International Journal of Pharmaceutics 505 (2016) 234–245

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

# Drug loaded composite oxidized pectin and gelatin networks for accelerated wound healing

Mythili Tummalapalli[a], Morgane Berthet[b], Bernard Verrier[b], B.L. Deopura[a], M.S. Alam[c], Bhuvanesh Gupta[a,*]

[a] Bioengineering Lab, Department of Textile Technology, Indian Institute of Technology, New Delhi 16, India
[b] Laboratory of Tissue Biology and Therapeutic Engineering, IBCP, UMR 5305 CNRS-UCBL-Lyon Cedex 07, France
[c] Department of Chemistry, Jamia Hamdard, New Delhi 62, India

ARTICLE INFO

*Article history:*
Received 4 January 2016
Received in revised form 2 April 2016
Accepted 4 April 2016
Available online 5 April 2016

*Keywords:*
Oxidized pectin
Gelatin
Infection control
Cell compatibility
Wound healing
Biocomposite

ABSTRACT

Biocomposite interactive wound dressings have been designed and fabricated using oxidized pectin (OP), gelatin and nonwoven cotton fabric. Due to their inherent virtues of antimicrobial activity and cytocompatibility, these composite structures are capable of redirecting the healing cascade and influencing cell attachment and proliferation. A novel *in situ* reduction process has been followed to synthesize oxidized pectin-gelatin-nanosilver (OP-Gel-NS) flower like nanohydrocolloids. This encapsulation technology controls the diffusion and permeation of nanosilver into the surrounding biological tissues. Ciprofloxacin hydrochloride has also been incorporated into the OP-Gel matrix to produce OP-Gel-Cipro dressings. While OP-Gel-NS dressings exhibited 100% antimicrobial activity at extremely low loadings of 3.75 $\mu g/cm^2$, OP-Gel-Cipro dressings were highly antimicrobial at 1% drug loading. While NIH3T3 mouse fibroblasts proliferated remarkably well when cultured with OP-Gel and OP-Gel-Cipro dressings, OP-Gel-NS hindered cell growth and Bactigras® induced complete lysis. Full thickness excisional wounds were created on C57BL/6J mice and the wound healing potential of the OP-Gel-NS dressings led to accelerated healing within 12 days, while OP-Gel-Cipro dressings healed wounds at a rate similar to that of Bactigras®. Histological examination revealed that OP-Gel-NS and OP-Gel-Cipro treatment led to organized collagen deposition, neovascularization and nuclei migration, unlike Bactigras®. Therefore, the OP-Gel-NS and OP-Gel-Cipro biocomposite dressings exhibiting good hydrophilicity, sustained antimicrobial nature, promote cell growth and proliferation, and lead to rapid healing, can be considered viable candidates for effective management.



## 1. Introduction

The modern concept of interactive wound dressings envisages a wound healing process in which the bioactive components incorporated in the dressing influence the physiological processes and direct the healing cascade. It has been proved that healing under a wet environment is augmented through easy migration of the extracellular matrix proteins and enhanced activity and availability of growth factors (Eaglstein, 2001; Balakrishnan et al., 2005). Hydrogels and hydrocolloids are being widely used in this respect to maintain a moist environment at the wound site and aid the healing process. Due to their high water contents, skin like consistency and biocompatibility, hydrogels are expected to promote healing and enhance reepithelialization (Singh and Pal, 2008). Furthermore, such hydrophilic wound dressings would preclude trauma to the patient during ambulation or dressing removal. A hydrogel material that can influence the healing cascade and provide sustained antimicrobial activity is expected to lead to accelerated wound healing (Vowden and Vowden, 2014; Mogoşanu and Grumezescu, 2014; Broughton et al., 2006). Taking this as our objective, in the current study, we attempted to develop polysaccharide-protein network structures that can inherently influence the healing cascade and enhance the tissue regeneration process.

Pectin is an anionic polysaccharide obtained from the cell walls of terrestrial plants. In addition to being biocompatible, biodegradable and non-cytotoxic, pectin is a hydrocolloid in nature and therefore is suitable for wound healing applications. The presence of hydroxyl, carboxyl and carboxymethyl groups on the backbone chain renders it readily susceptible to functionalization and

* Corresponding author.
*E-mail address:* bgupta@textile.iitd.ernet.in (B. Gupta).

http://dx.doi.org/10.1016/j.ijpharm.2016.04.007
0378-5173/

modification. Pectin and its derivatives are considered highly suitable for wound healing applications. The high level of esterified galacturonic acid residues in pectin is responsible for its strong anti-inflammatory effect leading to the suppression of inflammatory enzymes such as iNOS and COX-2 (Birch et al., 2015). Chemically sulfated citrus pectin fractions with good antithrombogenic properties have been developed which could be used for wound care (Cipriani et al., 2009). The sulphated pectins directly inhibited α-thrombin even in the absence of heparin co-factor II and exhibited 100% effectiveness against venous thrombosis. When papin was immobilized in pectin and used for wound debridement, a 20% faster healing was observed in the first four days alone (Jáuregui et al., 2009). Oxidized pectin and carboxymethyl chitosan membranes were developed by *in situ* crosslinking were found to be non-hemolytic and cytocompatible (Fan et al., 2012). Pectin is a natural anti-glycation agent and has been used beneficially to treat healing impaired wounds. Aerosols of collagen-pectin microparticles were developed using an electrospraying method for diabetic wound treatment and lung regeneration (Jayakumar et al., 2014). They exhibited high and sustained antimicrobial activity and good cell adhesion and proliferation. In our previous communication, we have reported the *in situ* synthesis of nanosilver within an oxidized pectin matrix (Tummalapalli et al., 2015). Such core-shell materials are expected to exhibit long term antimicrobial activity due to their controlled release behaviour. Therefore, these nanohydrocolloids have been used in the current study to synthesize nanosilver based wound dressings.

Gelatin, on the other hand, is a proteinous derivative deemed suitable for skin regeneration applications due to its non-immunogenicity, cell adhesion behaviour and blood coagulation characteristics. Gelatin sponges intended for rapid haemostasis in acute injury and surgical situations are available commercially. Hajosch et al. developed gelatin sponges capable of inducing haemostasis in less than a minute during surgeries (Hajosch et al., 2010). The haemostatic action is based on platelet damage at the contact of blood with gelatin, which activates the coagulation cascade. Gelatin has been used in combination with various natural and synthetic polymeric systems to fabricate wound dressings. Gelatin and gelatin-dendrimer nanofiber constructs were designed for drug delivery and wound care (Dongargaonkar et al., 2013). These networks were found to be capable of high antibacterial efficiency and anti-inflammatory effect. Gelatin-montmorillonite composite hydrogels were prepared in which ciprofloxacin was intercalated in the layered silicate structure (Kevadiya et al., 2014). The authors observed a sustained drug release profile from the nanocomposite structures and these hydrogels induced wound healing progression by enhanced cell migration and proliferation. Chitosan, honey and gelatin sheets were developed as burn dressings (Wang et al., 2012). Honey and chitosan were responsible for good antibacterial activity and low inflammation, while gelatin promoted cell migration and growth.

Polysaccharide-protein conjugates are expected to exert a synergistic effect on the mechanical properties, cytocompatibility and tissue regeneration features. Therefore, in the current study, we have developed *in situ* crosslinked oxidized pectin and gelatin (OP-Gel) matrices loaded with bioactive agents such as nanosilver or ciprofloxacin for accelerated wound healing. While ciprofloxacin is a broad spectrum antibiotic, nanosilver is an antimicrobial agent that precludes the issue of antibiotic resistance and is effective against more than 200 strains of bacteria, viruses and fungi (Madhumathi et al., 2010). These antimicrobial biopolymeric networks were then coated on nonwoven cotton to fabricate "biocomposite" wound dressings, where the cotton substrate acts as a support layer or reinforcement and the biopolymer coating is the matrix. The *in vitro* and *in vivo* behaviour of the dressings thus designed has been compared against those of a commercially available dressing, Bactigras®.

## 2. Experimental

### 2.1. Materials, cells and animals

Citrus pectin (Mw∼30,000 g/mol, degree of esterification∼72%) and ciprofloxacin hydrochloride were purchased from CDH Fine Chemicals, India. Gelatin, from porcine skin (high gel strength) was procured from Fluka Analytical, Germany. Periodic acid and silver nitrate were purchased from Merck Chemicals, India. Glycerol, nitric acid and isopropanol were obtained from Fisher Scientific, India. Luria broth and agar-agar, were obtained from Hi Media Laboratories, India. Bacterial strains of *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) were provided by IIT Delhi. Ultrapure water (18 MΩcm) from Millipore Milli-Q system, India was used in all experiments. All chemicals were of analytical grade and used without further purification. Nonwoven cotton fabric (21.24 GSM and 67% porosity) was isolated from Surgipad dressings manufactured by Johnson & Johnson Limited, India. Bactigras®, a commercial dressing containing chlorhexidine acetate, was procured from Smith & Nephew Healthcare Pvt. Ltd., India.

Mice NIH3T3 fibroblast cells were purchased from CelluloNet (Lyon, France). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with a solution of fetal bovine serum (10%, v/v, FBS) and antibiotics (penicillin and streptomycin, 5%, v/v) at 37°C under 5% $CO_2$. NIH3T3 were harvested with trypsine EDTA solution at 80% confluence.

Twenty-five C57BL/6J mice (male, age 10 weeks, 20–25 g) from Janvier Laboratories (Le Genest St Isle, France) were used in this study. The ambient temperature and relative humidity were maintained constant in the animal facility. Mice were acclimatized for one week before the experiments, and kept with free access to food and water. All animal experiments conducted at IBCP, Lyon were approved by the Ethics Committee for Animal Experimentation of the University of Lyon 1, France and complied with French legislation and ethics committee guidelines (Permission number BH2011-49, dated January 9, 2012).

### 2.2. Fabrication of biocomposite dressing

Periodate oxidized pectin (OP) of aldehyde content 2.101 mmol/g was synthesized according to our previously reported work (Gupta et al., 2013b). The OP thus synthesized was then crosslinked with gelatin at optimized reaction conditions to prepare OP-Gel matrices, as described in our previous study (Gupta et al., 2014). A 4% OP-Gel (70/30) crosslinked matrix was prepared according to the protocol for 16 h at 60°C, pH 6.4 and with 40% glycerol. Two different types of wound dressings were developed, with nanosilver or ciprofloxacin. Silver nitrate (1–4 wt%) was added to the OP-Gel solution at the end of 16 h and allowed to react for 5 min to obtain oxidized pectin-gelatin-nanosilver solutions (OP-Gel-NS). For the oxidized pectin-gelatin-ciprofloxacin dressings (OP-Gel-Cipro), ciprofloxacin hydrochloride (0.5-2.5 wt%) was added to the OP-Gel solution at the end of 16 h crosslinking time and allowed to react for 5 min. A nonwoven cotton fabric was dipped into the OP-Gel and OP-Gel-drug solutions, taken out and dried in the dark at room temperature to fabricate biocomposite structures.

### 2.3. High resolution transmission electron microscopy (HRTEM)

The HRTEM imaging of the OP-Gel-NS matrices was conducted on a TECNAI 200 kV TEM (Fei, Electron Optics, Netherlands) machine. A few drops of the OP-Gel-NS solutions obtained at the