Materials Letters 132 (2014) 34–37

Contents lists available at ScienceDirect

Materials Letters

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

Wound healing analysis of pectin/carboxymethyl cellulose/ microfibrillated cellulose based composite scaffolds

Neethu Ninan [a,b,*], Muthunarayanan Muthiah [c], In-Kyu Park [c], Nandakumar Kalarikkal [b], Anne Elain [a], Tin Wui Wong [d], Sabu Thomas [b], Yves Grohens [a]

[a] Université de Bretagne Sud, Laboratoire Ingénierie des Matériaux de Bretagne (LIMatB), Rue de St Maudé, BP 92116, 56321 Lorient Cedex, France
[b] Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Priyadarsini Hills PO, Kottayam 686560, Kerala, India
[c] Center for Creative Biomedical Scientists, Chonnam National University Medical School, Gwangju 501-757, South Korea
[d] Non-Destructive Biomedical and Pharmaceutical Research Centre, Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 16 October 2013
Accepted 9 June 2014
Available online 14 June 2014

Keywords:
Pectin
Carboxymethyl cellulose
Microfibrillated cellulose
Wound healing
Scaffold

ABSTRACT

In our previous study, we have synthesised pectin/carboxymethyl cellulose/microfibrillated cellulose composite scaffolds by lyophilisation and investigated its morphological, mechanical, thermal properties and tested their cytotoxicity. In this work, we explored the wound healing ability of pectin/carboxymethyl cellulose/microfibrillated cellulose based composite scaffolds. The pore size of the prepared scaffold was ideal for the growth of dermal fibroblasts. The *in vivo* studies conducted on Sprague Dawley rats showed that it could promote skin regeneration within ten days. The histological examination revealed excellent collagen deposition and complete re-epithelialisation in case of rats treated with composite, confirming its potential as excellent wound dressing material.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Wound healing is a multifarious regenerative process encompassing four phases namely, haemostasis, inflammation, proliferation and maturation. During haemostasis, blood vesssels constrict and form a clot [1]. Vasodilation occurs during inflammatory phase in which antibodies, leucocytes, growth factors and enzymes migrate to the site of injury to engulf bacteria and debris. Wound is reconstructed with new granulation tissue which mainly comprises of collagen and extracellular matrix during the proliferation phase [2]. Maturation is the final phase in which remodelling of collagen III–I occurs along with formation of cellular connective tissues [3].

An ideal tissue engineerng scaffold for wound healing should be highly porous allowing cells to grow, absorb excess wound exudates, maintain a moist environment and allow gas exchange. Several biopolymers are used for the fabrication of scaffolds [4]. Pectin is a heteropolysaccharide found mainly in the cell walls of plants composed of α-(1→4) linked D-galacturonic acid residues. Carboxymethyl cellulose (CMC) is a derivative of cellulose consisting of β-(1→4) glucopyranose residues, which is water soluble [5]. Microfibrillated cellulose (MFC) is a cellulosic material obtained by homogenisation consisting of short microfibrils of diameters ranging from 20 to 60 nm and are used as fillers in the manufacture of composites [6]. Since pectin has poor mechanical properties, it is blended with carboxymethyl cellulose (CMC). The incorporation of fillers like MFC within the matrix of pectin and CMC, will improve the mechanical integrity of composite scaffold.

In our previous article, we synthesied lyophilised pectin/CMC/MFC scaffold by varying the concentrations of MFC. Among the different samples, composite scaffold with 0.1% (w/v) of MFC (C1) was found to be the optimised scaffold which showed the highest mechanical strength and glass transition temperature, controlled swelling and degradation and excellent cell viability [7]. In the present study, we have investigated its ability to enhance wound healing in Sprague Dawley rats.

2. Materials and methods

Materials: Low methoxyl (LM) pectin and carboxymethyl cellulose (CMC) were bought from Central Drug House Private Limited (Delhi, India). Vitacel® microfibrillated cellulose (MFC) was acquired from Rettenmair France SARL (Saint Germain En Laye, France) and sodium hydroxide pellets were obtained from Acros Organics (Illkirch Cedex, France). Glycerol (purity-99%), endotoxin free water, calcium chloride, and absolute ethanol were procured

* Corresponding authors at: Université de Bretagne Sud, Laboratoire Ingénierie des Matériaux de Bretagne (LIMatB), Rue de St Maudé, BP 92116, 56321 Lorient Cedex, France. Tel.: +33 751464109.
E-mail address: neethuninan85@yahoo.co.in (N. Ninan).

http://dx.doi.org/10.1016/j.matlet.2014.06.056
0167-577X/© 2014 Elsevier B.V. All rights reserved.

from Sigma Aldrich (Saint-Quentin Fallavier, France). Harris hematoxylin and eosin (staining reagents) were bought from Leica Biosystems Richmund Inc. (Germany). Analgesics like ketamine hydrochloride and xylazine hydrochloride were acquired from Troy Laboratories, Australia. All the reagents were used without any further purification.

Fabrication of pectin/CMC/MFC porous scaffolds: As mentioned in our previous paper, 2% (w/v) of LM pectin and 0.8% (w/v) of CMC were dissolved in endotoxin free water separately under constant magnetic stirring [7]. Then LM pectin and CMC were mixed together along with 4% (v/v) of glycerol and left for overnight stirring. 0.1% (w/v) of MFC in endotoxin free water was sonicated for 30 min and added to pectin/CMC mixture using syringe and immediately crosslinked by 1% (w/v) of $CaCl_2$. The polymeric suspension was stirred well and transferred to petridishes, which were then kept at −20 °C. These frozen samples were freeze dried in Christ Alpha 1-2 LD Plus Freeze Dryer at −50 °C for 48 h to prepare porous scaffold (C1). The control scaffold without MFC is C0.

Characterisation: The morphology of the optimised scaffold was examined using scanning electron microscope (SEM) (JEOL, JSM 6031, Japan). Thin sections of C0 and C1 were excised using a razor blade, gold sputtered using Polaron sputtering apparatus and then observed under SEM. Porosity and pore size distribution of prepared scaffold was determined using microcomputed tomography (microCT) from Morlaix lab, France. Monochromatic X rays generated from V(TOMEX) 240D X-ray tube (at 240 kV and 320 W) were used to scan sample with thickness of 2.5 mm.

Wound healing studies: Wound healing studies were conducted on eight male Sprague Dawley rats purchased from Genetic Improvement and Farm Technologies Sdn Bhd Malaysia. On the day of experiment (day 0), rats weighing 200–250 g, were anesthetised by giving an intramuscular injection of a mixture of 90 mg/kg of ketamine and 10 mg/kg of xylazine. Around 5 ml of hot deionised water boiled to 80 °C was added through a circular plastic ring, affixed to the dorsal area of rats using adhesive glue, in 9 repetitive cycles, in order to induce partial thickness wound. Then the induced wound was covered with composite scaffold (C1), using standard gauze and 3 M adhesive tape. The rats were divided into two groups, namely, Group 1 (control rats with open wounds) and Group 2 (rats treated with C1). Photographs of the wound were taken and the wound area was redressed with C1 every day. The wound area was outlined on a transparent polyethylene sheet and the size of wound was measured using digital micrometre (Mitutoyo, Japan). The percentage of wound area closure was estimated using Eq. (1).

$$\text{Percentage of wound area closure } (\%) = 100 \times (A1 - A)/A1 \qquad (1)$$

where, $A1$ is the initial wound area calculated on day 0 and A is the wound area on day '*t*'. Significance was estimated using Student's *t* test and a probability level of $p < 0.05$ is recognised to be statistically significant.

Histochemistry: The animals were sacrificed on tenth day and the skin was excised into thin sections, using cryostat (Leica CM 1850 UV, Germany). The sliced skin was mounted on glass slides and stained by hematoxylin and eosin (H&E) staining reagents in Autostainer XL (Leica, Germany) and visualised under compound microscope (Leica DF2500, Germany), equipped with a camera to take digital images of stained sections.

3. Results and discussions

In our previous article, we synthesied lyophilised pectin/CMC/MFC scaffold by varying the concentrations of MFC. Among the different samples, composite scaffold with 0.1% (w/v) of MFC (C1) was found to be the optimised scaffold which showed the highest mechanical strength and glass transition temperature, controlled swelling and degradation and excellent cell viability [7]. In the present study, we have investigated its ability to enhance wound healing in Sprague Dawley rats.

Morphology analysis using SEM: Fig. 1 depicted the SEM images of C0 and C1. The pore size was found in the range of 30–300 μm in case of C0 and 10–250 μm in case of C1. Also, C1 contained well interconnected pores when compared to C0. The minor decrease in the pore size may be due to strong reinforcing effect of MFC incorporated in polymer matrix as reported previously. The architectural features like pore size, shape and interconnectivity are vital for cell seeding, migration, growth, mass transport and tissue formation [8].

Porosity estimation using microCT: Using microCT, the porosity of C1 was estimated to be 88%, which may enable easy diffusion of nutrients and gases and promote cell proliferation. The pore size was found in the range of 15–280 μm (Fig. 2). The pore size is measured to be higher compared to SEM as it is measured in terms of pixel in case of microCT [4]. Around 60% of pores have pore size below 20 μm and 38% of pores were found in the range of 20–40 μm.

in vivo studies: Previously, we have conducted cytotoxicity tests of samples on NIH 3T3 cell lines and C1 was found to have the highest

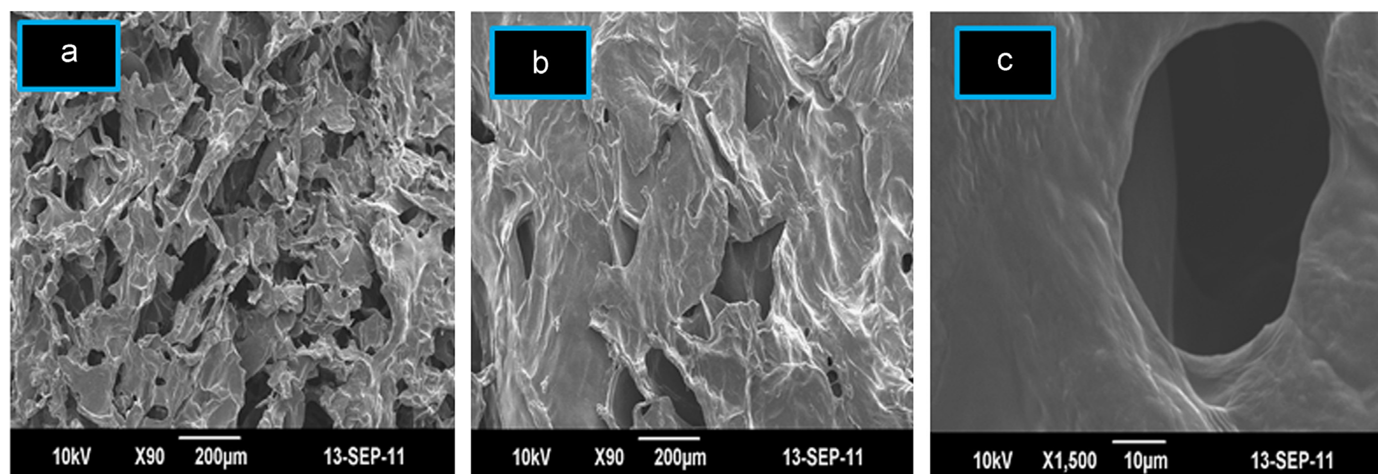

Fig. 1. SEM images of (a) C1, (b) C0 and (c) elliptical pore of C1.

Download English Version:

https://daneshyari.com/en/article/1643886

Download Persian Version:

https://daneshyari.com/article/1643886

Daneshyari.com