Carbohydrate Polymers 137 (2016) 584–593

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Highly biocompatible collagen–*Delonix regia* seed polysaccharide hybrid scaffolds for antimicrobial wound dressing

Kalirajan Cheirmadurai, Palanisamy Thanikaivelan*, Ragothaman Murali

Advanced Materials Laboratory, Centre for Leather Apparel & Accessories Development, Central Leather Research Institute (Council of Scientific and Industrial Research), Adyar, Chennai 600020, India

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 26 October 2015
Accepted 6 November 2015
Available online 10 November 2015

Keywords:
Collagen
Gulmohar seed polysaccharide
Cinnamon bark extract
Cross-linking
Antimicrobial

Chemical compounds studied in this article:
Ethanol (PubChem CID: 702)
Methanol (PubChem CID: 887)
Acetone (PubChem CID: 180)
Acetic acid (PubChem CID: 176)
Chloroform (PubChem CID: 6212)
Cinnamaldehyde (PubChem CID: 637511)
D_2O (PubChem CID: 24602)

ABSTRACT

Biomaterials based entirely on biological resources are ideal for tissue engineering applications. Here we report the preparation of hybrid collagen scaffolds comprising gulmohar seed polysaccharide (GSP) and cinnamon bark extract as cross-linking agent. ^{1}H NMR spectrum of GSP confirms the presence of galactose and mannose in the ratio of 1:1.54, which was further corroborated using FT-IR. The hybrid scaffolds show better enzyme and thermal stability in contrast to pure collagen scaffold probably due to weak interactions from GSP and covalent interaction through cinnamaldehyde. Gas permeability and scanning electron microscopic analysis show that the porosity of the hybrid scaffolds is slightly reduced with the increase in the concentration of GSP. The infrared and circular dichroic spectral studies show that the secondary structure of the collagen did not change after the interaction with GSP and cinnamaldehyde. The hybrid scaffolds stabilized with cinnamaldehyde show good antimicrobial activity against the common multi-drug resistant wound pathogens. These results suggest that the prepared hybrid scaffolds have great potential for antimicrobial wound dressing applications.

1. Introduction

Biomaterials play an important role in the wound healing process by providing rapid healing with reasonable cost and minimal inconvenience to the patient. Use of collagen (C) based biomaterials for wound healing applications is gaining importance since collagen represents the main structural protein of the skin matrix. Connective tissue derives its prominent features such as mechanical strength and activation of the blood clotting cascade from the collagen and its architectural arrangement (Purna & Babu, 2000). During wound healing process, collagen is known to play important role in the induction of clotting, cell proliferation, migration and the appearance of final scar. Though various types of collagen available in nature, type I collagen has been extensively used to prepare medical devices because of its abundance (Pachence, 1996). Different types of chemical cross-linkers such as glutaraldehyde (GA) (Ma et al., 2003), epoxy compounds (Van Wachem et al., 1999), and sulfhydryl reagents (Soares, Maia, Rayas-Duarte, & Soldi, 2009) were used to improve the physical properties of the collagen scaffolds (Charulatha & Rajaram, 2003; Park, Lee, Lee, & Suh, 2003). The use of chemical cross-linkers may lead to the presence of unreacted molecules in the collagen matrix, which can result in cytotoxic reaction. It is known that GA cross-linked collagen-based biomaterials release cytotoxic molecules, which may result from unreacted GA present in the samples or from hydrolytic or enzymatic degradation products (Olde Damink et al., 1996). Currently, natural or bio-based cross-linking agents such as genipin (Yan et al., 2010), proanthocyanidin (Han, Jaurequi, Tang, & Nimni, 2003), citric acid, malic acid (Reddy, Li, Yang, 2009), ferulic acid (Balaguer, Gomez-Estaca, Gavara, & Hernandez-Munoz, 2011), tannic acid (Natarajan, Krithica, Madhan, & Sehgal, 2013) are being explored and investigated.

Delonix regia belongs to the flowering plant from the Fabaceae family, noted for its fern-like leaves and flamboyant display of flowers. It contains pod type of fruits and the tree is commonly known as Gulmohar in India (Krishnaraj, Chandrasekar,

* Corresponding author. Tel.: +91 44 24910953; fax: +91 44 24910953.
E-mail addresses: thanik8@yahoo.com, thanik8@gmail.com (P. Thanikaivelan).

http://dx.doi.org/10.1016/j.carbpol.2015.11.015
0144-8617/

Nanjan, Muralidharan, & Manikandan, 2012). The seed contains galactomannan as a storage polysaccharide. Seed galactomannans attracted more research interest due to their property of forming viscous solutions or gels in aqueous media. The different chemical properties of these gums make them as a potent material for various applications like water holding, thickening, gelling, binding, emulsifying and formation of films. The gum can be synergistically interacted with other monomers and polymers due to the numerous OH group present in it (Srivastava & Kapoor, 2005).

Cinnamon zeylanicum bark is widely used as a spice. It is principally employed in cooking as a condiment and flavouring material, being largely used in the preparation of some desserts, chocolate, spicy candies, tea, hot cocoa, and liqueurs. In medicine, it acts like volatile oils and was once used as a cure for colds. It has also been used to treat diarrhoea and other problems of the digestive system. *C. zeylanicum* bark has high antioxidant activity. The essential oil of cinnamon also has antimicrobial properties, which can aid in the preservation of certain foods. It also has remarkable pharmacological effects in the treatment of type II diabetes and insulin resistance (Sathishkumar et al., 2009). Up-regulation of both mRNA and protein expression levels of type I collagen using cinnamon extract containing cinnamaldehyde was reported (Takasao, Tsuji-Naito, Ishikura, Tamura, & Akagawa, 2012). Cinnamaldehyde is a naturally occurring aromatic α,β-unsaturated aldehyde derived from cinnamon. Recently, it has been used as an antimicrobial/antifungal agent in active packaging applications (Becerril, Gomez-Lus, Goni, Lopez, & Nerin, 2007; Arfa, Preziosi-Belloy, Chalier, & Gontard, 2007; Rodriguez, Nerin, & Batlle, 2008).

Here, we prepared hybrid scaffolds comprising collagen and gulmohar seed polysaccharide (GSP) employing cinnamon bark extract as cross-linking agent. It is proposed that cinnamaldehyde present in the cinnamon bark extract would induce cross-linking in the collagen molecules. Further, the prepared scaffolds were analyzed for morphology, thermal stability, chemical interactions, swelling ability, enzymatic degradation and antimicrobial properties.

2. Materials and methods

2.1. Materials

Trimmed waste from cowhide was obtained from a local tannery at Central Leather Research Institute, Chennai. Gulmohar seeds were collected from Central Leather Research Institute (CLRI), Chennai, India. Cinnamon bark was purchased from local market and ground into powder. Ethanol and chloroform were purchased from SRL Pvt. Ltd., India. Collagenase was procured from Sigma-Aldrich.

2.2. Preparation of collagen solution

Hide powder from the raw cowhide trimming waste was prepared by previously reported method (Ashokkumar et al., 2012). The raw hide trimming pieces were collected from local tannery at Chennai and soaked in 300% (v/w) water for 5 h with three changes. The soaked trimming pieces were limed, dehaired, relimed, fleshed, and delimed using the conventional procedures to remove unwanted keratin, elastin, reticulin, albumin, globulin, proteoglycan and fats. The delimed hide pieces were soaked in 35 and 70% acetone for 3 h respectively, followed by 100% methanol for five times each 3 h duration to completely remove the moisture. Finally, the hide pieces were thoroughly dried in a vacuum drier (Stokes Vacuum Shelf Dryer, Model 138-H) and made into fine powder using a Willy mill (Model no. 1, Arthur H. Thomas Co., Philadelphia, PA, USA) of mesh size 2 mm with yield of 15% (on trimming waste weight). The solvent ratio and yield are shown in Table 1. About 1 g of hide powder was weighed and mixed with pre-chilled 0.5 M acetic acid. It was solubilized using a blender at 4 °C. After solubilization the collagen solution was stored at 4 °C for further use. The purity of obtained type-I collagen was analysed using hydroxyproline assay (Murali, Anumary, Ashokkumar, Thanikaivelan, & Chandrasekaran, 2011) and it was found to be 90 ± 2%. The structural, chemical and morphological properties of the pure collagen were analysed using Fourier Transform Infrared (FT-IR), circular dichroic (CD) spectral analysis and scanning electron microscopic (SEM) analysis as described below.

2.3. Isolation of gulmohar seed polysaccharide

Polysaccharide was extracted using a method reported for isolating galactomannans from seeds of Caesalpinia pulcherrima from the Fabaceae family (Thombre & Gide, 2013). The gulmohar seeds were soaked in 99% ethanol in a weight to volume ratio 1:3 at 60 °C for 3 h to inactivate enzymes. The seeds were soaked in warm water for 24 h and then kept at 100 °C for 3 h. The seeds were washed with water and the cotyledon portion was separated from the seed coat manually. Distilled water was added in a weight to volume ratio of 1:5 (cotyledon:water) and kept in an overhead stirrer for 24 h. The suspension was filtered through muslin cloth and centrifuged at 10,000 × *g* to remove insoluble matter. The galactomannan was precipitated by adding ethanol in a volume to volume ratio of 1:3 (aqueous seed extract:ethanol) and the precipitate was lyophilized (Lark, Penguin classic plus freeze dryer) to obtain GSP powder with a yield of 10% (on seed weight). The solvent ratios and yield are shown in Table 1.

2.4. Preparation of cinnamon bark extract

Cinnamon bark powder (2 g) was extracted with 100 ml of chloroform at 30 °C for 2 h. The extract was filtered through filter paper and concentrated to 50 ml at 50 °C (Takasao et al., 2012). The concentration of cinnamaldehyde was determined using UV–visible spectral analysis as described below.

2.5. Scaffold preparation

Collagen solution (10 mg/ml) was used for scaffold preparation. GSP solutions (25, 50, 75 and 100 wt.% to collagen) were prepared by dissolving respective quantity of GSP powder in 10 ml warm distilled water. Prepared GSP solution was added to 30 ml of collagen solution and 5 ml of chloroform extract of cinnamon bark was added as a cross-linking agent. The hybrid solution was stirred for 2 h at 30 °C to allow cross-linking and the evaporation of chloroform. The hybrid solution was poured into a mould and lyophilized. A control scaffold was prepared as mentioned above without the addition of GSP.

2.6. Characterization of GSP

For ^{1}H NMR analysis, the spectrum was obtained on JEOL 500 MHz spectrometer. The GSP (2%) was dissolved in D_2O at 80 °C with continuous stirring for 5 h, and the solution was examined. The ^{1}H NMR spectra was recorded at 80 °C.

Thermogravimetric analysis (TGA) of GSP was carried out using a thermogravimetric analyser (TA instruments, TGA Q50) employing 10 mg sample in aluminium pans. The experiment was carried out under nitrogen flow (50 ml/min), at heating rate of 10 °C/min over a temperature range of 25–800 °C.

The prepared GSP was analysed using the X-ray diffraction (Rigaku miniflex II, Desktop model with a Cu*K*α radiation source,