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ScienceDirect
Acta Biomaterialia 5 (2009) 3441–3452
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Biodegradable hydrogels based on novel photopolymerizable guar gum–methacrylate macromonomers for in situ fabrication of tissue engineering scaffolds

Ashutosh Tiwari [a], Jamison J. Grailer [b], Srikanth Pilla [a], Douglas A. Steeber [b], Shaoqin Gong [a,c,*]

[a] Department of Mechanical Engineering, University of Wisconsin-Milwaukee, 3200 North Cramer Street, Milwaukee, WI 53211, USA
[b] Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53211, USA
[c] Department of Materials, University of Wisconsin-Milwaukee, 3200 North Cramer Street, Milwaukee, WI 53211, USA

Received 25 February 2009; received in revised form 8 May 2009; accepted 1 June 2009
Available online 6 June 2009

Abstract

Guar gum (GG) is a non-ionic polysaccharide that is found abundantly in nature and has many properties desirable for biomedical applications. In the present work GG with molecular weights ranging from 74 to 210 kDa was modified with glycidyl methacrylate (GMA) to produce a series of water-soluble photopolymerizable guar gum–methacrylate (GG–MA) macromonomers of different molecular weights. We investigated the effects of molecular weight of GG–MA macromonomers from 102 to 216 kDa and with percent degree of methacrylation (%DM) ranging from 14% to 56% on the properties of GG–MA hydrogels. GG–MA hydrogels exhibited a three-dimensional open cell microstructure with an average pore size ranging from ~10 to 55 μm and an average pore density of from $\sim 2.4 \times 10^6$ to 8.6×10^7 pores cm^{-3}. The hydrogels exhibited equilibrium swelling ratios ranging from ~22% to 63%. The degree of *in vitro* enzymatic biodegradation of the hydrogels decreased linearly with increasing gel content and the degree of methacrylation of the respective macromonomers. The human endothelial cell line EA.hy926 was photo-encapsulated in the GG–MA hydrogels. Cells remained viable at low macromonomer concentrations, but cell viability decreased sequentially as the macromonomer concentration increased. GG–MA hydrogels with a 0.05 wt.% GG–MA macromonomer concentration revealed excellent endothelial cell proliferation, similar to that of the Matrigel™ control.

Keywords: Guar gum–methacrylate; Photopolymerizable macromonomer; Biodegradable hydrogels; Cytocompatibility; Tissue engineering scaffold

1. Introduction

Hydrogels derived from biomacromolecules such as polysaccharides have emerged as promising scaffolding materials for tissue engineering applications owing to their three-dimensional porous structure, biodegradability and biocompatibility, ability to imbibe a large amount of water, as well as biological fluids, and good mechanical integrity [1–7]. Such hydrogels can typically offer positive interactions with cells.

Although a number of hydrogels have been explored as scaffolding materials for cartilage, corneal and heart valve tissue engineering applications using a range of ionic polysaccharides, such as chitosan, alginate, hyaluronic acid and other glycosaminoglycans [8–11], certain limitations exist with ionic hydrogels due to the nature of the ionizable groups [12]. The dynamic swelling equilibrium of ionic hydrogels is closely related to pH, ionic strength, temperature and composition of the external solutions. Since ionic hydrogels are pH responsive, they may form biologically stable ionic networks due to the strong ionic interaction.

* Corresponding author. Address: Department of Mechanical Engineering, University of Wisconsin-Milwaukee, 3200 North Cramer Street, Milwaukee, WI 53211, USA. Tel.: +1 414 229 5946; fax: +1 414 229 6958.
E-mail address: sgong@uwm.edu (S. Gong).

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doi:10.1016/j.actbio.2009.06.001

A high degree of ionic cross-linking in ionic gels is likely to significantly reduce the network mesh size and the degree of swelling [13,14]. Thus, we explored non-ionic polysaccharide-based hydrogels for tissue engineering scaffold applications.

Guar gum (GG) is a non-ionic, water-soluble, biodegradable and biocompatible hetero polysaccharide composed of a $\beta(1 \rightarrow 4)$ D-mannopyranose backbone linked with $\alpha(1 \rightarrow 6)$ D-galactopyranose units in a 1:2 M ratio [15]. GG has various commercial applications due to its unique ability to alter rheological properties [16]. In the biomedical field GG and modified GG have both been used as a carrier for colon targeted [17] and transdermal drug delivery [18], however, its potential applications for tissue engineering scaffolds have not yet been explored.

In addition, previous research on polysaccharide-based tissue engineering scaffolds generally involved two steps: (1) fabrication of the three-dimensional (3-D) porous scaffold, and (2) cell seeding [19,20]. This approach has limitations in integrating bioactive components such as cells and growth factors within the scaffolds, including difficult and inconsistent cell seeding and, sometimes, an imbalanced environment for desired cell functions. Thus, in recent years there has been a growing interest in fabricating tissue engineering scaffolds using photopolymerizable macromonomers [21–23].

Photo-cross-linking allows macromonomers to be gelled in situ and enables gels to conform to the shape of the implantation site [24]. More importantly, bioactive components such as cells and growth factors can be mixed directly with the water-soluble and photopolymerizable macromonomers before forming hydrogels, thereby avoiding the difficult and inconsistent cell seeding process and resulting in a uniform cell distribution and an optimum environment for desired cell functions in the hydrogel matrices. One potential concern with this approach is the potential damage to the cells encapsulated within the photopolymerizable macromonomers due to extensive exposure to UV-light and a high concentration of photo-initiator during the gel formation process, however, previous studies indicated that a relatively short exposure to UV illumination and a mild concentration of the proper photo-initiator do not have notable adverse effects on human cells [2,25]. Moreover, the hydrogel properties can be conveniently engineered by varying the time and intensity of UV exposure and the concentrations of photo-initiator and macromonomers.

In this study we fabricated water-soluble and photopolymerizable guar gum–methacrylate (GG–MA) macromonomers. The goal of the present research is to combine the benefits of photo-cross-linked hydrogels with the advantageous properties of GG for tissue engineering scaffold applications. In order to optimize the GG–MA hydrogel matrices for 3-D cell cultivation the effects of molecular weight, degree of methacrylation and concentration of GG–MA macromonomers on the properties of GG–MA hydrogels were investigated extensively, including swelling behavior, porosity and rate of biodegradation. Cell viability and proliferation were studied using human endothelial cell line EA.hy926 as preliminary tests for the potential application of GG–MA hydrogels in tissue regeneration.

2. Materials and methods

2.1. Materials

Commercially available GG (mol. wt. 445 kDa, MP Biomedicals Inc.), 4-dimethyl-amino-pyridine (DMAP) (99%, Acros), glycidyl methacrylate (GMA) (97%, Acros) and 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propanone (I2959, Ciba) were used after purification. Fetal calf serum, penicillin, streptomycin, L-glutamine, propidium iodide and Calcein-AM were supplied by Invitrogen Corp. The Cellstripper™, Matrigel™, dialysis tubing and 96-well plates were purchased from Media Tech., BD Biosciences, Sigma and Corning, respectively.

2.2. Purification of GG

GG (mol. wt. 445 kDa) was purified with copper complexation [26]. One percent (w/v) GG solution was mixed with Fehling's solutions at 1:1 (v/v) to form a GG–copper complex. Next, the GG–copper complex was dissolved in 1 M HCl. The resulting decomplexed GG was precipitated with absolute ethanol. The GG precipitate was further dissolved in deionized water (DI H_2O), which was subsequently dialyzed with a dialysis tube (mol. wt. cut-off 12 kDa) against DI H_2O. Then, the resulting GG solution was filtered with 0.2 μm PTFE membranes. Finally, GG was solidified with acetone and dried under vacuum.

2.3. Preparation of GG samples of different molecular weights

To prepare GG samples of different molecular weights the acidic solutions of purified GG were exposed in a microwave oven for different time intervals [27]. Typically, 1.0 g GG was dissolved in 100 ml 0.025 N H_2SO_4 and subjected to irradiation in a microwave oven (0.95 kW, General Electric Co.) for a specified period: 0.5, 1.0, 1.5, 2.0 and 2.5 min. The resulting GG solutions were neutralized and individually precipitated with ethanol.

2.4. Synthesis of GG–MA macromonomers

Photopolymerizable GG–MA macromonomers were prepared by attaching methacryloyl groups onto a GG backbone using DMAP as catalyst (Fig. 1) [28]. Briefly, each GG was dissolved in anhydrous dimethyl sulfoxide (DMSO) to make a 40 mg ml^{-1} solution. Next, 3.5% (w/v) DMAP and 5 mM GMA were added separately to the GG solution and thoroughly mixed with stirring. The reaction was allowed to proceed at room temperature in a nitrogen atmosphere for 48 h. The solution was precipitated in a large excess of ethanol. The collected GG–MA

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