form polymeric nanoparticles with desired properties when mediated by reversible addition–fragmentation chain transfer (RAFT) polymerization. In this study, previously fabricated oligo(ethylene glycol) methyl ether methacrylate (OEGMEMA) and styrene based polymeric nanoparticles prepared via PISA have been inspired and a new strategy is demonstrated to overcome possible surface interaction and aggregation problems. ABC type triblock copolymer based polymeric nanoparticles were fabricated via RAFT mediated PISA technique. For the fabrication of such nanoparticles firstly, poly oligo(ethylene glycol) methyl ether methacrylate (POEGMEMA) polymer was synthesized using RAFT polymerization as macro-chain transfer agent (MCTA) with repeating unit number 15. Then glycidyl methacrylate (GMA) monomer was polymerized using this MCTA to get POEGMEMA-b-PGMA diblock copolymer as second level MCTA. Finally, styrene (ST) monomer was polymerized using obtained second level MCTA in methanol to synthesize POEGMEMA-b-PGMA-b-PST triblock copolymer for performing PISA. Obtained nanoparticles were characterized using dynamic light scattering analysis. Having functionalizable PGMA segment as inner shell of the nanoconstructs, these nanoparticles are suitable for covalent bonding of drug molecules or diagnostic reagents for cancer therapy.
Keywords: Nanoparticle, self-assembly, PISA, RAFT

INTRODUCTION

The last few decades have witnessed the increase in the usage of nanoparticles for the diagnosis and therapy for several diseases [1]. Having many superior chemical and physical characteristics compared to molecule or macro state, the nanosized particles have become useful tools for biomedical applications [2]. The number of these nanoparticles called nanopharmaceuticals on the world market increase day by day [3]. There are also lots of nanoscaled drug delivery and diagnosis systems in clinical trials for different diseases, especially cancer [4]. Morphological and the physiological nature of the cancer cells and tumor tissues make them available targets for nanoparticles [5]. While Enhanced Permeability and Retention (EPR) effect makes nanoparticle passively target the tumor tissue, physiological pH difference and over expressed proteins and peptide sequences in cell membrane enables nanoparticles to release the drug molecules in a controlled way and actively target the tissue. Beside these main strategies, there are different types of strategies that nanoparticle designers use for the diagnosis and the therapy of cancer diseases [6].

Designing a smart nanoparticle depends on the reasonable material choice as well as taking morphological and physiological parameters of the target tissue [7]. Tunable and functionalizable materials such as polymeric nanoparticles have great advantage for designing such smart vehicles [8]. There are various types of strategies for designing polymeric nanoparticles. One of these strategies called Polymerization Induced Self-Assembly (PISA) technique makes easy to get polymeric nanoparticles with different size and shape. The PISA technique based on chain extension of soluble polymer block using a second monomer to end up with an insoluble polymer block in polymerization solution [9].

Reversible addition–fragmentation chain transfer (RAFT) polymerization is a convenient polymerization technique enabling to get such block polymers [10, 11]. Pan and Armes research groups showed various types of PISA examples using RAFT polymerization [12-16]. Davis and co-workers performed PISA technique to get nanoparticles with different size and shape using mainly oligo(ethylene glycol) methyl ether methacrylate (OEGMEMA) and styrene (ST) monomers as hydrophilic and hydrophobic blocks, respectively. They showed various biological properties of fabricated nanoparticles such as cell internalization and biodistribution to understand effect of size and shape over these properties [17, 18]. In one of their latest examples they designed a nanoparticle morphology having and an additional functionalizable poly glycidyl methacrylate (PGMA) segment as outer shell. They further functionalized this segment with a radiolabel molecule and investigated biodistribution profile of nanoparticles with different size and shape [19]. Their study showed high potential of these nanoparticles for drug delivery and diagnostic applications besides elucidating their biodistribution profiles. However, having a functionalizable peripheral group may not be suitable in all cases such as when using hydrophobic drug and imaging molecules.

This study suggests and alternative synthetic route for the fabrication of these nanoparticles to have PGMA segment between POEGMEMA and PST as functionalizable moiety. Briefly, oligo(ethylene glycol) methyl ether methacrylate

(OEGMEMA) monomer was firstly used to get poly oligo(ethylene glycol) methyl ether methacrylate polymer with repeating unit number 15 via RAFT polymerization. The obtained macro chain transfer agent MCTA was used to polymerize glycidyl methacrylate (GMA) monomer to give POEGMEMA-*b*-PGMA block copolymer. This diblock copolymer was chain extended with styrene (ST) monomer in methanol to get nanoparticles composed of POEGMEMA-*b*-PGMA-*b*-PST triblock copolymer. Synthesized polymers were characterized using ^{1}H NMR (proton Nuclear Magnetic Rezonans) spectroscopy and size exclusion chromatography (SEC). Nanoparticles obtained at different polymerization time points were characterized via dynamic light scattering (DLS) analysis.

MATERIALS AND METHODS

Materials

Oligo(ethylene glycol) methyl ether methacrylate, (Mn: 300), glysidyl methacrylate and styrene monomers, 4-cyanopentanoic acid dithiobenzoate (chain transfer agent, CTA), 12,400 molecular weight cut-off dialysis membrane were purchased from Sigma Aldrich. All solvents purchased from Merck and used without distillation. For characterization of polymers ^{1}H NMR spectroscopy (400 MHz, Bruker) and size exclusion chromatography SEC (Schimadzu) was used. Size of nanoparticles was determined using dynamic light scattering (DLS, Malvern Zetasizer).

Synthesis of POEGMEMA and POEGMEMA-b-PGMA polymers

RAFT polymerization was used for the synthesis of POEGMEMA and POEGMEMA-*b*-PGMA. To a solution of OEGMEMA monomer (500 mg, 1.67 mmol) in acetonitrile (2.5 mL) was added CTA (23.3 mg, 0.084 mmol) and 2,2'-Azobis(2-methylpropionnitrile) (initiator, 1.71 mg, 0.010 mmol). The solution mixture was purged with N_2 and polymerization was allowed at 70 °C for 4 h. The reaction was ceased by cooling and open atmosphere exposure. The polymer was purified via several precipitations in diethyl ether. The obtained POEGMEMA with repeating unit number 15 was used as macro chain transfer agent in the following reaction. To synthesize POEGMEMA-*b*-PGMA diblock polymer to a solution of glysidyl methacrylate (171 mg, 1.21 mmol) in 2.5 mL acetonitrile was added POEGMEMA (300 mg, 0.060 mmol) and initiator (0.984 mg, 0.012 mmol). Reaction mixture was purged with N_2 and reaction was allowed for 4 hours at 70 °C. Reaction was stopped and obtained POEGMEMA-*b*-PGMA diblock polymer was purified as described above.

Formation of nanoparticles via PISA technique

Polymerization Induces Self-Assembly technique was performed for the formation of polymeric nanoparticles. To a solution of styrene (7.576 mg, 145.3 mmol) in methanol 15 mL was added POEGMEMA-*b*-PGMA (100 mg, 0.018 mmol) and initiator (0.59 mg, 0.0036 mmol). Reaction mixture was purged with N_2 and reaction was started at 70 °C. At different polymerization time points 2.5 mL polymerization solution samples were collected under N_2 atmosphere to get nanoparticles with different size. Milky white turbid solution samples were dialyzed against methanol with dialysis membrane (12,400 molecular weight cut-off).

RESULTS AND DISCUSSION

Davis and co-workers firstly, synthesized PGMA block and their route was followed by chain extension of this MCTA with POEGMEMA and PST blocks, respectively. Finally, they ended up with functional group at the surface of the nanoconstracts [19]. In this study, to end up with functional glysidyl group block as inner shell of the nanoparticles firstly, POEGMEMA block was synthesized and this reaction was followed by chain extension with PGMA and PST polymeric blocks. At the end of the last chain extension, as a result of insolubility PST segment in methanol, polymeric nanoparticles with functional moiety at the inner shell as a result of PISA approach. Successful synthesis and purification steps of polymers were confirmed by ^{1}H NMR spectroscopy. The proton resonances around 7.4 and 7.9 ppm belonging to the aromatic group of chain transfer agent and 3.39 ppm belonging to methyl ether group showed the successful polymerization of POEGMEMA polymer. The chain extension with PGMA segment confirmed by the chemical shifts around 3.23-2.84 ppm corresponds to the epoxide group of GMA group. According to the integral calculation it was revealed that POEGMEMA-*b*-PGMA has averagely 15 OEGMEMA and 3 GMA repeating units. Chain extension with styrene monomer at different polymerization durations under dispersion condition is confirmed by the increased proton resonances around 7.30-6.27 due to aromatic group of styrene monomer. Size Exclusion Chromatography (SEC) traces revealed that chain extension of POEGMEMA-*b*-PGMA with styrene monomer succeeded under dispersion environment. Molecular weights (Mn) of the POEGMEMA-b-PGMA-PST triblock copolymers were found to be 18.500 (after 7 h) and 22.400 (after 20 h) with poly dispersity index (PDI) values of 1.22 and 1.35, respectively. Dynamic Light Scattering (DLS) analysis of these nanoparticles also confirmed size change of the nanoparticles as a function of time. The number based average size values of the nanoparticles were found to be 54 nm (after 7 h) and 128 nm (after 20 h) with the PDI values of 0.18 and 0.42, respectively. The dramatic increase in PDI values nanoparticles after 20 h was probably resulted due to morphological change of the particles from spherical to filamentous shape.

As a result, ^{1}H NMR, SEC and DLS results showed that polymeric nanoparticles suitable for post functionalization were successfully fabricated using PISA technique. Having functionalizable groups at inner shell of the nanostructure makes these nanoparticles more suitable than the ones having peripheral functional groups. Since the surface chemistry is very important for the biodistribution and cell internalization character of the nanoparticles, the particles with non-biocompatible agents at periphery have problems with overcoming biological barriers such as hepatic and reticulo-endothelial system based clearance.

CONCLUSION

In this study, successful formation of functional polymeric nanoparticles composed of POEGMEMA-*b*-PGMA-*b*-PST block copolymer was shown via PISA technique. These post- functionalizable nanoparticles with different size and shape are alternative vehicles for new drug delivery and diagnosis systems.

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