Carbohydrate Polymers 152 (2016) 271–279

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Modified glycogen as construction material for functional biomimetic microfibers

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ARTICLE INFO

Article history:
Received 8 May 2016
Received in revised form 21 June 2016
Accepted 28 June 2016
Available online 2 July 2016

Chemical compounds studied in this article:
Glycogen (PubChem CID 439177)
Sodium hydroxide (PubChem CID 14798)
Acetic acid (PubChem CID 176)
Allyl bromide (PubChem CID 7841)
Propargyl bromide (PubChem CID 7842)

Keywords:
Glycogen
Fibers
Irradiation crosslinking
Wound healing dressing
Tissue

ABSTRACT

We describe a conceptually new, microfibrous, biodegradable functional material prepared from a modified storage polysaccharide also present in humans (glycogen) showing strong potential as direct-contact dressing/interface material for wound healing. Double bonds were introduced into glycogen *via* allylation and were further exploited for crosslinking of the microfibers. Triple bonds were introduced by propargylation and served for further click functionalization of the microfibers with bioactive peptide. A simple solvent-free method allowing the preparation of thick layers was used to produce microfibers (diameter ca 2 μm) from allylated and/or propargylated glycogen. Crosslinking of the samples was performed by microtron beta-irradiation, and the irradiation dose was optimized to 2 kGy. The results from biological testing showed that these highly porous, hydrophilic, readily functionalizable materials were completely nontoxic to cells growing in their presence. The fibers were gradually degraded in the presence of cells.

1. Introduction

Significant effort was recently invested into the development of materials for tissue engineering (TE) and wound healing purposes in regenerative medicine (Langer & Vacanti, 1993). The scaffolds for TE must fulfill specific requirements, such as biocompatibility, nontoxicity, controlled biodegradability, good mechanical properties and suitable surface chemistry for the successful attachment, migration, proliferation, and differentiation of the cells. In addition, the majority of native tissues have established architecture that ensures their specific functions or properties (West-Mays & Dwivedi, 2006 Wolinsky & Glagov, 1964), therefore, the particular scaffold must be, in fact, an artificial extracellular matrix, which will support growth of the cells to form the desired tissue (Murugan & Ramakrishna, 2007).

Advanced wound dressings promoting healing processes should possess similar, yet specific properties (Lee, Jeong, Kang, Lee, & Park, 2009; Mogosanu & Grumezescu, 2014). They should be: essentially nontoxic and non-immunogenic, so as not to disturb the wound healing process; highly permeable, to allow both drainage of exudate and oxygen penetration from the outer atmosphere (open wounds) or from outer vasculature (internal use) to the lesion; hydrophilic, to maintain humidity allowing wet healing; and functionalizable, to enable controlled delivery of healing promoters. It is advantageous if these materials are slowly biodegradable and do not attract cells significantly, allowing closing of the wound space with the newly formed tissue. A fibrous nature is especially advantageous for wound healing dressings due to the

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http://dx.doi.org/10.1016/j.carbpol.2016.06.107
0144-8617/

inherently extremely high porosity and permeability given by all-communicating pores occupying the overwhelming part of the material volume.

Therefore, appropriate 3D architecture, material and technique for the preparation of biomimetic TE materials have to be selected. Various techniques have been studied and applied for the preparation of TE scaffolds, e.g., electrospinning (Li et al., 2005; Liang, Hsiao, & Chu, 2007), solvent casting/particulate leaching (Mikos et al., 1994; Xiang, Liao, Kelly, & Spector, 2006), gas foaming (Sachlos & Czernuszka, 2003), rapid prototyping (Jeong et al., 2012), and phase separation (Ma, 2008).

The materials that are used for TE play a crucial role here. Most of these materials are polymer-based and are natural, synthetic or hybrid in origin. Due to biocompatibility, biodegradability, biological activity and abundance, polymers of natural origin have become increasingly recognized for application in this field. The most intensively studied natural or natural-derived materials include starch-based polymers (Gomes, Ribeiro, Malafaya, Reis, & Cunha, 2001), chitosan (Guo et al., 2006; Lee et al., 2002; Xuebing, Peixing, George, & Shuihong, 2011), alginate (Oliviera & Reis, 2011; Tilakaratne, Hunter, Andracki, Benda, & Rodgers, 2007), hyaluronic acid (Chung et al., 2006; Collins & Birkinshaw, 2013; Ji et al., 2006), dextran (Levesque & Shoichet, 2006), cellulose (Fang, Wan, Gao, Tang, & Dai, 2009; Vinatier et al., 2009), collagen (Koch et al., 2006; Xiao, Qian, Young, & Bartold, 2003; Yow, Quek, Yim, Leong, & Lim, 2009), gelatin (Holland, Tabata, & Mikos, 2005; Park, Temenoff, Holland, Tabata, & Mikos, 2005) and fibrin (Miller, Fisher, Weiss, Walker, & Campbell, 2006; Mol et al., 2005).

One prospective natural polymer that has not been studied in the area of wound healing is glycogen (GG). GG is the main storage form of D-glucose in mammalian organisms, including humans. The highest concentrations in humans are present in the liver and muscles. GG is hyperbranched poly(D-glucose), where D-glucose units are connected with each other by $\alpha(1 \rightarrow 4)$ bonds and branching is *via* $\alpha(1 \rightarrow 6)$ bonds (Shearer and Graham, 2002; Tirone & Brunicardi, 2001). Recently, we have shown that GG forms different 3D nano- and microarchitecture structures depending on the initial concentration of its aqueous solution *via* freeze-drying from water (Vetrik et al., 2013). A mostly fibrous nature is adopted due to the highly amorphous nature and the spherical shape of the GG macromolecules. This is an organic solvent-free process that is easy to perform and allows the possibility to prepare even bulk layers of fibrous material, which is quite problematic by the widely used electrospinning technique. Nevertheless, these GG structures remain water soluble, so they cannot be considered as potential TE-dedicated materials without additional modification. The presence of a high number of hydroxyl groups in a polymer backbone allows us to perform a variety of substitutions in GG to adjust the required properties.

In this report, we show for the first time that modified GG may be used for the construction of biodegradable hydrophilic biomimetic microfibers with properties suitable for biomedical applications, such as wound healing. We describe the modification of GG through simultaneous alkylation of hydroxyl groups in GG with allyl bromide and propargyl bromide. The obtained GG derivatives were used to fabricate fibers and sponge-like structures followed by electron irradiation to obtain a water insoluble "pre-scaffold" crosslinked by radical polymerization of the allyl groups. The presence of triple bonds in the propargyl moieties in the structure of the obtained material provides a possibility to perform alkyne-azide click reactions to attach different biological active moieties, e.g., peptides, proliferation agents, and growing factors, which is a significant advantage compared to our previous system (Vetrik et al., 2013), which uses grafting of poly(ethyl cyanoacrylate) to the nanofibers from the vapor phase for stabilization, not allowing surface functionalization, and turning the hydrophilic polysaccharide surface into a hydrophobic polycyanoester. Copper-catalyzed click reaction with RGD peptide (known to promote the attachment and growth of several different types of cells) (Ruoslahti, 1996) was performed to investigate the amount of available alkyne groups in the fabricated pre-scaffold. The biological behavior of the obtained materials was evaluated in *in vitro* cell culture and has shown that the material fulfils all criteria and is thus highly prospective for advanced wound healing dressings and/or interfaces in direct contact with bone tissue.

2. Materials and methods

2.1. Materials

Sodium hydroxide (98.9%) and acetic acid (98%) were obtained from Lach-Ner Ltd. (Neratovice, Czech Republic). All other reagent grade chemicals were purchased from Sigma-Aldrich Ltd. (Prague, Czech Republic) and were used as received. Ultrapure Q-water that was ultra-filtered on a Milli-Q Gradient A10 system (Millipore, Molsheim, France) was used throughout the work. Dialysis tubing (Spectra/Por 3, molecular weight cut-off 3500 Da) was purchased from Serva Electrophoresis GmbH (Heidelberg, Germany).

2.2. Modification of glycogen

In a typical experiment, glycogen (from oyster, type II, catalogue number Sigma-Aldrich G8751, weight-average molecular weight 10 MDa, 4.00 g, 22 mmol glucose units) and sodium hydroxide (1.97 g, 49 mmol) were dissolved in water (136 mL), and the solution was cooled to 0 °C. Allyl bromide (640 μL, 7.39 mmol) and propargyl bromide (107 μL, 1.4 mmol) were added, and the mixture was stirred for 10 h at 0 °C and then overnight at room temperature. Acetic acid (3.66 mL, 64 mmol) was added to neutralize the residual sodium hydroxide, the resulting solution was dialyzed against water using membrane tubing Spectra/Por 3 with a MWCO 3500 Da for 48 h and freeze-dried. Yield: 3.80 g (95%) of allyl propargyl glycogen (APG).

2.3. Fabrication of fibrous glycogen structures

Aqueous solutions of APG (concentrations of 0.5 or 5 wt.%) were placed into crystallization dishes (forming a layer approximately 10 mm thick) and were frozen in dry ice (freezing rate was approximately 2 °C min^{-1}). The frozen samples (initial temperature −18 °C) were then lyophilized on a Scanvac Coolsafe 110-4 Pro (MERCI Ltd., Brno, Czech Republic) freeze drier, shelf temperature −10 °C, pressure 20 Pa, duration 48 h.

2.4. Irradiation of the samples

The fibrous structures from APG were exposed to an electron beam (10 MeV electron energy, electron current 25 μA, 2 kGy/min) on a Microtron MT25 accelerator with high frequency source to cause radiation crosslinking. The dose of radiation used was in the range of 2–150 kGy.

2.5. Characterization of fibrous structures

Fourier transform infrared (FT-IR) spectra were obtained on a Perkin-Elmer Paragon 1000PC spectrometer equipped with the Specac MKII Golden Gate single attenuated total reflection (ATR) system (PerkinElmer Co., U.S.A.) with a diamond crystal and angle of incidence of 45°. The ^{1}H NMR spectra were obtained on a Bruker Avance DPX-300 spectrometer (Bruker Co., Austria) at 310 K operating at 300.13 MHz. 4,4-Dimethyl-4-silapentane-1-sulfonic acid was used as an internal standard, and D_2O was used as the solvent.