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Morphology and immunological activities of genistein-modified poly(ethylene glycol) diacrylate networks

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ABSTRACT

By virtue of well-known antioxidant, anti-inflammatory, and antibacterial activities, a phytochemical called 'genistein' is incorporated into polymer network membrane for wound dressing. Binary phase diagram of genistein/polyethylene glycol diacrylate precursor (PEGDA) blends was established both experimentally and theoretically. It was found that genistein crystals developed in the matrix of the PEGDA network, especially at high genistein contents. Biological activities including cytotoxicity, antioxidant, and anti-inflammatory of the networks were evaluated. The genistein-modified PEGDA network revealed improved antioxidant and anti-inflammatory functions desirable for multifunctional wound dressing.

1. Introduction

Polymer gels have attracted considerable attention in biomedical and pharmaceutical applications including contact lenses, membranes or biosensors, linings for artificial hearts, tissue scaffolds, and drug delivery devices [1–3]. Due to the chemical (e.g., covalent, ionic bonding) and/or physical (e.g., hydrogen bonding and crystallization) crosslinking, polymer gels are insoluble in aqueous media, but capable of retaining large amounts of water or biological fluids. Recently, hydrogels have found their ways to wound dressing. Swollen hydrogels resemble natural living tissues, providing better feel for the skin in comparison to conventional ointments and patches [1]. In addition, these networks are permeable to oxygen, nutrients, and other water-soluble metabolites, while providing a barrier to secondary infection from the environment [4]. However, in some severe wounds such as chronic wounds and ulcers, active gel dressings with a combination of moisturizing and healing benefits such as antioxidant, anti-inflammatory, and anti-microbial activities have been highly sought [5–9].

Presently, natural substances derived from plants have garnered considerable interest in pharmaceutical arena by virtue of their non-toxicity and diverse biological functions, which are capable for treating human diseases. Genistein is a phytochemical, which is abundant in soybean and sugar beet. Neat genistein is a crystalline solid, which melts at 305 °C and its glass transition temperature (T_g), as estimated from the movement of a single T_g with composition of its completely miscible poly(vinyl pyrrolidone) (PVP) and poly(ether sulfone) (PES) blends, to be in the vicinity of 150–170 °C [10]. Of particular importance is that genistein exhibits diverse biomedical properties, e.g., anti-microbial, antioxidant, and anti-inflammatory activities that promote beneficial effects on wound healing. In addition, genistein affords protection to the skin from the adverse effect of ultraviolet (UV) radiation [11,12].

In this article, photopolymerization has been carried out to convert mixtures of genistein and poly(ethylene glycol) diacrylate (PEGDA) to a cross-linked network with the aid of a photoinitiator such as camphorquinone, curable with blue light. The choice of blue light is that genistein is inherently an excellent radical scavenger exhibiting strong UV absorption corresponding to the UV-A and UV-B regions, and thus the photo-efficiency of photo-crosslinking

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agent is rather poor. Moreover, the present approach provides significant adaptability of light source for *in situ* polymerization to meet clinical and dental conditions [13]. Under physiological conditions, the above non-invasive method produces minimal heat release during crosslinking. It should be emphasized that the present genistein/PEGDA system, unlike a conventional gel, is a solid network by virtue of high T_g of the genistein constituent; but it swells in water or biological fluids (please see TOC).

Miscibility and phase morphology of the genistein/PEGDA blends have been investigated by means of differential scanning calorimetry (DSC), optical microscopy (OM), scanning electron microscopy (SEM), and wide angle X-ray diffraction (WAXD). To elucidate the phase behavior, solid-liquid phase diagrams of the starting genistein/PEGDA mixtures were solved self-consistently in the context of the combined free energies of Flory-Huggins (FH) and phase field (PF) theories. Subsequently, *in vitro* biocompatibility tests were performed by evaluating cytotoxicity and immunological properties of genistein/PEGDA networks, specifically antioxidant and anti-inflammatory activities.

2. Experimental and theoretical methods

2.1. Materials and sample preparation

Photo-crosslinkable poly(ethylene glycol) diacrylate (PEGDA) was purchased from Sigma-Aldrich. The number-average molecular weight ($\overline{M}_n$) and the polydispersity index ($\overline{M}_w/\overline{M}_n$) of PEGDA were 847 g/mol and 1.10, respectively. The photo-oxidant camphorquinone (CQ, $C_{10}H_{14}O_2$) and the photo-reductant ethyl 4-(dimethylamino) benzoate (4EDMAB, $C_{11}H_{15}NO_2$), received from Sigma-Aldrich, were mixed in the ratio of 1:4 by weight and used as photo-initiator. Genistein ($C_{15}H_{10}O_5$) was purchased from MDidea Exporting Division (YinChuan, China). The reported purity value of genistein was greater than 98%. Tetrahydrofuran (THF) and dimethysulfoxide (DMSO) obtained from Sigma-Aldrich were used as received.

Various compositions of the starting genistein/PEGDA mixture were dissolved in THF to form 5 wt% polymer solutions and subsequently miscibility behavior and phase morphology were examined. The thin films (~10 μm thick) were obtained by solvent casting on glass slides at ambient temperature and dried for 24 h. Subsequently, these films were placed in a vacuum oven controlled at 50 °C for 2 days to remove any remaining solvent. These dried films were used in the determination of phase diagram of genistein/PEGDA blend by means of DSC and POM.

For the fabrication of photopolymerizable mixtures in THF, the 25 wt% precursor solutions, consisting of genistein and PEGDA, were prepared at various blend ratios, i.e., 10/90, 20/80, 30/70, 40/60, and 50/50 by weight. A mixture of 1:4 CQ:4EDMAB (i.e., 10 wt% relative to the precursor weight) was added to the precursor solutions by rigorously stirring at room temperature. The uniformly mixed solutions were then cast in a rectangular mold on transparent Teflon sheets. Subsequently, photopolymerization was carried out by exposing them to visible blue light generated by a dental photo-curing unit (Densply, SmartLite® IQ2, Model 200) at 450–475 nm and 700 mW/cm [2] for 20 min. The resulting membranes were washed with deionized water to remove unreacted precursors and then further dried in the vacuum oven at 40 °C for 24 h.

2.2. Methods

2.2.1. Characterization of Genistein/PEGDA blends

UV-visible absorption spectrum of neat genistein in DMSO solution was acquired using UV-vis spectrophotometer (Hewlett-Packard, Model 8453) in the range of 250–900 nm. The glass transition and crystal melting temperatures of genistein/PEGDA blends before and after photopolymerization were determined using differential scanning calorimetry (Model Q200, TA Instruments). The sample encapsulated in hermetic pan was scanned at a heating/cooling rate of 2 °C/min under nitrogen atmosphere. Thermogravimetric analysis (Model Q-50, TA Instruments) thermograms of the blend samples (~10 mg) were scanned from 30 to 600 °C at a heating rate of 10 °C/min under nitrogen gas circulation.

The emerging microstructures were examined using optical microscopy (Model BX60, Olympus) under the crossed polarization modes and photographed as a function of temperature using a digital camera (Canon, EOS 400D) at a magnification of 50×. Surface morphology of the genistein/PEGDA networks were further observed by means of scanning electron microscope (SEM) (Jeol, model JSM-7401F). The specimens were coated with silver for 90 s using a sputtering machine (model K575X, Emitech). SEM micrographs were taken at least three different locations that are representative of the whole specimen.

To investigate the crystalline structure, wide-angle X-ray diffraction (WAXD) measurements were carried out on neat genistein powder as well as various compositions of genistein/PEGDA networks using a diffractometer (Bruker, AXS D8) operated at 40 kV and 40 mA equipped with a monochromatic Cu-Kα X-ray source ($\lambda = 1.5417$ Å). A 2 *theta* Bragg's scans were acquired over an angular range from 5 to 40°.

2.3. In vitro biocompatibility evaluation of genistein-modified PEGDA networks

2.3.1. Blood samples

Venous blood, collected into heparinized tubes, was obtained from healthy adult donors and used immediately after collection. Caution was exercised to ensure that the amount of collected blood from each volunteer met specific criteria for exemption from the IRB oversight. Biological tests on genistein-modified PEGDA networks were sterilized in a laminar flow hood under UV light for 1 h on each side at room temperature for measurements of cell viability, reactive oxygen species (ROS) level, and blood serum level of proinflammatory cytokines, i.e., interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). All measurements were undertaken in at least three times.

2.3.2. Cytotoxicity assay

A cell viability assay was performed to evaluate the effect of the genistein-modified PEGDA networks on whole blood cells using viability staining solution. Briefly, the freshly collected whole blood was pipetted into individual tubes containing the genistein/PEGDA network. The red blood cells were lysed by adding ammonium chloride buffer solution followed by incubation for 10 min at room temperature. The tubes were then centrifuged for 5 min at 1800 rpm. The supernatant was discarded and the remaining leukocytes were washed twice using PBS. After the supernatant was decanted, a nucleic acid dye, 7-aminoactinomycin D (7-AAD) (BD-Via-Probe; BD Bioscience, CA), was added to the leukocyte preparation. Note that 7-AAD cannot penetrate an intact cell membrane; it only stains dead cells that underwent membrane burst. The tubes were incubated in the dark for 10 min to permit dye uptake. The cells were resuspended in PBS and then the cell suspension was analyzed using a flow cytometer (Beckman Coulter, Model FC500) with the aid of CXP Analysis software. The fluorescence signal from the dead cells was measured on the FL3 red channel (wavelength 650 nm). The detailed procedure may be found elsewhere [13–15].