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Enzymatically cross-linked gelatin-phenol hydrogels with a broader stiffness range for osteogenic differentiation of human mesenchymal stem cells

Li-Shan Wang, Chan Du, Joo Eun Chung, Motoichi Kurisawa *

Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, The Nanos, Singapore 138669, Singapore

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ABSTRACT

An injectable hydrogel system, composed of gelatin–hydroxyphenylpropionic acid (Gtn–HPA) conjugates chemically cross-linked by an enzyme-mediated oxidation reaction, has been designed as a biodegradable scaffold for tissue engineering. In light of the role of substrate stiffness on cell differentiation, we herein report a newly improved Gtn hydrogel system with a broader range of stiffness control that uses Gtn–HPA–tyramine (Gtn–HPA–Tyr) conjugates to stimulate the osteogenic differentiation of human mesenchymal stem cells (hMSCs). The Gtn–HPA–Tyr conjugate was successfully synthesized through a further conjugation of Tyr to Gtn–HPA conjugate by means of a general carbodiimide/active ester-mediated coupling reaction. Proton nuclear magnetic resonance and UV–visible measurements showed a higher total phenol content in the Gtn–HPA–Tyr conjugate than that content in the Gtn–HPA conjugate. The Gtn–HPA–Tyr hydrogels were formed by the oxidative coupling of phenol moieties catalyzed by hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP). Rheological studies revealed that a broader range of storage modulus (G') of Gtn–HPA–Tyr hydrogel (600–26,800 Pa) was achieved using different concentrations of H_2O_2, while the G' of the predecessor Gtn–HPA hydrogels was limited to the range of 1000 to 13,500 Pa. The hMSCs on Gtn–HPA–Tyr hydrogel with G' greater than 20,000 showed significantly up-regulated expressions of osteocalcin and runt-related transcription factor 2 (RUNX2) on both the gene and protein level, with the presence of alkaline phosphatase, and the evidence of calcium accumulation. These studies with the Gtn–HPA–Tyr hydrogel with G' greater than 20,000 collectively suggest the stimulation of the hMSCs into osteogenic differentiation, while these same observations were not found with the Gtn–HPA hydrogel with a G' of 13,500.

1. Introduction

Hydrogels are widely used as biomaterial formulations for drug delivery or as scaffolds in tissue engineering because their highly hydrophilic characteristics provide an excellent environment for bioactive agents that include therapeutic proteins, growth factors and cells [1–3]. Injectable hydrogel systems are of particular interest for biomedical applications because bioactive agents and cells can be easily encapsulated in hydrogels with a simple injection of a mixture of gel precursors and bioactive agents in solution [4,5]. Thus, no surgical procedures are required for the implantation of hydrogels, or their removal in the case of the biodegradable ones. Hydrogels can be formed in situ either via chemical and/or physical cross-linking reaction mechanisms. It is generally accepted that chemically cross-linked hydrogels are superior to physically cross-linked hydrogels in terms of stability and control in mechanical strength, although the latter has the advantage of being free of cross-linkers. Several strategies have been adopted to prepare chemically cross-linked hydrogels using either natural or synthetic polymers. Chemically cross-linked hydrogels are formed by radical polymerization that adopts redox- or photo-initiators, Michael-type addition reactions, disulfide bond formations, and aldehyde-mediated cross-linking [6–11].

Recently, an enzymatic cross-linking strategy has attracted intensive attention in the preparation of chemically cross-linked hydrogels [12–21]. Hydrogels composed of biopolymer-phenol conjugates were formed using the oxidative coupling of phenol moieties catalyzed by hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP). In our previous reports, we have developed an injectable hydrogel scaffold system composed of gelatin–hydroxyphenylpropionic acid (Gtn–HPA) conjugates. This injectable hydrogel scaffold had tunable stiffness for controlling the proliferation and differentiation of human mesenchymal stem cells (hMSCs) in a two-dimensional (2-D) and three-dimensional (3-D) cell culture environment [16,17]. The stiffness of the hydrogels was readily tuned by varying the H_2O_2 concentration without changing the concentration of its polymer precursor. In 2-D cell culture systems, the hMSCs on a softer hydrogel (storage modulus, G' = 600 Pa)

* Corresponding author. Tel.: +65 6824 7139; fax: +65 6478 9083.
E-mail address: mkurisawa@ibn.a-star.edu.sg (M. Kurisawa).

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