Polymer Degradation and Stability 133 (2016) 312–320

Contents lists available at ScienceDirect

Polymer Degradation and Stability

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

Synthesis and characterization of MMP degradable and maleimide cross-linked PEG hydrogels for tissue engineering scaffolds

Jingjing Yu [a], Feng Chen [a], Xichi Wang [b], Nianguo Dong [b, **], Cuifen Lu [a, *], Guichun Yang [a], Zuxing Chen [a]

[a] Hubei Collaborative Innovation Center for Advanced Organochemical Materials & Ministry-of-Education Key Laboratory for the Synthesis and Application of Organic Functional Molecules, Hubei University, Wuhan 430062, China
[b] Department of Cardiovascular Surgery, Union Hospital, Tongji Medical College, Huazhong Science and Technology University, Wuhan 430030, China

ARTICLE INFO

Article history:
Received 25 June 2016
Received in revised form
22 August 2016
Accepted 6 September 2016
Available online 7 September 2016

Keywords:
PEG hydrogels
MMP degradable
Maleimide
Michael-type addition

ABSTRACT

A series of poly(ethylene glycol) (PEG) hydrogels were successfully prepared *via* Michael-type addition between 4-arm PEG-maleimide (PEG-4MAL) and MMP degradable peptide. The gelation time was significantly short (~10 min) in a low concentration of TEA (4 mM). Thermogravimetric analysis indicated the thermal stabilities of hydrogels. SEM images confirmed a porous structure and the pore size increased with the increase of the PEG chain length. Rheological measurements indicated that all the hydrogels exhibited the characteristics of elastomer and the cross-linking density had a correlation to the polymer weight percentage. After immersing in 0.9% sodium chloride injection, PEG hydrogels exhibited a good water absorption capacity, and their swelling ratio were directly related to the amount of cross-linking. Biological activities of the hydrogels were evaluated by *in vitro* enzymatic degradation and *in vitro* cell compatibility on mesenchymal stem cells (MSCs) and the results showed that the hydrogels were biocompatible and could be degraded by exogenously delivered MMPs or cell-secreted MMPs. Thus, PEG hydrogels exhibited the potential for tissue engineering scaffolds.

1. Introduction

Poly(ethylene glycol) (PEG) hydrogels are attractive for use as tissue engineering scaffolds [1–4] because they are intrinsically biocompatible, resist non-specific protein adsorption and permit the rapid encapsulation of cells in cytocompatible conditions.

The mechanical property of PEG hydrogels can be regulated by changing the molecular weight and concentration of PEG [5,6]. Moreover, the biodegradability of PEG hydrogels can be conferred by the incorporation of degradable components via enzymatic, hydrolytic or environmental pathways. A matrix metalloproteinase (MMP) degradable peptide is often chosen to crosslink PEG chains to create a hydrogel network that is degraded by cell-secreted MMPs [7–9].

The reported PEG hydrogels were formed by either Michael-type addition polymerization [5,10] or free-radical initiated polymerization [3,8]. A major drawback of free-radical initiated polymerization is that it can significantly reduce encapsulated cell viability. In contrast, Michael-type addition polymerization avoids the use of cytotoxic free-radicals and UV light, but instead requires a nucleophilic buffering reagent [11] to facilitate the addition reaction. However, PEG hydrogels formed by Michael-type addition polymerization with the end functional group of acrylate or vinyl sulfone in the presence of high concentrations of nucleophilic buffering reagent have cytotoxic effects on several sensitive cells [12]. The maleimide group is extensively used in peptide bioconjugate chemistry because of its faster reaction kinetics than acrylate and vinyl sulfone, high specificity for thiols at physiological pH and requiring a low concentration of nucleophilic buffering reagent [13].

Although much research effort has been directed towards PEG hydrogels crosslinked by MMP degradable peptide as scaffolds, but a comprehensive investigation of mechanical property, degradation property and biocompatibility of PEG hydrogels crosslinked by MMP degradable peptide has not previously been reported. Therefore in this study, a series of PEG hydrogels were prepared from 4-arm PEG-maleimide (PEG-4MAL) macromer crosslinked

* Corresponding author.
** Corresponding author.
E-mail addresses: dongnianguo@hotmail.com (N. Dong), lucf@hubu.edu.cn (C. Lu).

http://dx.doi.org/10.1016/j.polymdegradstab.2016.09.008
0141-3910/

with MMP degradable peptide under physiological conditions by Michael-type addition polymerization. Moreover, the FTIR spectra, morphologies, thermal properties, mechanical properties, swelling properties, degradation properties and *in vitro* cell compatibility of the hydrogels were characterized.

2. Experimental

2.1. Materials

PEG-4MAL (5, 10 and 20 kDa, >95% end-group substitution) was purchased from the Jenkem Technology Co., Ltd. (Beijing, China). Recombinant Human MMP-2 (62 kDa, 98% purity by HPLC analyses) was purchased from the PeproTech (USA). MMP degradable peptide (Ac-G**C**RD-GPQG↓IWGQ-DG**C**G-NH_2, 1.7 kDa) was obtained from the GL Biochem Ltd. (Shanghai, China). Plastic cell culture dishes and plates were purchased from Wuxi NEST Biotechnology Co Ltd (Wuxi, China). Bone marrow-derived mesenchymal stem cells (MSCs) were obtained from Sprague-Dawley (SD) rats in Tongji Medical College (Wuhan, China). All other chemicals were purchased from Sinopharm Chemical Reagent (Wuhan, China) and were used without further purification.

2.2. Preparation of PEG hydrogels

A schematic of the hydrogel formation is presented in Fig. 1. Hydrogels were prepared by Michael-type addition of thiol-containing peptide (Ac-GCRD-GPQG↓IWGQ-DGCG-NH_2) onto PEG-4MAL, as described by García et al. [13]. First, the pre-polymer solutions were prepared by dissolving PEG-4MAL and thiol-containing peptide (molar ratio of MAL/SH was 1:1) into triethanolamine solution (TEA; pH 7.4, 4 mM) at a specific concentrations, e.g. 5%, 7.5% wt/v. The pre-polymer solutions were mixed by vortexing and then transferred to an injection syringe for gelation at 37 °C. The gelation time was determined as the time when the hydrogel would no longer flow by the force of gravity.

2.3. Chemical structure identification

FTIR spectra were obtained on dried samples at room temperature using a Nicolet IS 50 spectrometer (ThermoFisher Scientific Co.), equipped with a diamond Attenuated Total Reflection (ATR). Spectra were obtained at a resolution of 2 cm^{-1} in the range 4000–600 cm^{-1} for a total of 16 scans. The swollen hydrogels were frozen rapidly at −55 °C and then dried in a freeze-dryer.

2.4. Thermal properties

To examine thermal stability of hydrogels, the hydrogel samples were measured by thermal analysis system (TG/DTA), using a Mettler Toledo equipment (thermobalance sensitivity: 0.1 μg), which was previously calibrated in the temperature range of 30–800 °C by running tin and lead as melting standards, at a heating rate (ϕ) of 20 °C/min, using open alumina crucibles and a dry nitrogen purge flow of 40 mL/min. Sample weights ranging from 8 to 10 mg were used.

2.5. Scanning electron microscopy

To observe the interior morphologies of hydrogels, the swollen

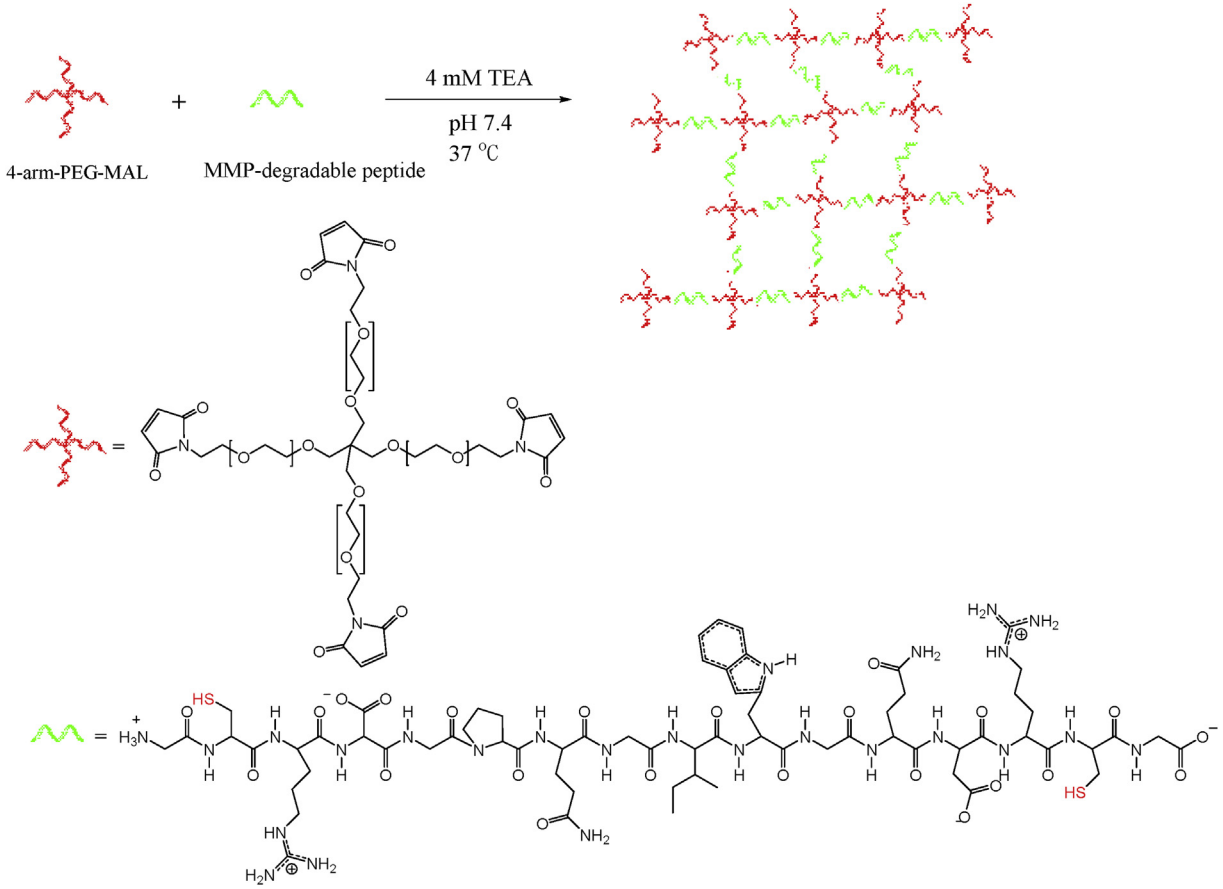

Fig. 1. Schematic of PEG hydrogel formation.