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Preparation of macro-, micro-, and nano-sized poly(Tannic acid) particles with controllable degradability and multiple biomedical uses

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ABSTRACT

Different size ranges of poly(Tannic acid) (p(TA)) particles, 2000–500 μm, 500–200 μm, 200–20 μm, and 20–0.5 μm, were successfully synthesized by using lecithin/gasoline microemulsion media. Macro, micro, and nano sized p(TA) particles were crosslinked via poly(ethylene glycol) diglycidyl ether (PEGGE) with 85 ± 7% gravimetric yield. The hydrolytic degradation of different sizes of p(TA) particles in physiological pH conditions, in pH 5.4, 7.4, and 9.0 buffer solutions at 37.5 °C, were investigated. It was found that p(TA) particles with 20–0.5 μm size distribution are more stable than the other sized particles due to the higher amounts of crosslinker used during synthesis. Furthermore, macro size p(TA) particles (2000–500 μm) were totally degraded at pH 9 within 12 days, whereas a linear and sustained degradation profile was obtained at pH 7.4 with 75 ± 4% weight loss for 24 days. The antioxidant capacity of p(TA) particles was also tested and 20–0.5 μm sized p(TA) particles demonstrated the highest antioxidant capacity with 0.1305 ± 0.0124 mg gallic acid equivalency and 145 ± 21 mM trolox equivalent g^{-1}. It was also further demonstrated that the degraded p(TA) particles showed high antimicrobial activity against a wide spectrum of bacteria and yeast strains such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*. *In vitro* blood compatibility of p(TA) particles was also examined by hemolysis % and blood clotting index and micrometer sized p(TA) particles are more hemocompatible with enhanced blood clotting capability. In addition, WST-1 cytotoxicity test results showed that 200–20 μm and 20–0.5 μm sized p(TA) particles were biocompatible up to 50 μg/mL concentration with 74 ± 3 and 68 ± 2% cell viabilities for L929 fibroblast cells.

1. Introduction

Macro, micro, and nano sized hydrogels are soft crosslinked particles forming a three dimensional polymer network with different dimensions that can be swollen in suitable solvents such as water [1,2]. They can be prepared from different original material like natural sources with diverse attractive properties including biocompatible [3], biodegradable [4], non-toxic, modifiable [5], biomimicry nature [6]. Biopolymer-based particles are one of the most important materials for clinical and biomedical applications such as biosensors [7], drug carrier and delivery systems [8], tissue engineering [9], wound dressing/healing [10], gene therapy [11], and so on. Tannic acid (TA) is a naturally occurring phenolic compound existing in the bark and fruits of many plants, known as hydrolysable polyphenol [12]. TA-based materials are likely to have antibacterial and antioxidant capabilities due to the existence of these phenolic groups resulting in superior biomaterial designs. Therefore, TA has biological activities including vasodilatation [13], antibacterial, anti-viral [14], anti-inflammatory [15], antioxidant, anticarcinogenic, and antimutogenic properties [16], and has been extensively investigated in medicine, leather, agriculture, food, and biomedical applications [17]. TA can directly interact with various biomacromolecules such as collagen, albumin, gelatin, chitosan [18], enzymes, and carbohydrates depending on the pH values and temperatures, offering wide spectrum of application potentials [19,20]. Crosslinked bulk and micron sized p(TA) hydrogels were prepared by our group in earlier studies [21–24]. Here, we report a single step method for the preparation of crosslinked p(TA) macro,

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micro, and nanoparticles by using poly(ethylene glycol) diglycidyl ether (PEGGE) as crosslinker in the same media with high yield (85 ± 7%). The control of particle size and their degradation are very vital parameters in design, fabrication and use of natural polymeric particles for specific applications. Different size ranges of p(TA) particles were separated by a simple filtration technique e.g., via 500, 200, and 20 μm pore sized filters. Four different size ranges of p(TA) particles; 2000–500 μm, 500–200 μm, 200–20 μm, and 20–0.5 μm, were synthesized using the same lecithin/gasoline emulsion system.

For many years, degradable biomaterials containing drug-like molecules have attracted much attention for pharmaceutical and clinical applications owing to their controllable drug release mechanism [25]. Especially, different sizes of hydrogel are highly sensitive to environmental changes such as pH, temperature, ionic strength, and so on and they can readily degrade in a controlled fashion based on these environmental factors [26,27]. TA can be used as medicine in burn, analgesic, diarrhea, and poison treatments [17,28]. Therefore, the hydrolytic degradation profiles of different size ranges of p(TA) particles were investigated in biological conditions; at pH 5.4 (skin), pH 7.4 (blood), and pH 9 (intestine) and 37.5 °C. Controlled degradation of p(TA) particles with changing particle size distributions and pHs were also measured. Also, as TA is a well known antimicrobial and antioxidant material against a broad spectrum of microorganisms [15], the effects of size range of degradable p(TA) particles against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* were determined via disc diffusion test. Because of its high antioxidant properties, TA can regulate biological functions or inhibit pathogenesis. Therefore, *in vitro* antioxidant capacity of different size ranges of p(TA) particles were evaluated by using total phenol content values and trolox equivalency antioxidant capacity methods. Furthermore, the blood compatibility of the prepared p(TA) particles were tested on red blood cells generally evaluated by *in vitro* hemolysis ratio and blood clotting tests. Thus, the effects of size distribution on the blood compatibility of p(TA) particles were determined by *in vitro* hemolysis ratio and blood clotting tests. Furthermore, the cytotoxicity of p(TA) particles on L929 fibroblast cells were investigated by WST-1 test. Apoptotic and necrotic cell death indexes of L929 fibroblast cells exposed to p(TA) particles were also determined by the double staining method.

2. Material and methods

2.1. Materials

Tannic acid (TA, ACS grade, 97% Sigma-Aldrich) as oligomer, poly(ethylene glycol) diglycidyl ether (PEGGE, M_n:500, Aldrich) as crosslinker, triethylamine (TEA, 99.5%, Sigma-Aldrich) as an accelerator, L-alpha-Lecithin (granular, 98%, Acros Organic) as surfactant, and gasoline (95 octane, local vender) as solvent were used as received. Acetone (99%) and absolute ethyl alcohol (99%) were purchased from Kimetsan. Folin-Ciocalteau's phenol reagent (FC), 2,2′-Azino-bis-(3-ethylbenzothioazoline-6-sulfonic acid) (ABTS) and gallic acid (GA, 97.5–102.5%) were obtained from Sigma-Aldrich for antioxidant studies. *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 10145 strains were obtained from Microbiology Department of School of Medicine at Canakkale Onsekiz Mart University. Nutrient agar (Merck), and potato dextrose agar (Merck) were chosen as microbial growth media. Ultra-pure distilled water 18.2 MΩ cm (Millipore-Direct Q UV3) was used throughout the study. L929 fibroblast cell line was obtained from ŞAP Institute (Ankara, Turkey) for biocompatibility studies. Sodium bicarbonate, calcium chloride, ethyl alcohol, trypan blue, phosphate buffer solution (PBS), and hydrogen chloride (0.1 M) were purchased from Sigma-Aldrich. Fetal bovine serum (FBS, Serva, Israel), Dulbecco Modified Eagles Medium (DMEM, BD., USA), and trypsin-EDTA (Sigma-Aldrich) were used as growth media. The cell culture flasks and other plastic materials were obtained from Corning (NY, USA). Hoechst 33342, propidium iodide (PI), caspase 3 antibody, and chromogen kit were purchased from Roche (Germany).

2.2. Synthesis of macro-, micro-, and nanometer-sized p(TA) particles

P(TA) macro-, micro-, and nanoparticles were synthesized via a single step reaction using water-in-oil microemulsion technique. In brief, 1 g TA was dissolved in 4 mL 0.5 M NaOH solution. Then, 1 mL of this solution was added to 50 mL 0.1 M lecithin in gasoline solution at 50 °C under vigorous stirring at 600 rpm. Immediately, crosslinker (PEGGE, % 200 mol ratio with respect to TA) and accelerator (TEA, 15 μL) were added to the reaction medium and mixing continued for 12 h. At the end of the crosslinking reaction, the reaction medium was filtered with filters with different pore sizes; 500 μm, 200 μm, and 20 μm, to separate different sizes of particles. Then, the p(TA) nanoparticles were collected by centrifugation at 35544*g* for 2 min. The obtained p(TA) particles with different size ranges were washed with gasoline and cyclohexane two times to remove surfactant and unreacted chemicals. The p(TA) particles were then dried with a heat gun and kept in a closed container for further use.

2.3. Characterization of p(TA) particles

FT-IR spectra of the prepared p(TA) particles were recorded with Nicolet iS10, Thermo instrument in transmission mode in the frequency range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1} using the ATR technique.

The size, shape and the surface structure of particles were visualized by scanning electron microscope (SEM, Jeol JSM-5600 LV) images with an operating voltage of 20 kV. The p(TA) particles were placed on carbon tape that was attached to aluminum SEM stubs. Then, particles were coated with gold to a few nanometers thickness in a vacuum.

Thermogravimetric analysis (TGA) was conducted via a thermogravimetric analyzer (SII TG/DTA 6300) at a heating rate of 10 °C min^{-1} under a dry flow of N_2 of 100 mL min^{-1}. Nearly 5 mg samples were put into ceramic crucibles and the thermal degradation of particles were determined in the range of 50–1000 °C.

2.4. Hydrolytic degradation of p(TA) particles

To investigate the effect of size on particle degradation, hydrolytic degradation of p(TA) macro-, micro-, and nanoparticles were determined in three different pH buffers, pH 5.4 (citrate buffer), pH 7.4 (phosphate buffer) and pH 9 (phosphate buffer). Briefly, 30 mg of p(TA) particles were put into 30 mL buffer solutions at 37.5 °C placed in constant temperature water bath shaker under slow shaking. Then, the degraded TA or released TA amount was measured by using UV–Vis spectrometer (T80 + UV/VIS Spectrometer, PG Ins. Ltd) at 280 nm wavelength according to previously prepared calibration curves for TA solutions in pH 5.4, 7.4, and 9 buffer solutions. The weight loss % of particles was determined.

2.5. Antioxidant properties of p(TA) particles

2.5.1. Total phenol content

Total phenol content of samples was investigated by Folin-