Materials Letters 214 (2018) 224–227

Contents lists available at ScienceDirect

Materials Letters

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

Biological activity of radiation-induced collagen–polyvinylpyrrolidone–PEG hydrogels

Maykel González-Torres [a,*], Gerardo Leyva-Gómez [b,1], Margarita Rivera [c], Edgar Krötzsch [b], Rogelio Rodríguez-Talavera [d], Ana Leonor Rivera [a,e], Alejandro Cabrera-Wrooman [b,*]

[a] Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México, Ciudad de México 04510, Mexico
[b] Laboratorio de Tejido Conjuntivo, Instituto Nacional de Rehabilitación "Luís Guillermo Ibarra Ibarra", Ciudad de Mexico 14389, Mexico
[c] Instituto de Física, Universidad Nacional Autónoma de México, Ciudad de Mexico 04510, Mexico
[d] Centro de Física Aplicada y Tecnología Avanzada, Universidad Nacional Autónoma de México, Querétaro 76230, Mexico
[e] Instituto de Ciencias Nucleares, Universidad Nacional Autónoma de México, Ciudad de México 04510, Mexico

ARTICLE INFO

Article history:
Received 13 June 2017
Received in revised form 21 November 2017
Accepted 3 December 2017
Available online 5 December 2017

Keywords:
Hydrogels
Collagen
Polyvinylpyrrolidone
Polyethylene glycol
Cell viability
Fibroblasts

ABSTRACT

The synthesis of novel degradable hydrogels, with designable structures and good biocompatibility, produces attractive materials for use in connective tissue regeneration applications. However, the way in which human fibroblast cells respond to terpolymer hydrogels such as collagen–polyvinylpyrrolidone–polyethylene glycol (C–PVP–PEG) remains largely unknown. The aim of this study was to synthesize a gamma-radiation-induced C–PVP–PEG hydrogel and to evaluate its structure, morphology and cell viability in fibroblasts. Fourier transform infrared spectroscopy showed shifting of amide I to higher frequencies, as evidence of crosslinking produced by the radiation. The morphology of the hydrogel varied with the ratio of the polymers. We show a proposal for the mechanism of the hydrogel synthesis. The *in vitro* assessment of the hydrogels suggested that at 50 kGy, the presence of collagen decreased the cell viability for all samples, except for C–PVP. However, when PEG and PVP were added to the collagen, the cell's viability increased with respect to the irradiated collagen sample.

1. Introduction

Radiation-induced crosslinking is an environmentally friendly method used to prepare hydrogels [1]. The main advantages of this method are that it can be carried out without catalyst or chemical initiation, it is an effective and fast way to synthesize homogeneous three-dimensional networks in one step, and it can be controlled by varying the radiation doses and dose rate. Additive-free collagen (C) hydrogels have been successfully obtained by this method. At low doses and high concentrations, the hydrogel was found to be conducive to the growth of fibroblasts (*in vitro*) and showed excellent biodegradability and biocompatibility (*in vivo*) [2]. The biological effect of fibrillogenesis on crosslinked collagen has also enabled it to be utilized as scaffolds for tissue engineering [3]. Other chemical substrates of interest are polyvinylpyrrolidone (PVP) and polyethylene glycol (PEG). Previous reports showed that PVP can be blended via gamma-irradiation with methyl hydroxyethyl cellulose [4] and carrageenan [5] to produce hydrogels for use in biomedicine and antimicrobial wound dressing. Besides reports claiming that PEG is a versatile compound that might be used to prepare hydrogels under mild conditions [6], other studies have confirmed collagen mimetic peptide-PEG as a novel class of polymer for use as a synthetic collagen mimic [7]. Moreover, gamma-radiation played an interesting role in the development of tissue-engineered hydrogels to form membranes [8] and valves with acceptable recellularization and durability [9]. Thus, the preparation of γ-irradiated C-PVP, as well as its physicochemical and functional characterization [10], and the network structure of superabsorbent hydrogels have been studied [11]. However, it is not yet known whether the C–PVP–PEG hydrogels can be generated by radiation-induced polymerization and what is the nature of the biological activity of these new biomaterials. To the best of our knowledge, this terpolymer has not been reported on. The aim of this work is to evaluate the synthesis of the proposed hydrogel, to survey its structure, and to investigate the cell viability in fibroblasts.

* Corresponding authors.
E-mail addresses: mikegcu@gmail.com (M. González-Torres), acabrera@inr.gob.mx (A. Cabrera-Wrooman).
[1] Current address: Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de Mexico, Ciudad de México 04510, Mexico.

https://doi.org/10.1016/j.matlet.2017.12.006
0167-577X/

2. Experimental section

2.1. Materials

The pepsinized porcine (type I) collagen (C) was dialyzed before use (5 mM, acetic acid, DMS Branch Pentapharm, Aesh, Switzerland). Polyvinylpyrrolidone (PVP, ISP Investments, Inc., Wilmington, DE, U.S.A.) and polyethylene glycol (PEG, Sigma-Aldrich, St. Louis, MO, U.S.A.) of 8000 and 400 Da respectively were used. The polymers were mixed in a phosphate buffer solution (PBS, 0.1 M, pH = 7.4, Sigma-Aldrich, St. Louis, MO, U.S.A.). Standard autosampler vials with cap and septa, 12 × 32 mm, 8–425 thread (Sigma-Aldrich) were employed to prepare the solutions.

2.2. C–PVP–PEG hydrogel preparation

Several solutions in the PBS were prepared; the first one (labeled M1) was roughly 0.35 mg/mL of collagen; the second one (M2) was a mixture of C + PEG (0.35 mg/ml; 50 mg/ml); the third sample (M3) contained C + PVP (0.35 mg/ml; 25 mg/ml), while the samples M4, M5 and M6 consisted of C–PVP–PEG mixtures with increasing concentrations of PVP/PEG polymers keeping the collagen concentration constant for all samples: M4 (0.35 mg/ml; 50 mg/ml, 25 mg/ml), M5 (0.35 mg/ml; 125 mg/ml, 50 mg/ml), and M6 (0.35 mg/ml; 150 mg/ml, 75 mg/ml). The synthesis of the hydrogel was achieved via the simultaneous irradiation method, where the mixture of polymers is subjected to the source of ^{60}Co-γ-radiation in air (Gamma Beam 651PT, Nordion International), at a dose rate of approximately 3.6 kGy/h and a dose of 50 kGy (measured with a Fricke dosimeter). Argon flushing was carried out to assist in degassing the samples before irradiation.

2.3. Hydrogel characterization

Fourier transform infrared (FTIR) spectra were recorded on a Spectrum Two™ spectrophotometer (Perkin Elmer, U.S.A.) in order to estimate the functional groups presented in the wet and dry hydrogels. We prepared previously the hydrogels for scanning electron microscope (SEM) study. The polymers were dried in acetone solution by critical point drying (Critical Point Dryer CPD, K850, Quorum). The morphological aspects of the sputter-coated (carbon) dehydrated hydrogels were studied by SEM (JEOL, JSM-5600 LV).

2.4. Cytotoxicity study

For this experiment, human fibroblast (BJ1) cell line (generously donated by PhD. Iván Velasco UNAM, México) with different hydrogels was cultivated in 96 well plates. 2x10^4 cells were plated in each well with 200 μl of Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 5% of antibiotic for 24 h at 5% CO_2 and 37 °C. The hydrogels were removed and added to the medium of 10 μl of 5 μg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and incubated for 3 h [12]. The supernatant was removed, and each well was washed with saline solution at room temperature. Subsequently, the formazan precipitate was dissolved with 200 μl of DMSO/isopropanol 1:1, and then measured by colorimetry at 570 nm with an iMARK™ microplate Absorbance Reader (Bio-Rad Laboratories, Inc). Microscopy images were obtained using an Axion Observer Z1, Carl Zeiss inverted light microscope with a 10 × N.A.

3. Results and discussion

Fig. 1 shows the FTIR of the PVP, PEG, and M1–M6 samples. As can be seen in Fig. 1a, the signals at 3000–2800 cm^{-1}, 1651 cm^{-1}, 1436 cm^{-1}, and 1282 cm^{-1} correspond to C-H stretching, carbonyl absorption peak, characteristic C-H stretching vibrations (1493–1423 cm^{-1}) and C-N stretching of PVP, respectively. The figure also shows the hydroxyl signal (3445 cm^{-1}) and the skeletal vibration of PEG (1100 cm^{-1}) [13]. It is of note that collagen displays a broad band, centered at 3290 cm^{-1} and assigned to amide A (Fig. 1a and b). The stretching vibrations at 3074 cm^{-1} and 2941 cm^{-1} are associated with the amide B. The signal at 1628 cm^{-1} (amide I) is particularly useful for assessing conformational changes. Two other bands of interest are observed at 1528 cm^{-1} and 1229 cm^{-1}, which are attributed to amide II (bending) and amide III (stretching) vibrations. The shifting towards higher wavenumbers, for instance, from 1628 cm^{-1} to 1641 cm^{-1}, reveals an evidence of crosslinking of C + PEG (M2; Fig. 1a). The most striking feature of the M3 sample is the decrease of the bending vibration (C-N) and also the shifting of amide I (1628–1658 cm^{-1}, Fig. 1a; Fig. S1, supplementary data) [11]. The M4–M6 spectra correspond to the prepared terpolymer (C–PVP–PEG). As shown, they follow the same trend as the M2 and M3 samples, which indicates that the polymers are crosslinked. In addition, a decrease can be observed in the relative intensity of the amide II and III and the broadening of amide A and B. This result suggests that PVP and PEG are forming intermolecular interactions through hydrogen bonds with collagen, and also that the collagen backbone has chemically reacted with these polymers [14].

The morphology of the samples was characterized by SEM (Fig. 2a–f). As seen, the hydrogels have a porous structure induced by the critical point dryer. The size of the pores decreased for sample M3 (Fig. 2c). The increase of the PVP/PEG ratio caused the absence of pores in samples M5 and M6 (Fig. 2e and f) [15].

Here, we show the proposal for the mechanism of synthesis of the C–PVP–PEG (Fig. S2, supplementary data). First, the presented species are formed by the radiolysis of water. Second, the PEG, PVP and collagen radical are produced by hydrogen abstraction. Third, crosslinked species and macro-radicals can be obtained by radical coupling and propagation reactions. Finally, the macro-radicals of PVP and PEG react with collagen macro-radical to yield complex structures as shown at the end of the reaction. We represent the collagen as Gly-X-Y, where Gly stands for glycine, while X and Y symbolize proline and hydroxyproline.

Measurement of the cell viability in fibroblasts showed a decrease of 78% in the cellular viability value for irradiated collagen (Fig. 3, M1). These results are corroborated with the phase contrast images (Fig. 4b). A compact collagen cylinder was formed in the solution (see graphical abstract). It is suggested that the high-dose radiation affected irreversibly the collagen structure [10]. The addition of PEG produced an increase in cell viability with respect to collagen (Fig. 4c, M2). Previous reports indicate that PEG can be used to protect against the toxic effect on the cells at high radiation doses [16]. Interestingly, the combination of C–PVP (M3) showed a better cell viability (Fig. 4d). In an earlier work, it was demonstrated that radiation-induced C–PVP hydrogel can be obtained and structurally characterized [10]. Furthermore, the addition of different proportions of PEG + PVP to collagen (M4–M6) showed a reduction of cell viability to levels similar to that of C–PEG. The samples did not show significant differences in the absorbance values for cell viability. The new polymer configuration seems to be adapting better to the cell line than irradiated collagen (Fig 3, M1). However, with a greater increase in the amount of PVP–PEG there is a marked alteration in the cell morphology (Fig. 4f and g).