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Novel chitin/chitosan-glucan wound dressing: Isolation, characterization, antibacterial activity and wound healing properties

A.M. Abdel-Mohsen[a,b,c,*], J. Jancar[a,b,d], D. Massoud[e], Z. Fohlerova[a], H. Elhadidy[f,g], Z. Spotz[a], A. Hebeish[c]

[a] CEITEC-Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic
[b] SCITEG, a.s., Brno, Czech Republic
[c] National Research Centre, Textile Research Division, Department of Pre-treatment and Finishing of Cellulosic Fibers, Dokki, Cairo, Egypt
[d] Faculty of Chemistry, Institute of Materials Chemistry, Brno University of Technology, Brno, Czech Republic
[e] Zoology Department, Faculty of Science, Fayoum University, Fayoum, Egypt
[f] CEITEC–Central European Institute of Technology, Institute of Physics of Materials, Academy of Sciences of Czech Republic, Brno, Czech Republic
[g] Faculty of Science, Physics Department, Mansoura University, Mansoura, Egypt

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ABSTRACT

Chitin/chitosan-glucan complex (ChCsGC) was isolated from *Schizophyllum commune* (*S. commune*) and dissolved for the first time in precooled (−15 °C) 8 wt.% urea/6 wt.% NaOH aqueous solution. Novel nonwoven microfiber mats were fabricated by wet-dry-spinning technique and evaluated the mechanical of fabrics mats and surface morphology. Isolated and nonwoven mat were characterized employing FTIR-ATR, Optical microscope, TGA, DSC, H/C NMR, SEM and XRD techniques. According to the physical/chemical characterization measurements we can assumed that, the net and the novel dressing mats have the same chemical structure with slightly changes in the thermal stability for the dressing mats.The biological activity of the nonwoven ChCsGC fabric was tested against different types of bacteria exhibiting excellent antibacterial activity. Cell viability of the plain complex and nonwovens mats were evaluated utilizing mouse fibroblast cell line varying concentrations and treatment time. ChCsGC did not show any cytotoxicity against mouse fibroblast cells and the cell-fabrics interaction was also investigated using fluorescence microscope. The novel ChCsGC nonwovens exhibited excellent surgical wound healing ability when tested using rat models.

1. Introduction

Wounds are commonly classified as without and/or with tissue loss, acute and chronic (Lee et al., 2000) and the surgical wounds represent over 40% of all the wound cases worldwide. Wound infection is one of the primary complications of today's wound care management able to impede the healing process and can keep the wound from complete closure (Guo and DiPietro, 2010). The application of biological materials, in different form like solutions, creams, for drug delivery to the wounds are not very effective as they rapidly absorb fluid during the process and lose their physical, mechanical properties and become unstable (Boateng et al., 2008). For this reasons, the use of wound dressing mats is preferred as they provide better exudate management and prolonged residence at the wound site. The advanced wound dressing such as chitin/chitosan-glucan complex have many advantage as solid form (physical stability), antibacterial activity, healing character as well as drug carriers compared with the traditional dressing such as

Abbreviations: ChGC, chitin-glucan complex; ChCsGC, chitin/chitosan-glucan complex; TGA, thermal gravimetric analysis; SEM, scanning electron microscopy; TEM, transmission electron microscopy; DSC, differential scanning calorimetry; IPA, iso-propyl alcohol; *S. commune*, *Schizophyllum commune*; NMR, nuclear magnetic resonance; S/L, solid to liquid ratio; FTIR-ATR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; *E. coli*, Escherichia coli; *K. pneumoniae*, *Klebsiella pneumoniae*; *B. subtilis*, *Basillus subtilis*; *S. aureus*, *Staphylococcus aureus*; NIH, mouse fibroblast cell line; 3T3, standard fibroblast cell line; hr, hour; min, minute; MTT, 3-(4-5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide; IR, inflammatory reaction; SC, beneath the scab; GT, granulation tissue; HF, hair follicles.

* Corresponding author at: Central European Institute of Technology (CEITEC), Brno University of Technology, Technicka 3058/10, 616 00 Brno, Czech Republic. Tel.: +420 773063837.
E-mail addresses: abdel-mohsen@ceitec.vutbr.cz, abdo_mohsennrc@yahoo.com (A.M. Abdel-Mohsen).

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gauze and cotton wool that take no active part in wound healing process. Chitosan is the second most abundant natural polysaccharide routinely obtained by deacetylation of chitin. Chitosan exhibits unique properties such as biocompatibility, biodegradability and bacteriocidity (Abdel-Mohsen et al., 2012; Abedini et al., 2013; Fan et al., 2014; Lee and Mooney, 2001) and process-ability into micro and nano-fibers. Due to the antibacterial activity, healing properties, haemostasis and anesthetic effect, it has attracted enormous attention in biomedical material research as a component of wound dressing (Angspatt et al., 2011; Arockianathan et al., 2012; Bagheri-Khoulenjani et al., 2009; Bellini et al., 2012; Boateng et al., 2008; Bueno and Moraes, 2011; Chen et al., 2015; Fan et al., 2014; Valle et al., 2014).

Fungal polysaccharides and polysaccharide-protein complexes, found in cell walls as a part of the extracellular matrix, have been investigated due to a variety of biologically attractive functions such as anti-tumor, anti-nociceptive, anti-inflammatory, anti-oxidative and immune stimulatory activities (Burkatovskaya et al., 2006; Cheng et al., 2012; Cho et al., 1999; Chorvatovičová and Šandula, 1995; Cordeiro et al., 2012; Valasques Junior et al., 2014). The β-D-glucans are polymers of D-glucose monomers linked by β-glyosidic bonds (with or without (1→6)-β-D-glucose side chains) found in the cell walls of many bacteria, plants, fungi and yeasts (Ogawa et al., 2014; Reis et al., 2002). A variety of β-D-glucan differing in structures have been isolated from various sources and their biological activity can be regulated by various structural parameters, such as the primary structure, molecular weight, functionalization and conformation (Ogawa et al., 2014).

Chitosan and glucan are the main components of the cell walls of fungi occurring in two forms, as a free chitosan and a chitosan covalently bonded to β-glucan (Kanetsuna and Carbonell, 1970). These branched β-1,3-glucans are accompanied by different amounts of glucans with β-1,6 and β-1,3 links only or alternating β-1,3 and β-1,6 links together with chemical bonding among glucan chains. This results in forming a cross-linked network which confers rigidity to the cell wall (Mislovičová et al., 2000). The individual polysaccharide chains aggregate into micro-fibrils held together by hydrogen bonds and this, together with the cross-linked network of glucan, results a very strong and rigid cell wall structure (Dergunova et al., 2009).

Chitin–glucan complexes from *Komagataella pastoris* (Farinha et al., 2015), *Komagataella Pichia* (Chagas et al., 2014), *Aspergillus niger* (Skorik et al., 2010), cell wall of fungus *Gongronella butleri* (Nwe et al., 2008), yeast bud scars (Yamaoka et al., 1989) was isolated by chemical and enzymatic processes. The resultant chitosan–glucan complexes from chitin-glucan were used as flocculation agent and as support for cultivation of mammalian cells (Skorik et al., 2010). Moreover, chitosan–glucan complex was tested for removal of selected metal ions from waste water (Chorvatovičová and Šandula, 1995). In addition, the reduced molecular weight carboxymethylated derivative of a chitosan–glucan complex was investigated for its anti-mutagenic activity. The disadvantage of the chitin/chitosan-glucan complex is the poor solubility in water and in other types of solvents greatly reducing its applicability. The lack of applications of chitosan fibers were the poor mechanical and stability of dressing chitosan fibers/fabrics as well as to prepare 3D structure of chitosan has to be chemically cross-linked by different hazardous cross-linker (Lee et al., 2004; Schiffman and Schauer, 2007; Yang et al., 2005). Almost all studies of chitosan based wound dressing only used on form of fibers (micro/nano) (Annur et al., 2015; Schiffman and Schauer, 2007; Toivonen et al., 2015; Zhang et al., 2008), films (Ligler et al., 2001; Mi et al., 2006; Suginta et al., 2013; Yuan et al., 2007) or hydrogel (Jungst et al., 2015; Kumar et al., 2004; Suginta et al., 2013), but chitin/chitosan-glucan microfibers/nonwoven mats not been reported. Therefore, in the present research we dissolve the complex for the first time in green solvent, and to innovate complex-based wound dressing with improved the bacteria killing and to enhance the healing properties.

In this work, we present results on isolation, physico-chemical characterization of chitin/chitosan-glucan complex (ChCsGC), and preparation of novel ChCsGC microfiber nonwoven mats using wet-spun technology. We report on using urea/sodium hydroxide aqueous solution to dissolve the complex for the first time. The surface morphology, chemical stability, biological activity and mechanical properties of a novel nonwoven mat flat wound dressing were investigated in relation to their structural variables. The antibacterial activity, cytotoxicity, *in vitro* and *in vivo* effect of this wound dressing in closing surgical wounds were assayed employing rat models.

2. Experimental

2.1. Materials

Mycelium as a source of chitin/chitosan-glucan complex was produced using *Schizophyllum commune* strain from collection of microorganism (Contipro Biotech Ltd., Czech Republic). Sodium hydroxide, acetic acid, ethanol, and isopropyl alcohol (IPA) were purchased from Lach-Ner, s.r.o., Czech Republic; MilliQ water was prepared by Millipore Elix instrument was used for all experiments.

2.2. Methods

2.2.1. Isolation of ChGC and ChCsGC

5 g (dry weight) mycelium fermented from *Schizophyllum commune* (*S. commune*) was treated with 1% of NaOH and stirred for 5 h at room temperature (25 °C). The biomass was treated with 2% of NaOH at 90 °C for 1–10 h, cooled down to room temperature and then diluted with distilled water to (1:100 S/L) ratio. The suspension solution of mycelium was filtered and the insoluble part was collected, washed three times in distilled water with neutral pH, washed in 75% isopropyl alcohol and pure isopropyl alcohol. The product was dried at 50 °C in oven, yielding 70% of chitin-glucan complex (ChGC). 1 g of chitin-glucan complex (ChGC) was dispersed in 25–60% sodium hydroxide, heated up to 90 °C and stirred for 5 h. The product was filtered and washed with distilled water until neutral pH, followed by drying at 50 °C. The alkali insoluble material of ChCsGC was treated with 1 M acetic acid at 50 °C for 2 h removed acid soluble chitosan, filtered, washed with distilled water until pH neutral, and then dried at 50 °C for 24 h. Increased the pH of the supernatant, chitosan started to precipitated, collected and dried at 60 °C for 5 h. The yield percent was 72% of ChCsGC and the degree of deacetylation (DDA) of the obtained chitin/chitosan-glucan complex (ChCsGC) was 71%.

2.2.2. Dissolution of ChCsGC

Chitin/chitosan-glucan complex (ChCsGC) with 71% degree of deacetylation (DDA) was used in all experiments. 6/8/86 wt.% NaOH/urea/H_2O mixture solution precooled to (−15 °C). ChCsGC sample in the desired amount was dispersed immediately in the solution under vigorous stirring for 30 min at room temperature to obtain a transparent dope of ChCsGC with different concentrations ranging from 0.1 to 5%.

2.2.3. ChCsGC fibers and nonwoven mats

ChCsGC fibers were prepared by wet-dry-spinning technique. Three grams of the complex was dissolved in urea/sodium hydroxide aqueous solution (see Section 2.2.2) until well flowing viscous and homogenous solution was obtained. The prepared solution was spun using the lab nozzle with needle diameter of