Carbohydrate Polymers 159 (2017) 29–38

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Polyethyleneimine modified poly(Hyaluronic acid) particles with controllable antimicrobial and anticancer effects

Nurettin Sahiner[a,b,*], Selin Sagbas[a], Mehtap Sahiner[c], Ramesh S. Ayyala[d]

[a] Faculty of Science & Arts, Chemistry Department, 17100 Canakkale, Turkey
[b] Nanoscience and Technology Research and Application Center (NANORAC), Canakkale Onsekiz Mart University, Terzioglu Campus, 17100 Canakkale, Turkey
[c] Department of Leather Engineering, Ege University, Bornova, 35100, Izmir, Turkey
[d] Department of Ophthalmology, School of Medicine, Tulane University, 70112, New Orleans, LA, United States

ARTICLE INFO

Article history:
Received 6 October 2016
Received in revised form 6 December 2016
Accepted 8 December 2016

Keywords:
Hyaluronic acid microgel/nanogel
PEI-modified HA particles
Antimicrobial HA particles
Biocompatible p(HA)-PEI particles
Cancer treatment

ABSTRACT

Poly(hyaluronic acid) (p(HA)) particles with sizes from few hundred nm to few tens of micrometer were synthesized by using epoxy groups containing crosslinker glycerol diglycidyl ether (GDE) with high yield, 94 ± 5%. P(HA) particles were oxidized by treatment with sodium periodate and then reacted with cationic polyethyleneimine (PEI) at 1:0.5, 1:1, and 1:2 wt ratio of p(HA):PEI to obtain p(HA)-PEI particles. From zeta potential measurements, isoelectronic points of bare p(HA) particles increased to pH 8.7 from 2.7 after modification with cationic PEI. New properties, such as antibacterial property, were attained for p(HA)-PEI after modification. The highest minimum bactericidal concentration (MBC) values were 0.5, 1, and 0.5 mg/mL against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* species for 1:0.5 ratio of p(HA)-PEI at 72 h incubation time. Moreover, the p(HA)-PEI particles were found to be biocompatible with L929 fibroblast cells, and interestingly, p(HA)-PEI particles were found to inhibit MDA-MB-231 breast and H1299 cancer cell growth depending on amount of PEI in p(HA)-PEI particles.

1. Introduction

Hyaluronic acid (HA), a natural biopolymer present in animal tissues and microorganism membranes, is associated with many biological activities such as inhibition of tumor growth and metastasis, regulation of the immune system, improving wound healing, and so on and is widely used in the biomedical and biotechnology industries (Chen et al., 2015; Shen et al., 2014). Especially, HA-derived materials have high pharmacological demand and are used for medical applications such as filler (Monheit & Coleman, 2006), drug carrier and delivery material (Mero & Campisi, 2014; Purcell et al., 2014), in ocular medicine, plastic surgery and for tissue scaffolds related to their biodegradability and high biocompatibility with tissues (Boussif et al., 1995; Scanté, Zuber, Herlin, & Vandamme, 2011). HA is an intracellular molecule that can influence cell attachment, migration and proliferation in the human body (Iskandar et al., 2009; Kogan, Šoltés, Stern, & Gemeiner, 2007; Patil, Patil, Chaudhari, & Chincholkar, 2011). Moreover, HA has anti-inflammatory and anti-asthmatic properties (Iskandar et al., 2009) and possesses an effective regenerative property (Noble, 2002). Recently, nano- and micron-sized poly(hyaluronic acid) (p(HA)) hydrogel particles have become preferred materials due to their valued properties such as high surface area, biocompatibility, controlled biodegradability, modifiable surface characteristics, and adjustable sizes for various pharmacological applications including injectable materials, drug carrier and delivery, tissue engineering, and so on (Burdick & Prestwich, 2011; Collins & Birkinshaw, 2013; Ekici, Ilgın, Butun, & Sahiner, 2011).

In previous studies, we synthesized micron-sized p(HA) particles with divinyl sulfone crosslinker (Ekici et al., 2011; Sahiner et al., 2012). In this work, p(HA) particles were synthesized with epoxide crosslinkers e.g., GDE and TMPGDE for the first time with a high yield (94 ± 5%). P(HA) particles are suitable materials for chemical modification to generate various functional groups due to the existence of –COOH and –OH groups in the polymeric backbone. Especially amine-based cationic polymers are very resourceful due to their modifiable nature (Demirci & Sahiner, 2014a; Sahiner, 2013a, 2013b). Modifiable amine groups provide significant new properties such as tunable functional groups, surface charges, antimicrobial property, etc (Demirci & Sahiner, 2014a; Sahiner, 2013b). Therefore, many researchers pay great atten-

* Corresponding author at: Faculty of Science & Arts, Chemistry Department, 17100 Canakkale, Turkey.
E-mail address: sahiner71@gmail.com (N. Sahiner).

http://dx.doi.org/10.1016/j.carbpol.2016.12.024
0144-8617/

tion to amine-based polymers such as polyethyleneimine (Cai et al., 2013; Demirci & Sahiner, 2014b; Schneider, Breinkmann, & Möhvald, 2003), chitosan (Agudelo, Nafisi, & Tajmir-Riahi, 2013; Onishi, 2010; Remaut et al., 2007; Richard, Thibault, De Crescenzo, Buschmann, & Lavertu, 2013; Schiffman & Schauer, 2007; Vanamudan & Pamidimukkala, 2015), melamine (Hang et al., 2011; Schwab et al., 2009; Wang et al., 2015) and poly(4-vinly pyridine) (Sahiner, 2013b) in the literature. In recent years, many researchers paid attention to polyethyleneimine (PEI) due to its great DNA transfection efficiency (Liu, Zhang, Zhou, & Jiao, 2010; Xia et al., 2009; Vinogradov, Zeman, Batrakova, & Kabanov, 2005) and antimicrobial activity (Demirci & Sahiner, 2014a; Sahiner, 2013a, 2013b; Shvero, Zaltsman, Hazan, Weiss, & Beyth, 2015). The most valuable cationic polymer for DNA transfection is PEI (Boussif et al., 1995; Schafer, Hobel, Bakowsky, & Aigner, 2010) due to its relatively efficient and high capacity to complex with DNA (Dey et al., 2011). Besides, PEI has been used to transfect a variety of cells in vitro and in vivo for anticancer studies (Loh, Yao, Yap, & Chung, 1997; Rajesh, Rekha, & Sharma, 2012).

The aim of this study is to combine polycationic modified PEI and polyanionic HA as p(HA)-PEI particles for promising new synergic applications such as antimicrobial agent and effective antioxidant and anti-cancer material. The prepared p(HA)-PEI particles with different ratios of p(HA) particles to PEI (1:0.5, 1:1, 1:2) may provide controlled antimicrobial and anticancer properties. Morphological and physicochemical properties of p(HA)-based particles were investigated via optic microscope, SEM images, FT-IR spectroscopy, TGA, DLS, zeta potential measurements and with titration experiments. PEI release studies from PEI loaded p(HA) particles and modified p(HA)-PEI particles were investigated under physiological conditions at pH 7.4 and 37.5 °C. As PEI is a well-known antimicrobial material due its polycationic nature (Demirci & Sahiner, 2014a; Sahiner, 2013a, 2013b; Shvero et al., 2015), the antimicrobial susceptibility of p(HA)-PEI particles containing different amounts of PEI were tested against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 6633 strains by using micro dilution method. The biocompatibility of biomaterials is one of the most important properties in biomedical applications, and previously HA and p(HA) particles were shown to be highly biocompatible for *in vivo* and *in vitro* applications to be used in tissue engineering and drug delivery applications (Burdick & Prestwich, 2011). Therefore, the cytotoxicity of p(HA), and p(HA)-PEI particles prepared at different mole ratios (1:0.5, 1:1 and 1:2) were evaluated by measuring cell viability of L929 fibroblast, MDA-MB-231 breast cancer, and H1299 lung cancer cells via WST-1 assay. PEI-containing particles possess cationic charge can be a potential material for cancer therapy due to simple penetration mechanism into a tumor cell (Loh et al., 1997; Rajesh et al., 2012). Herein, the cell viability of the p(HA)-PEI particles containing different ratios of PEI were investigated on MDA-MB-231 breast cancer and H1299 lung cancer cell lines and their apoptotic and necrotic indices were determined.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium salt from *Streptococcus equi* (HA, MW: 1200 kDa, Sigma-Aldrich), glycerol diglycidyl ether (GDE, technical grade, Aldrich) as crosslinker, sodium bis(2-ethylhexyl) sulfosuccinate (AOT, 98%, Sigma-Aldrich) as surfactant, and 2,2,4-trimethylpentane (isooctane, Merck) as solvent were used as received. Sodium (meta)periodate ($NaIO_4$, >99%, Sigma-Aldrich) as an oxidizing agent and polyethyleneimine (PEI, 50% in water M_n:1800, Sigma Aldrich) were used for modification of p(HA) particles. Nutrient agar (Merck), potato dextrose agar (Merck), and nutrient broth (Merck) were used as microbial growth media. *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 6538 strains were obtained from the Microbiology Department of the School of Medicine at Canakkale Onsekiz Mart University. MDA-MB-231 cancer cell line was obtained from Hacettepe University, Biochemistry Division of the Medical Faculty (Ankara, Turkey). H1299 cancer cell line was obtained from Uludag University, Biochemistry Division of Medical Faculty (Bursa, Turkey). L929 fibroblast cell line was obtained from the Tissue and Cell Culture Bank of the Foot and Mouth Disease Research Institute (Ankara, Turkey). Cell culture flasks and other plastic material were purchased from Corning (USA). The growth medium, Dulbecco Modified Medium (DMEM) without L-glutamine supplemented with fetal calf serum (FCS) and Trypsin-EDTA was purchased from Biological Industries (USA). 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2H-tetrazolium, monosodium salt (WST-1) was purchased from Roche (Germany). Hoechst 33342 and propodium iodide (PI) were purchased from Serva (Israel). Phosphate buffer solution (PBS) was purchased from Sigma-Aldrich (USA). All the solvents, acetone and ethanol were highest purity available. All aqueous solutions were freshly prepared using ultrapure distilled water 18.2 M Ω cm (Millipore-Direct Q UV3).

2.2. Synthesis of p(HA) particles

Linear HA weighing 100 mg was dissolved in 2 mL 0.2 M NaOH solution. Then 1.08 mL of this solution was dispersed in 30 mL of 0.2 M AOT solution in isooctane under vigorous stirring at 12,000 rpm for 1 h. The crosslinker, GDE (50 mol% relative HA repeating unit), was subsequently added to this mixture under vigorous stirring for 1 h. To precipitate the formed p(HA) particles, an excess amount of acetone was added to this solution. The p(HA) particles were washed and purified with acetone and water mixture (1:1 by volume) at least 3 times by centrifugation for 10 min at 10,000 rpm. The prepared p(HA) particles were dried with a heat gun, and stored in a closed container for modification, and further studies.

2.3. Modification of p(HA) particles with PEI

P(HA) particles weighing 1 g were dispersed in 100 mL 10 wt% of $NaIO_4$ aqueous solution in order to oxidize the proximal –OH groups to generate two aldehyde groups on the p(HA) particle network according to previous studies (Ilgin et al., 2010; Jia et al., 2004). This mixture was stirred at 500 rpm for 12 h. Then, the particles were precipitated with excess amount of acetone and washed with acetone by centrifugation at least two times. The aldehyde group-containing p(HA) particles were treated with various concentrations of PEI solution in the chemical modification procedure. Briefly, 0.5 g p(HA) particles treated with $NaIO_4$ were put into 20 mL aqueous solution containing different concentrations of PEI (0.5, 1, and 2 mL) for 12 h. The p(HA)-PEI particles obtained using 1:0.5, 1:1, and 1:2 wt ratio of HA:PEI treatments were precipitated with excess amount of acetone. The p(HA)-PEI particles were washed with acetone-water mixture at least two times by centrifugation for 10 min at 10,000 rpm, dried with a heat gun and stored in a closed container for drug loading and release studies.

2.4. Absorption of PEI onto p(HA) particles

Bare p(HA) particles weighing 0.5 g were placed into 20 mL aqueous solution containing 1 mL PEI for 12 h at room temperature. The prepared PEI-absorbed p(HA) particles were precipitated with excess amount of acetone. Particles were washed with ace-