European Polymer Journal 108 (2018) 57–68

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Redox-responsive phosphonate-functionalized poly(β-amino ester) gels and cryogels

Hatice Betul Bingol[a], Fatma Demir Duman[b], Havva Yagci Acar[b], Mustafa Baris Yagci[c], Duygu Avci[a,*]

[a] Department of Chemistry, Bogazici University, 34342 Bebek, Istanbul, Turkey
[b] Department of Chemistry, Koc University, 34450 Sariyer, Istanbul, Turkey
[c] Surface Science and Technology Center (KUYTAM), Koç University, 34450 Sariyer, Istanbul , Turkey

ARTICLE INFO

Keywords:
Macromonomers
Phosphonate
Poly(β-amino)ester
Biomaterials
Degradation

ABSTRACT

Poly(β-amino ester) networks have gained attention as a class of degradable polymers for biomedical applications, particularly as scaffolds for tissue engineering. In this work, two novel phosphonated diamines (one containing a redox-responsive disulfide group) are reacted with diacrylates via aza-Michael addition reaction to form acrylate terminated poly(β-amino ester)s which are subsequently used as macromolecular precursors for fabrication of degradable gels and cryogels. The degradation rates of the gels and cryogels are monitored in phosphate buffer saline (PBS) and dithiothreitol (DTT), and the degradation times are found to range from hours to months depending on the design of chemical structure. The *in vitro* cytotoxicities of the degradation products are assessed with mouse embryonic fibroblast cells (NIH 3T3) and human osteosarcoma cells (Saos-2). The tailorability of the degradation rates and the non-toxicity of the degradation products make these poly(β-amino ester) gels and cryogels good candidates as scaffolds for tissue engineering applications.

1. Introduction

Biodegradable polymers have been an extensively studied area of research for a wide scope of bioapplications including tissue engineering, drug delivery and gene delivery [1,2]. Especially in tissue engineering applications, biodegradability of the polymer into nontoxic materials under physiological conditions is considered a key factor since it prevents the necessity for a second surgery to remove the polymer after the treatment.

Poly(β-aminoester)s (PBAEs) are a class of biodegradable polymers first synthesized by Langer et al.[3,4]. PBAEs are synthesized via aza-Michael addition of diamines and diacrylates by step-growth polymerization. This versatile procedure is tolerant to many functional groups, does not require complex protection/deprotection steps and does not produce byproducts. Using the diacrylate in a slight molar excess ensures that the end groups contain acrylates readily available for polymerization. These PBAE macromers have been polymerized to obtain biodegradable network polymers whose properties such as degradation rate, mechanical strength and cellular interaction can be easily tuned particularly suited for tissue engineering applications [5–10]. They have also been used to crosslink monofunctional methacrylates for synthesis of biodegradable PBAE based hydrogels [11]. PBAEs were successfully electrospun into fibrous scaffolds to obtain novel materials for tissue engineering applications [12,13]. PBAEs are widely used as non-viral vectors for gene delivery applications due to their capability to complex with the negatively charged DNA [14–17]. Amphiphilic copolymers of PBAEs have the ability to form pH sensitive micelles to be used as drug delivery agents [18–23]. PBAE based structures such as self-assembled nanoparticles, microspheres and mesoporous silica nanoparticles have been utilized as platforms for drug delivery [24–27]. Co-delivery systems based on PBAEs for simultaneous delivery of chemotherapeutic agents, proteins and nucleic acids were also studied [28–30]. Recently PBAEs have been used for various applications such as preparation of semi-interpenetrating networks and shape memory materials, logic gates and optical imaging probes [31–34].

The degradation of PBAEs is largely governed by the chemical structure and it is possible to enhance the degradation by incorporation of trigger responsive domains within the structure. Recently, PBAEs which can be degraded on demand by triggers such as pH, UV light and redox state have been reported [35–37]. Disulfide bonds are incorporated into polymers to serve as redox dependant cleavage sites [38]. Disulfide bond is stable against hydrolysis but gets cleaved selectively through thiol–disulfide exchange reactions in the reducing

* Corresponding author.
E-mail address: avcid@boun.edu.tr (D. Avci).

https://doi.org/10.1016/j.eurpolymj.2018.08.029
Received 11 April 2018; Received in revised form 20 July 2018; Accepted 19 August 2018
Available online 20 August 2018

environment of tumor tissue and intracellular compartments due to elevated levels of glutathione [39]. Other than glutathione, synthetic reagents such as 1,4-dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP) can also be used for reducing disulfides [40]. Studies demonstrated that using disulfide containing PBAEs for gene delivery increased transfection efficiency and decreased cytotoxicity [41–43]. Redox-responsive PBAE hydrogels showed excellent control over the release of antioxidants and proteins [44,45]. pH and redox dual-responsive PBAE micelles and nanoparticles were designed for controlled delivery of chemotherapy agents [46,47].

Phosphorus based molecules such as phosphates, phosphonates and bisphosphonates provide biodegradability, hemocompatibility and protein adsorption resistance to polymers which they are incorporated into [48,49]. Strong interactions of these materials with hydroxyapatite (HAP)-based tissues such as dentin, enamel, and bone make them promising candidates for applications in dentistry, bone targeted imaging and drug delivery, and bone tissue engineering applications [50–61].

Recently we have reported the effect of the bisphosphonate and phosphonate group on degradation properties, biocompatibility and cell interaction properties of PBAEs [62,63]. Herein we aim to combine the desirable properties of phosphonate group and redox-responsiveness of disulfide groups to obtain degradable PBAE based network polymers designed for bone tissue engineering applications. Our PBAE macromers were synthesized from aza-Michael addition reaction between a disulfide containing phosphonate-functionalized diamine and diacrylates poly(ethylene glycol) diacrylate (PEGDA) or 1,6-hexanediol diacrylate (HDDA). These macromers were used to fabricate degradable gels and cryogels. The degradation behaviour of the hydrogels and cryogels in PBS vs DTT; and the cytotoxicity of degradation products on mouse embryonic fibroblast cells (NIH 3T3) and human osteosarcoma cells (Saos-2) were investigated.

2. Materials and methods

2.1. Materials

1,6-hexanediamine, cystamine dihydrochloride salt, diethyl vinylphosphonate, triethyl amine (TEA), poly(ethylene glycol) diacrylate (PEGDA, M_n = 575), 1,6-hexanediol diacrylate (HDDA), 2,2-dimethoxy-2-phenylacetophenone (DMPA), dithiothreitol (DTT) and all solvents were purchased from Sigma-Aldrich and used as received. Roswell Park Memorial Institute medium (RPMI 1640 medium) with l-glutamine and 25 mM HEPES, trypsin/EDTA and penicillin-streptomycin (Pen-Strep) solutions were obtained from Multicell, Wisent Inc. (Canada). Fetal bovine serum (FBS) was purchased from Capricorn Scientific GmbH (Germany). Thiazolyl blue tetrazolium bromide (MTT) and tablets of phosphate buffered saline (PBS) were provided by Biomatik Corporation (Canada). 96-well plates were obtained from Nest Biotechnology Co. Ltd. (China). Mouse embryonic fibroblast NIH 3T3 and human osteosarcoma Saos-2 cells were a generous gift from Dr. Halil Kavakli (Koc University, Istanbul, Turkey).

2.2. Characterization

The chemical structures of phosphonate-functionalized diamines and PBAE macromers were proved by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and FTIR spectroscopies. NMR spectra were recorded on Varian Gemini 400 MHz spectrometer using $CDCl_3$ as solvent and tetramethyl silane (TMS) as an internal reference. FTIR spectra were recorded on a Thermo Scientific Nicolet 380 spectrometer. Raman spectra were recorded using Renishaw Invia Raman microscope. The glass transition temperatures (T_g) of the macromers and gels were determined with differential scanning calorimetry (TA Instruments Q100): 5–10 mg sample was placed and sealed inside the pan and heated under nitrogen atmosphere from −80 °C to 80 °C with a scanning rate of 10 °C min^{-1}. Degradation studies were done using a VWR Incubating Mini Shaker operating at 37 °C and 200 rpm.

2.3. Synthesis of phosphonate-functionalized diamines

2.3.1. Synthesis of tetraethyl((hexane-1,6-diylbis(azanediyl))bis(ethane-2,1-diyl))bis(phosphonate) (A1)

1,6-Hexanediamine (0.23 g, 2 mmol) and diethyl vinylphosphonate (0.74 mL, 4.8 mmol) were mixed at room temperature for 2 days. The mixture was washed with petroleum ether to remove unreacted diethyl vinylphosphonate and the pure product was obtained as a light yellow viscous liquid in 94% yield.

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.31 (m, 16H; CH_3 and $CH_2CH_2CH_2CH_2$), 1.43 (m, 6H; $CH_2CH_2CH_2CH_2$, NH), 1.93 (dt, J = 16 Hz, J = 8 Hz, 4H; CH_2-P), 2.57 (t, J = 8 Hz, 4H; $CH_2CH_2CH_2NH$), 2.87 (4H; $NHCH_2CH_2P$), 4.08 (m, 8H; OCH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, δ): 16.33 (CH_3), 25.72, 27.10 (C—P), 27.20 ($CH_2CH_2CH_2CH_2$), 29.94 ($CH_2CH_2CH_2CH_2$), 43.28 ($NHCH_2CH_2$—P), 49.48 ($CH_2CH_2CH_2NH$), 61.63 (OCH_2); ^{31}P NMR (150 MHz, $CDCl_3$, δ): 30.55 ppm; FTIR (ATR): ν = 3487 (w; N—H), 2980 and 2927 (C—H), 1236 (P═O), 1022 and 951 (P—O) cm^{-1}.

2.3.2. Synthesis of tetraethyl (((disulfanediylbis(ethane-2,1-diyl))bis(azanediyl))bis(ethane-2,1-diyl))bis(phosphonate) (A2)

First, cystamine dihydrochloride salt was neutralized to cystamine using TEA. Cystamine dihydrochloride salt (0.45 g, 2 mmol) was dissolved in 2 mL of distilled water and TEA (0.62 mL, 4.4 mmol) was added dropwise in an ice bath. The solution was stirred for 15 min at room temperature. Diethyl vinylphosphonate (0.74 mL, 4.8 mmol) dissolved in 2 mL of distilled water was added to the reaction mixture which was then stirred at room temperature for 2 days. The mixture was extracted with diethyl ether (3 × 20 mL), the ether phase was dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was finally washed with petroleum ether to remove unreacted diethyl vinylphosphonate. The product was obtained as a light yellow viscous liquid in 27% yield.

^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.31 (t, J = 16 Hz, 12H; CH_3), 1.74 (s, 2H; NH), 1.93 (dt, J = 16 Hz, J = 8 Hz, 4H; CH_2—P), 2.80 (t, J = 8 Hz, 4H; S—CH_2), 2.92 (m, 8H; S—CH_2CH_2 and $NHCH_2CH_2P$), 4.10 (m, 8H; OCH_2); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 16.34 (CH_3), 25.73, 27.12 (C—P), 38.42 (S—CH_2), 42.93 ($NHCH_2CH_2$—P), 47.62 (S—CH_2CH_2), 61.61 (OCH_2); ^{31}P NMR (150 MHz, $CDCl_3$, δ): 30.20 ppm; FTIR (ATR): ν = 3425 (N—H), 2978 and 2943 (C—H), 1221 (P═O), 1020 and 961 (P—O) cm^{-1}.

2.4. Synthesis of PBAE Macromers

The diacrylates (PEGDA or HDDA) and phosphonated diamines A1 or A2 were mixed at a molar ratio of 1.1:1 at room temperature for 4 days while stirring. After the precipitation of the mixtures into diethyl ether (PEGDA-based ones) or petroleum ether (HDDA-based ones) to remove unreacted diacrylates and amines, the macromers were obtained as colorless to light yellow viscous liquids.

2.5. pH sensitivity of PBAE macromers

50 mg of macromer was dissolved in deionized water with a concentration of 1.0 mg mL^{-1} and the pH was adjusted to 2 by use of HCl. The macromer solution was titrated with 0.1 M NaOH aqueous solution with increments of 50 µL. The increase in pH was recorded with a pH meter (WTW Inolab pH 720) at room temperature. The pK_b value was determined from the inflection point of the titration curve which responds to the pH value where 50% of protonated amine groups are neutralized.