Materials Science and Engineering C 76 (2017) 417–424

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Cytoprotective effect of the enzyme-mediated polygallic acid on fibroblast cells under exposure of UV-irradiation

Roberto Sánchez-Sánchez [a], Alejandra Romero-Montero [b], Carmina Montiel [b], Yaaziel Melgarejo-Ramírez [a], Carmina Sánchez-Ortega [c], Haydée Lugo-Martínez [d], Beatriz Cabello-Arista [a], Roeb García-Arrazola [b], Cristina Velasquillo [a], Miquel Gimeno [b,*]

[a] Laboratorio de Biotecnología, Instituto Nacional de Rehabilitación Luis Guillermo Ibarra, Mexico DF, Mexico
[b] Facultad de Química, Departamento de Alimentos y Biotecnología, Universidad Nacional Autónoma de México, Ciudad Universitaria, México DF 04510, Mexico
[c] Unidad de Ingeniería de Tejidos Terapia Celular y Medicina Regenerativa, Instituto Nacional de Rehabilitación Luis Guillermo Ibarra, Mexico DF, Mexico
[d] Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, México DF 04510, Mexico

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 22 December 2016
Accepted 9 March 2017
Available online 14 March 2017

ABSTRACT

The poly(gallic acid), produced by laccase-mediated oxidation of gallic acid in aqueous media (pH 5.5) to attain a novel material with well-defined molecular structure and high water solubility (500 mg/mL at 25 °C), has been investigated to understand its potential biological activities. In this regard, a biomedical approach based on cytoprotective effect on human fibroblast cells exposed to UV-irradiation in the presence of the polymer has been demonstrated. The results also shows that 200 μg/mL of poly(gallic acid) inhibits the growth and migration of dermal fibroblasts and cancer cell lines without affecting cell viability. Poly(gallic acid) pretreatment with 10 μg/mL protects dermal fibroblasts from UV induced cell death and additionally, the cytoprotective effect reduce ROS presence in the cells. This property can be correlated with the antioxidant power (IC_{50} of 23.5 μg/mL) of this novel material, which was ascertained by electronic paramagnetic resonance spectroscopy and spectrophotometrically. Additionally, the antimicrobial activity of this material was corroborated with the inhibition of *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) strains (MIC = 400 mg/mL) common bacteria found in hospitals.
© 2017 Elsevier B.V. All rights reserved.

1. Introduction

The field of materials science and engineering is developing chemical compounds with adequate characteristics to induce or provide cell protection against damage. The skin is the organ responsible to protect the body against pathogen microorganisms, loss of water, ultraviolet (UV) damage and participates in thermoregulation. After skin damage, the wound healing process requires cell proliferation, migration, growth factors secretion, matrix remodeling, induction of cell death and other processes. Although keratinocytes are the first cells to receive the UV irradiation, dermal fibroblasts are responsible for production of collagen and secretion of growth factors involved in skin repair after suffering damage [1–3]. Fibroblasts are the major type of cells that constitute the dermis of skin. These cells are a suitable model system to test the protective effects of engineered materials. Skin damage by UV-irradiation induces oxidative stress, which is an alteration that leads to an increased production of free radicals beyond the capability of scavenging; which in turn, promotes interaction within cells leading to proteins, membrane and gene damage [4,5]. Additionally, oxidative stress is involved not only in wound healing but also in several metabolic syndromes and diseases such as diabetes, obesity, cancer and neurodegenerative disorders [6,7]. Noteworthy, over 200 epidemiological observations have shown that consumption of natural polyphenolic antioxidants from diet intake of fruits and vegetables has a protective effect on cancer [8]. On the biomedical field, several studies have shown that phenolic compounds reduce in vitro oxidation of low-density lipoprotein without toxic aspects associated to synthetic phenolic antioxidants [9–11]. Most of natural phenolic antioxidants have radical scavenging capacity but also can present antimicrobial activities and anticancer effect with the absence of undesired side effects for food and biomedical applications [6,12]. Nonetheless, natural polyphenols sources provide mostly hydrophobic extracts with limited or none water solubility. Additionally, these polyphenols display high UV sensitivity as they degrade easily under this type of irradiation loosing most of their properties; hence, limiting their practical applications [13]. Alternatively, several approaches have been reported by conjugation of phenols onto polymer matrices or their glycosylation employing enzymes in the sought for pharmacological applications, among others [14]. Recently, an enzyme-mediated polymer from the naturally

* Corresponding author.
E-mail address: mgimeno@unam.mx (M. Gimeno).

http://dx.doi.org/10.1016/j.msec.2017.03.068
0928-4931/© 2017 Elsevier B.V. All rights reserved.

abundant and low water-soluble gallic acid (GA), the poly(gallic acid) (PGAL), has been successfully synthetized by our research group in a non-toxic process using laccase from *Trametes versicolor* biocatalyst, with defined molecular structure having all pendanthydroxyl and carboxyl groups in a conjugated phenyl-phenyl propagation [15]. High water solubility and electric semiconductivity was proven for our enzymatically synthetized PGAL with an average number molecular weight (M_n) of approximately 7000 Da. Further work is presented herein focusing on the effect of PGAL on biological activities including cell proliferation, migration, viability and its potential biomedical application to provide a cytoprotective effect under UV irradiation, in addition to antioxidant and antimicrobial capacities. This work demonstrates that PGAL has potential skin protection applications.

2. Materials and methods

2.1. Materials

GA (3,4,5-trihydroxybenzoic acid, reactive grade), 1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) were purchased from Sigma Aldrich (US) and used as received. Laccase from *Trametes vesicolor* (LTV) was supplied by Fluka as a lyophilized powder with an enzyme activity (U) of 0.28/mg, which was determined as follows; 50 μL of enzyme in phosphate buffer (1 mg/mL) pH 5.0 (100 mM) was added to 2.9 mL of an ABTS solution (9.1 mM, pH 5) for 2 min at 25 °C and the mixture was spectrophotometrically measured in a Genesys 10S UV–vis spectrophotometer (Thermo scientific) at 405 nm ($E_{405} = 36.8\ M^{-1}\ cm^{1}$). U was expressed as the amount of enzyme needed to produce 1 μmol of oxidized ABTS per min. Ethanol (technical grade) was supplied by Química Barsa, S.A. de C.V. Dulbecco's Modified Eagle Medium (DMEM F12), fetal bovine serum (FBS; Gibco, USA), phosphate buffered saline (PBS), penicillin, streptomycin and amphotericin B were supplied by Gibco (USA) HCT 116 (colon cancer cell line, ATCC® CCL-247™) and HT-29 (colorectal adenocarcinoma cell line, ATCC® HTB-38™) were supplied by ATCC® (USA). All other reagents were used as supplied.

2.2. LTV-mediated synthesis of PGAL

Enzymatic synthesis of PGAL [15] was carried out in a 500 mL round-bottom amber flask containing 250 mL of acetate buffer (pH 5, 250 mM) and 8.5 g of GA. 20 mL of NaOH (2 M) were added and stirred for 20 min. to complete dissolution of GA. Then, LTV (0.95 g) was added and the mixture was stirred for 48 h at 25 °C with air bubbling using an air pump (Elite 800) to maintain 6 mg/L of oxygen concentration in the media throughout the reaction as monitored with an Apliensez-DO sensor (Applikon, USA). Product was precipitated into cold (5 °C) ethanol (1/10; v/v) under stirring, filtered and dried in a Stable temp Cole Palmer (USA) vacuum oven at 40 °C connected to a Vacuubrand PC3 RZ 2.5 systemat 10×10^4 mbar to give PGAL as black powder in 96% yield.

2.3. PGAL characterizations

M_n and polydispersity index (PDI) of the PGAL sample were determined by size exclusion chromatography (SEC) using a HPLC Agilent 1210 series, equipped with Refractive index detector using two Ultrahydrogel 500 (7.8 × 300 mm) columns in serie (Waters, USA) placed in a thermostat (25 °C) and calibrated with polyethylene glycol standards (Varian, USA). Samples were eluted with ultrapure deionized water (Symplicity UV Millipore, USA) with LiCl (0.1 M) in a 0.8 mL/min flow. All samples were dissolved in the mobile phase and filtered (0.45 μm) *prior to* injection in the chromatographer. Infrared (IR) spectra were acquired in an ATR-FTIR spectrometer (Perkin Elmer, spectrum 100) in a 4000–250 cm^{-1} range. ^1H NMR spectra of PGAL were recorded on a Varian Unity Innova spectrometer (USA) at 400 MHz using deuterated water.

2.4. Radical scavenging studies of PGAL by EPR spectroscopy and ABTS methods

Inhibition of the DPPH radical by PGAL was conducted in a Bruker E-500 Elexsys EPR spectroscope (USA). Samples of different concentrations of PGAL and Trolox (as reference compound) were mixed with distilled water (2 mL) solution containing PGAL (0.1 mM) and DPPH (0.5 mM). Samples were incubated for 30 min at room temperature *prior to* EPR analyses. The relative percentage of DPPH radical scavenging activity (RSA) was estimated according to Eq. (1):

$$RSA = (ho - hc)/hox\,100 \quad (1)$$

where hc is the peak height of EPR spectrum with PGAL and ho the peak heights of the EPR spectrum without PGAL. RSA determinations were carried out in triplicate. Experimental data were fitted to Probit statistical analysis in NCSS® programme (NCSS LLC. USA). IC_{50} value was the concentration of PGAL that inhibit 50% the RSA of DPPH. For spectrophotometric determinations, a concentration of ABTS (7 μM) was mixed in the dark with potassium persulfate (2.45 μM) to produce ABTS radical cation ($ABTS^+$). Then, $ABTS^+$ solution was diluted with PBS buffer (NaCl, KCl, Na_2HPO_4 and KH_2PO_4 at pH 7.4) up to absorbance of 0.70 at 734 nm as measured in a Genesys 10S UV–vis spectroscope (Thermoscientific, USA). Stock solutions of PGAL (120, 240, 360, 480, 600 ppm) were diluted in water and then 100 μL aliquots of each dilution were added into the assay tubescontaining 1.9 mL of the $ABTS^+$ solution. Absorbance was monitored for 7 min and the results were correlated to Trolox calibration curve. Percentage of inhibition was calculated and plotted as concentration of PGAL vs Trolox. All determinations were carried out in triplicate.

2.5. Antimicrobial assay

Minimum inhibitory concentrations (MIC) were determined in Mueller-Hinton broth (Becton Dickinson, USA) media according to the Clinical and Laboratory Standards Institute (CLSI) method for the pharmacological standard strains *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. Seven concentrations of PGAL were prepared in isotonic solutions (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 ppm). 100 μL of each solution were loaded in a microplate Star 96. Samples were inoculated with 100 μL of the bacterial suspensions (D.O 0.547) and grown at 37 °C for 24 h. The mixtures were monitored in a Microplate Reader FLUO star Omega, BMG Labtech at 600 nm. Minimal inhibitory concentration (MIC) for each strain was obtained at complete inhibition of the bacterium at the lowest concentration of PGAL after incubation for 20 h. All experiments were independently repeated at least three times. Statistics were determined by ANOVA with Tukey post-hoc test using Graph-Pad Prism 5. P values < 0.05 were considered to be statistically significant.

2.6. Isolation and culture of dermal fibroblasts

Dermal fibroblasts were isolated from skin biopsies from aesthetic surgeries undergoing elective abdominoplasties, previous informed consent signature. Briefly, dermis was digested with 30 mg/mL type I collagenase (Worthington Biochemical) during 2 h. Resulting supernatant was passed through a 70 μm nylon cell strainer (Corning) and solution was inactivated with DMEM media supplemented with 10% fetal bovine serum, 50 UI/mL penicillin and 50 mg/mL streptomycin. Cells were centrifuged at 1200 rpm for 5 min and then were counted and seeded at 1×10^4 cells/cm^2.

2.7. Proliferation assay

Cell proliferation was analyzed with CyQUANT® Cell Proliferation Assay Kit (Molecular Probes, Invitrogen). Briefly, cells were seeded at

Download English Version:

https://daneshyari.com/en/article/5435133

Download Persian Version:

https://daneshyari.com/article/5435133

Daneshyari.com