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The effects of varying poly(ethylene glycol) hydrogel crosslinking density and the crosslinking mechanism on protein accumulation in three-dimensional hydrogels

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ABSTRACT

Matrix stiffness has been shown to play an important role in modulating various processes determine cell fate, such as differentiation and cell cycle. Given that the stiffness can be easily tuned by varying the crosslinking density, poly(ethylene glycol) (PEG) hydrogels have been widely used as an artificial cell niche. However, little is known about how changes in the hydrogel crosslinking density may affect the accumulation of exogenous growth factors within 3-D hydrogel scaffolds formed by different crosslinking mechanisms. To address such shortcomings, we measured protein diffusivity and accumulation within PEG hydrogels with varying PEG molecular weight, concentration and crosslinking mechanism. We found that protein accumulation increased substantially above a critical mesh size, which was distinct from the protein diffusivity trend, highlighting the importance of using protein accumulation as a parameter to better predict the cell fates in addition to protein diffusivity, a parameter commonly reported by researchers studying protein diffusion in hydrogels. Furthermore, we found that chain-growth-polymerized gels allowed more protein accumulation than step-growth-polymerized gels, which may be the result of network heterogeneity. The strategy used here can help quantify the effects of varying the hydrogel crosslinking density and crosslinking mechanism on protein diffusion in different types of hydrogel. Such tools could be broadly useful for interpreting cellular responses in hydrogels of varying stiffness for various tissue engineering applications.

1. Introduction

Matrix stiffness has recently been recognized as playing an important role in regulating cell fate and tissue development. It has been shown that stem cells specify their lineage and commit to their fate by matrix stiffness, which mimics specific tissue-level elasticity [1–3]. Matrix stiffness has also been shown to directly influence other cellular fate processes, such as cell cycle, in a variety of cell types, including myofibroblasts [4], epithelial cells, vascular smooth muscle cells and osteoblasts [5]. Cancer cells are also known to be mechanosensitive, and increasing matrix stiffness resulted in an increase in cell growth, spreading and migration [6].

To study the effects of varying matrix stiffness on cell behavior, poly(ethylene glycol) (PEG) hydrogels have been widely used to create a biomimetic artificial niche with tunable biochemical and biomechanical cues [7]. The biomechanical cue, specifically stiffness of the PEG hydrogel, can be easily tuned by varying the molecular weight or concentration of the PEG [8]. Increasing the molecular weight or decreasing the concentration of PEG reduces the crosslinking density of the hydrogel, which results in a softer gel. However, varying the crosslinking density simultaneously changes the mesh size of the hydrogel network, which, in turn, can influence protein diffusion in 3-D hydrogels [9,10]. Previous studies have shown that increasing the gel crosslinking density by increasing the molecular weight or decreasing the concentration of PEG can lead to decreasing diffusivity of different solutes (vitamin B_{12}, insulin, myoglobin, trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin (BSA), IgG) [9–11].

However, varying the gel crosslinking density influences not only the protein diffusivity but also the protein accumulation within hydrogels. To date, most studies have only looked at protein diffusivity (how fast proteins can diffuse within hydrogels), ignoring protein accumulation (how much proteins can actually go into 3-D hydrogels), which can directly influence the encapsulated

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cells. A recent study has shown that varying the hydrogel crosslinking density can alter the stem cell differentiation, which is unlikely to be related to mechanotransduction [12]. Since cells are known to be sensitive to available soluble factors, it is important to characterize the effects of varying the hydrogel crosslinking density on exogenous protein accumulation within 3-D hydrogels, thereby allowing the correct interpretation of the mechanisms that regulate cellular responses in hydrogels with varying stiffness.

Protein accumulation can also be affected by the crosslinking mechanism of PEG hydrogels. Although it is known that the crosslinking mechanism can affect network homogeneity [13], it is poorly understood how different crosslinking mechanisms can affect protein accumulation in 3-D hydrogels. Typically, PEG hydrogels can be crosslinked via two mechanisms: chain-growth (CG) or step-growth (SG) polymerization (Fig. 1). CG polymerization is generally less controllable, and is known to form a more heterogeneous gel network. Because monomers are crosslinked via polymer kinetic chains, the crosslink functionality is hardly controlled and network defects (e.g. loop) are more likely to form. On the other hand, SG polymerization generally results in a more homogeneous hydrogel network that is formed by at least two different monomers with defined functionality and mutually reactive end groups [13]. Given that different crosslinking mechanisms can affect the network homogeneity, it is important to understand how the crosslinking mechanism affects protein accumulation in 3-D PEG hydrogels.

Therefore, the goal of this study was to investigate the effects of varying crosslinking density and crosslinking mechanism of PEG hydrogel on protein accumulation within the hydrogel. Specifically, we varied the hydrogel crosslinking density and the crosslinking mechanism, and investigated their effects on protein accumulation within hydrogels. To control the crosslinking density, the PEG molecular weight or concentration was varied. To study how the crosslinking mechanism affects protein diffusion, CG and SG polymerization were used to form PEG hydrogels. To mimic accumulation of exogenously supplemented soluble growth factor in 3-D tissue engineering scaffolds, premade PEG hydrogels were immersed in protein solution for 24 h to load the protein. After 24 h of loading, gels were placed in fresh PBS for 24 h, followed by gel homogenization to take into account any protein that remain entrapped in the hydrogel. Protein accumulation within hydrogels was calculated from the protein release accumulated over 24 h and the protein that remained entrapped in the hydrogels. Protein diffusivity was calculated by fitting the protein fractional release curve to a 3-D Fickian model.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) (PEG, mol. wt. 2, 3, 4, 10 kDa), K_2CO_3, dichloromethane (DCM), acryloyl chloride, potassium iodide (KI), Celite® 521, 4-(dimethylaminio)pyridine (DMAP), N,N′-diisopropylcarbodiimide (DIC), 5-norbornene-2-carboxylic acid, sodium hydride (NaH), allyl bromide, 2,2-dimethoxy-1,2-diphenylethan-1-one (DMPA), dithiothreitol, tetrahydrofuran (THF) and thioacetic

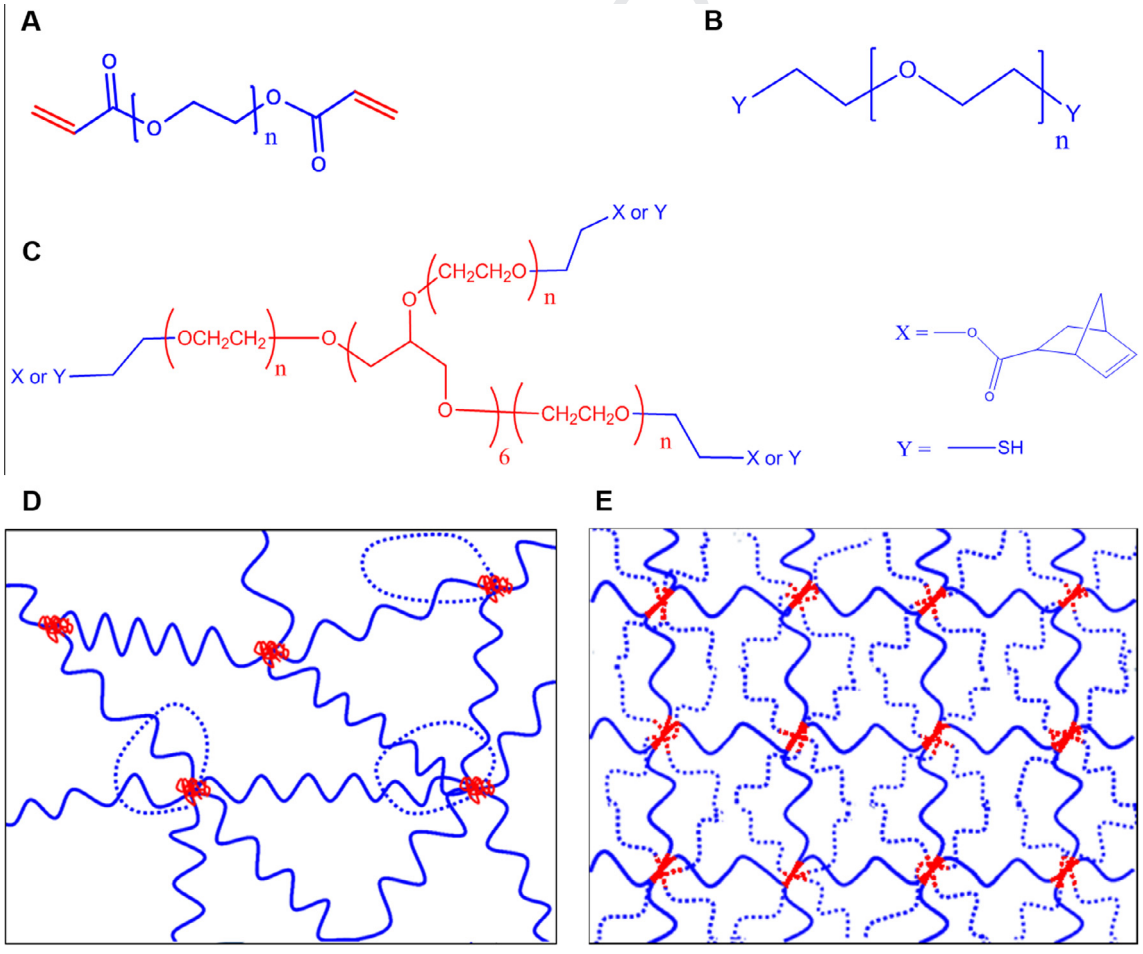

Fig. 1. Chemical structure of polymers and schematic of PEG hydrogel network formed by different crosslinking mechanisms. Chemical structure of (A) PEGDA, (B) PEG-dithiol, (C) 8-arm PEG-norbornene (X) (8-arm PEG-NB) or 8-arm PEG-thiol (Y) (8-arm PEG-SH). (D) CG gels using PEGDA. (E) SG gels using norbornene- and thiol-terminated PEG. Blue line: polymer chains between crosslinking points; red line: crosslinking points; solid line: on the same plane; dotted line: on different planes.

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