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Single step natural poly(tannic acid) particle preparation as multitalented biomaterial

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ABSTRACT

In this study, we report the preparation of poly(tannic acid) (p(TA)) particles by crosslinking with glycerol diglycidyl ether (GDE) and trimethylolpropane triglycidyl ether (TMPGDE). The p(TA) particles are negatively charged as obtained by the zeta potential measurements, −27 mV. P(TA) particles are found to be an effective antioxidant material as 170 mg L^{-1} of p(TA) particle demonstrated the antioxidant equivalency of 82.5 ± 7.2 mg L^{-1} of gallic acid (GA), used as standard in Folin–Ciocalteau (FC) method. Additionally, TA and p(TA) particles have a strong antimicrobial effect against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 6633. Furthermore, p(TA) particles were used as drug delivery materials by using model drugs such as TA itself, and GA in the release studies in PBS at pH 7.4 at 37.5 °C, and found that p(TA) particles can release 80.8 and 87.4% of the loaded TA and GA, respectively. Interestingly, p(TA) maintained its fluorescent property upon crosslinking of TA units. It is further demonstrated that p(TA) particles are as effective as cisplatin (a cancer drug) against A549 cancerous cells that both showed about 36 and 34% cell viability, respectively whereas linear TA showed 66% cell viability at 37.5 μg mL^{-1} concentration. Above this concentration p(TA) and cisplatin showed almost the same toxicity against A549 cancerous cells. Additionally, p(TA) particles are found to be much more biocompatible against L929 Fibroblast cells, about 84% cell viability in comparison to linear TA with about 53% at 75 μg mL^{-1} concentration.

1. Introduction

Tannic acid (TA) is hydrolyzable phenolic secondary metabolites obtained from plants [1,2], and consists of a central glucose unit and ten gallic acid molecules attached to it [3]. Tannic acid has many attractive biological functions such as antimutagenic, antimicrobial [4,5], antiviral, anticarcinogenic, anti-inflammatory, astringent [6], homeostatic [7], and is an antioxidant material [8–11]. Free radicals are highly reactive hazardous species that play a crucial role in several chronic and degenerative diseases such as allergies, diabetes, Parkinson's, Alzheimer's, cancer, and cardiovascular diseases [12]. Tannic acid is an important plant-based polyphenol [13], with many usages in pharmaceutical [7], coating [14], and cosmetic industries due to its free radical scavenger capability, and the ability to protect the body from damaged cells [15–21].

Microgels are crosslinked polymeric materials swollen by a good solvent e.g., water [22,23]. Smart microgels are considered as fast stimuli-responsive materials against stimuli such as pH, ionic strength [24], temperature [25], magnetic fields [26,27], electric field and so on [28,29]. Recently, there is increasing interest in various potential applications of microgels with additional functions such as drug or protein delivery [30–33], biosensing [34], chemical separation [35], and catalysis [36,37]. The design and synthesis of microgels from a biodegradable [38], and biocompatible [39] source as an environment-responsive material for use in tissue engineering [40,41], cosmetics, coatings, food, and pharmaceutical industries [38] have prominent significance. As biodegradability and biocompatibility of biomaterials are the two most important parameters for biomedical applications such as drug delivery and tissue engineering [42,43], materials with natural origin, with no toxicity of by-products, with additional characteristics such as antimutagenic, antimicrobial [4,5], antiviral, anticarcinogenic, anti-inflammation, and astringent properties are in great demand.

It has been reported that TA is a natural crosslinker owing to their hydroxyl and carboxyl groups, that can interact with biopolymers such as myosin, collagen, chitosan, and digestive enzymes and so on for different biomaterial designs [9,44]. Herein, we report for the first time the synthesis and characterization of p(tannic acid) hydrogel particles with a high yield (73 ± 6%) via simple one step microemulsion crosslinking of the natural molecule, TA. Morphological and physicochemical properties of TA and p(TA) particles were examined via SEM, TGA, FT-IR, DLS and zeta potential measurements. The degradability of p(TA) particles in PBS (pH 7.4) at 37.5 °C was assessed. Some of the

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reports in the literature demonstrated that TA possesses some synergetic effect with some drug compounds; such as with analgesic, anticarcinogenic [45], antibiotic, antiviral, antiseptic agents [20]. Therefore, gallic acid (GA) and tannic acid (TA) were used as model drugs for loading into p(TA) particles from ethanol and release into PBS (pH 7.4) was studied. Moreover, the antimicrobial effects of TA are well-known against the wide range of bacteria. In this study, the antimicrobial and antioxidant properties of TA molecules and p(TA) particles were evaluated via analysis of antimicrobial tests against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, and *Bacillus subtilis* ATCC 6633 using agar well diffusion and broth-micro dilution tests. Total phenolic content and DPPH assay methods were used to determine the antioxidant properties of TA and p(TA) particles. Moreover, optical and fluorescent properties of TA and p(TA) particles were investigated by UV–Vis and fluorescence spectrometers. Additionally, the cytotoxicity of linear TA and p(TA) particles against A549 cancerous cells, and L929 fibroblast cells were investigated. The apoptotic and necrotic indices against these cells were also determined. Here, the aim of the present study was to combine the versatile properties TA molecules synergistically by turning TA molecules into p(TA) particle form in a single step with additional therapeutic benefits and advantages to be used as biomaterials.

2. Materials and methods

2.1. Materials

Tannic acid (TA) (ACS grade) as monomer, glycerol diglycidyl ether (GDE) (technical grade) and trimethylolpropane triglycidyl ether (TMPGDE) (technical grade), as crosslinkers, L-alpha-lecithin, granular as surfactant, were purchased from Aldrich, and Acros chemical companies. Gasoline as an organic solvent was procured from a local vender. All the other chemicals including acetone (99%), absolute ethyl alcohol (99%), NaOH, and HCl were purchased from Merck, Aldrich, and Fluka chemical companies. All aqueous solutions were freshly prepared using ultra pure distilled water 18.2 M·Ω·cm (Millipore-Direct Q UV3). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Aldrich), Folin–Ciocalteau's phenol reagent (FC) (Sigma-Aldrich), and gallic acid (GA) (97.5–102.5% Aldrich) were used for antioxidant analysis. *E. coli* ATCC 8739, *S. aureus* ATCC 6538, and *B. subtilis* ATCC 6633 strains were used for antimicrobial tests. Nutrient agar (Merck) and nutrient broth (Merck) were used as microbial growth media. A549 cancer cells were obtained from the Division of Biochemistry of Medicinal Science Faculty, Uludag University, Turkey. L929 fibroblast cell lines were purchased from SAP Institute of Ministry of Agriculture (Ankara, Turkey). Cell culture flasks and other plastic material were purchased from Corning (NY, USA). Dulbecco Modified Eagle's Medium (DMEM) with L-glutamine, fetal calf serum (FCS), trypsin-EDTA, Hoechst 33342 and propidium iodide (PI) were purchased from Serva (Israel), WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] reagent was purchased from Roche (Germany).

2.2. Synthesis of tannic acid particles

Tannic acid, 2.5 g was dissolved in 5 mL 0.2 M NaOH solution. From this solution, 0.5 mL was dispersed in 15 mL of 0.1 M lecithin in gasoline under vigorous stirring at 12,000 rpm for 1 h. GDE (115 μL, 100 mol% of gallic acid) and TMPGDE (115 μL, 30 mol% of gallic acid) as crosslinkers were subsequently added as crosslinking agents to this mixture under vigorous stirring. The crosslinking reaction proceeded for 12 h at 50 °C. The obtained p(TA) particles were precipitated by centrifugation at 35544 *g* for 2 min at 20 °C. The supernatant solution was decanted and p(TA) particles were washed with cyclohexane to remove lecithin. Then the particles were washed with ethanol–water mixture two times to remove the unreacted species (TA, surfactant, crosslinker) by centrifugation at 35,544 *g* for 2 min. The prepared particles were dried with a heat gun and kept in a closed container for further use.

2.3. Characterization of p(TA) particles

The p(TA) particles were imaged by scanning electron microscopy (SEM) by placing the particles onto carbon tape-attached aluminum SEM stubs, after coating with gold to a thickness of a few nanometers, in a vacuum using SEM (Jeol JSM-5600 LV), operating at 20 kV.

The thermal characterization of crosslinked p(TA) particles was carried out using a thermogravimetric analyzer (SII TG/DTA 6300). About 5 mg of particles was placed in ceramic crucibles and the weight lost was recorded over the temperature range of 50–1000 °C at a heating rate of 10 °C min^{-1} under a dry flow of N_2 of 100 mL min^{-1}.

The functional groups of p(TA) particles were analyzed using FT-IR spectroscopy (Perkin Elmer spectrum 100) in the spectral range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1}, using the ATR technique.

Zeta potential measurements of p(TA) particles were conducted with Zeta-Pals Zeta Potential Analyzer BIC in different KCl solution concentrations: 0.1, 0.01, and 0.001 M KCl solutions at different pHs in DI water. The pH of a solution containing 100 mg p(TA) in 50 mL at different pHs (2–10) was adjusted using 0.2 M NaOH or HCl solution.

2.4. Degradation of p(TA) particles

Degradation studies of p(TA) particles were performed at three different pHs: 7.4, pH 11 in PBS, and 3 in citrate buffer solution prepared from 0.1 M sodium citrate and 0.1 M HCl. Briefly, 10 mg p(TA) particles was suspended in 1 mL of buffer solutions and transferred to a dialysis membrane (molecular weight cut off ≥12,000 Da, Aldrich). Then the dialysis membrane was placed into a closed beaker containing 50 mL of buffer solutions and this beaker was put into a shaking water bath at 37.5 °C. The released amounts of TA into pHs 3, 7.4, and 11 were determined with UV–Vis spectroscopy (T80 + UV/VIS Spectrometer, PG Ins. Ltd) at 280 nm. Then, the amount of degradation was determined from previously created calibration curves at these pH values for the corresponding buffer solutions.

2.5. Drug loading and release of p(TA) particles

Two model drugs, TA and GA, were used for in vitro drug loading and release studies of p(TA) particles. For loading, 200 mg p(TA) particles was put into 50 mL 300 ppm TA and GA solutions in ethanol under constant stirring at 250 rpm for 24 h. The drug-loaded p(TA) particles were purified by centrifugation and dried by heat gun. TA and GA loading amounts into p(TA) particles were determined with UV–Vis spectroscopy (T80 + UV/VIS Spectrometer, PG Ins. Ltd) at 278 nm and 272 nm in ethanol, respectively. For the release studies, 50 mg drug-loaded p(TA) particles was added into 1 mL of PBS (pH = 7.4) and transferred to a dialysis membrane (molecular weight cut off ≥12,000 Da, Aldrich). Then the dialysis membrane was placed into a closed beaker containing 25 mL of PBS buffer at 37.5 °C and stirred at 200 rpm. The amount of TA and GA released from p(TA) particles into the PBS buffer was determined with UV–Vis spectroscopy at 280 nm and 265 nm, respectively via their previously constructed calibration curves at the same wavelengths. Drug release measurements were done in triplicate and the results are given as the average values with standard deviations.

2.6. Antioxidant activity of TA and p(TA) particles

2.6.1. Total phenol content of TA and p(TA) particles

To determine antioxidant activity, the total phenol content of TA and p(TA) particles was determined by using the Folin–Ciocalteau (FC) method with some modifications. TA or p(TA) particle solutions were prepared at 0.17 mg mL^{-1} in DI water. And, 0.1 mL of this solution was added to 1.25 mL 0.2 N solution of FC phenol reagent and vortex