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Research Paper

# Regenerated chitin fibers reinforced with bacterial cellulose nanocrystals as suture biomaterials

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ABSTRACT

The objective of this work was to prepare a novel filament with good biocompatibility and mechanical performance which can meet the demands of surgical sutures. Bacterial cellulose nanocrystals (BCNCs) were used to reinforce regenerated chitin (RC) fibers to form BCNC/RC filaments. Mechanical performance measurements demonstrated that the strength of the BCNC/RC filament was increased dramatically over the RC analogue. A yarn made of 30 BCNC-loaded fibers also achieved satisfactory mechanical performance, with a knot-pull tensile strength of 9.8 ± 0.6 N. Enzymatic degradation studies showed the BCNC/RC materials to have good biodegradability, the rate of which can be tuned by varying the concentration of BCNCs in the yarn. The RC and the BCNC/RC materials had no cytotoxicity and can promote cell proliferation. *In vivo* experiments on mice demonstrated that suturing with the BCNC/RC yarn can promote wound healing without obvious adverse effects.

## 1. Introduction

Chitin and bacterial cellulose (BC) are both natural products. Chitin, an abundant and important polysaccharide material in nature, is extracted primarily from shellfish sources such as shrimp and crab. (Jayakumar et al., 2011); it is also found in small amounts in insects and other invertebrate shells. BC is a biopolymer with the same molecular structure as cellulose from plants, but is made from microbial fermentation (Amin, Abadi, & Katas, 2014). Chitin, BC and their derivatives have been widely studied in the field of biomaterials, often due to their excellent biocompatibility (Li et al., 2015; Nguyen et al., 2014; Skołucka-Szary et al., 2015; Wang et al., 2016).

Chitin is a biopolymer composed of β-1,4 glycans of *N*-acetyl-*d*-glucosamine units (Supplementary Information, Fig. S1a). It has low toxicity and biodegradability when implanted *in vivo* (Anitha et al., 2014; Deepthi et al., 2016; Pogorielov et al., 2017). Chitosan, also known as deacetylation chitin, is usually obtained by heating chitin with concentrated alkaline solutions, through which the acetyl groups are partially removed. As a result, the water insoluble chitin is converted into soluble chitosan. Because of the wound healing, anti-inflammatory and antibacterial properties of both chitin and chitosan, attempts have been made to use these materials for a range of applications (Ding et al., 2015; Teimouri & Azadi, 2016) including wound dressings (Huang et al., 2014; Xie et al., 2008), surgical sutures (Dobrovol'skaya, Kasatkin, Yudin, Ivan'kova, & Elokhovskii, 2015; Khor & Lim, 2003), and as scaffolds in tissue engineering (Dhivya, Saravanan, Sastry, & Selvamurugan, 2015; Liu, Ma, Mao, & Gao, 2011). In particular, chitin and chitosan can promote fibroblast proliferation and macrophage migration, and accelerate vascularization and granulation during wound healing processes (Riccardoaa, 2009). These properties make chitin a promising biomaterial for absorbable scaffolds and sutures.

However, controlled degradation is essential for a scaffold in tissue engineering applications (Teimouri, Ebrahimi, Emadi, Beni, & Chermahini, 2015), and is equally important for absorbable sutures. While chitin can be degraded by lysozyme present in the human body, in general it has low biodegradability – a major limiting factor for its use in absorbable sutures. As a result, chitosan has attracted more attention in this regard due to its much greater biodegradability. Unfortunately, the mechanical strength of chitosan is very poor, and hence it has mainly been explored for suture coating (Maslova, Uspenskii, Gal'Braikh, & Kil'Deeva, 2016; Viju & Thilagavathi, 2013). To improve the quality of chitin such that it can be used for sutures it is necessary to make chemical modifications, or to develop new fiber production (spinning) processes to prepare suturable

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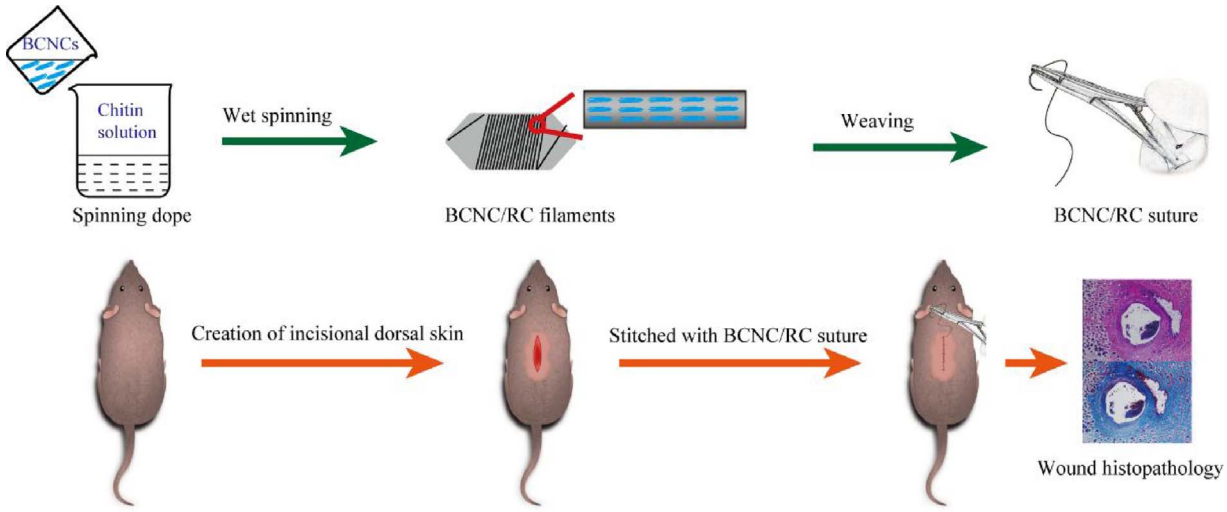


**Fig. 1.** The process of suture preparation and wound closure.

threads with appropriate properties. A study by Shao et al. (Shao et al., 2015) is an example of the former; these authors prepared a diacetyl chitin suture with good performance. The latter approach aims to improve the suture properties through adjusting the spinning parameters, especially through the development of novel solvent dissolution and composite formation methods.

Chitin and chitosan can be processed into a range of different forms, for instance membranes and films, pellets or particles, or fibers and filaments. The latter are most commonly prepared using wet spinning (where a polymer is dissolved into a solvent and then extruded into an anti-solvent where it precipitates to form fibers) or dry-jet wet spinning (in which the polymer solution is extruded under heat and pressure into an air gap before entering a coagulation bath). Since the chitin must be dissolved and then re-precipitated, chitin fibers prepared by wet spinning are termed regenerated chitin (RC) fibers.

The majority of studies exploring chitin focus on membranes/films and pellets/particles, with little work concerning spinning. Thus, there is a deficit of knowledge as to the most appropriate parameters to use in producing chitin-based filaments. This is important, because the properties of the spun fiber vary significantly with the processing parameters and solvents used. An optimization of the spinning process therefore offers a route to address the many points to be improved during manufacture if chitin or its derivatives are to be used as surgical sutures. For instance, RC materials spun using ionic liquids (Kai, Müller, Beyer, Hermanutz, & Buchmeiser, 2015; Singh et al., 2016; Singh et al., 2013) have excellent mechanical performance but low biodegradability *in vivo*. In contrast, RC fibers made using an aqueous acetic acid solution have excellent biodegradability but poor mechanical performance (Yan, Shen, Ji, Yang, & Shen, 2014). Since the chitin sutures reported to date have limitations in terms of their mechanical strength and/or degradation time, and cannot meet surgical requirements, it is necessary to find a more suitable solvent and to develop a spinning method to produce a fiber with both appropriate mechanical performance and biodegradability.

Cellulose nanocrystals (CNCs) offer a potential route to improving mechanical performance. They have been widely explored for applications such as reinforced composites (Gorgieva, Girandon, & Kokol, 2017; Ketabchi, Khalid, Ratnam, & Walvekar, 2016), drug delivery systems (Barbosa et al., 2016; Zainuddin et al., 2017), catalysis (An, Long, & Ni, 2016; Musa, Ahmad, Hussein, Saiman, & Sani, 2017), optical and electronic materials (Espinha et al., 2016; Gençer, Schütz, & Thielemans, 2016), enzyme immobilization (Kim et al., 2015; Sunasee, Hemraz, & Ckless, 2016), and as biosensors (Esmaeili et al., 2015; Schyrr et al., 2014), *inter alia*. CNCs are short rigid single crystals of cellulose, generally with a width of *ca.* 5–20 nm and length of 100–300 nm (Habibi, Lucia, & Rojas, 2010). The chemical structure of cellulose is shown in Fig. S1b (Supplementary Information). The mechanical properties and high length-diameter ratio of CNCs suggest great potential in the reinforcement of (nano)composites (Lee, Clancy, Kontturi, Bismarck, & Shaffer, 2016; Leung, Lam, Chong, Hrapovic, & Luong, 2013). Sources of CNCs include both plant (Chen, Chen, Wang, Yao, & Wang, 2017; Qing et al., 2016; Yang & Cranston, 2014) and bacterial cellulose (Pirich et al., 2015; Sacui et al., 2014; Vasconcelos et al., 2017; Yoon, 2016). Most CNCs have been obtained from wood pulp or cotton, but there is a problem common to both in that non-cellulose components such as hemicellulose and ash content present in the raw material must be removed before use. In contrast, BC is very pure, and hence using bacterial CNCs (BCNCs) can obviate the need to remove impurities (Sacui et al., 2014).

In this work, we aimed to fabricate a bioresorbable fiber with strong and elastic mechanical performance, and a controllable degradation period. This requires the preparation of a good spinning dope. In preliminary work (data not shown) we found that chitin can be dissolved successfully using a solvent system of NaOH–urea combined with a freeze–thaw process. However, the mechanical properties (*e.g.* tenacity and strength) of the resultant regenerated chitin fibers were much worse than those obtained using N,N-dimethylacetamide/lithium chloride as the solvent system. Unfortunately lithium salts have the potential to be toxic to humans, so an alternative approach is required. Here we explored the potential of BCNCs to reinforce chitin-based fibers, preparing BC/chitin blends, processing these into fibers, and then exploring the utility of the latter in wound healing. The experimental approach adopted is illustrated schematically in Fig. 1.

## 2. Experimental

### 2.1. Materials

Bacterial cellulose (BC) was provided by the Hainan Yida Co., Ltd. Chitin powder was purchased from Sigma-Aldrich. Lysozyme (biological grade, ≥20,000 U/g), sulfuric acid ($H_2SO_4$, 95%–98%), sodium hydroxide (NaOH, ≥97%), and carbamide (urea ≥ 99%) were supplied by Sinopharm Chemical Reagents. L929 cells (mouse fibroblast cells) were provided by the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences). Monofilament polyamide sutures (H501, 3-0, black) were obtained from Shanghai Jinhuan Medical Products Co. Ltd.