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Evaluation of biomaterial containing regenerated cellulose and chitosan incorporated with silver nanoparticles

M.I. Niyas Ahamed[a,*], S. Sankar[a], P.Mohammed Kashif[b], S.K.Hayath Basha[b], T.P. Sastry[a]

[a] Bio-products Laboratory, Central Leather Research Institute, Adyar, Chennai 600020, India
[b] Department of Biochemistry, Islamiah College (Autonomous), Vaniyambadi 635751, India

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ABSTRACT

Biomaterials are used in regenerative medicine, implantable materials, controlled release carriers or scaffolds for tissue engineering. In the present study, the composites containing regenerated cellulose (RC) and chitosan (Ch) impregnated with silver nanoparticles (AgNP) with and without antibiotic gentamicin (G) were prepared. The composites prepared were characterized for their physico-chemical and mechanical properties and the results have shown the composite nature. RC–Ch–Ag and RC–Ch–Ag–G composites were used as wound dressing materials in experimental wounds of rats. The healing pattern of the wounds was evaluated by planimetric studies, macroscopic observations, biochemical studies and mechanical properties. The results have shown faster healing pattern in the wounds treated with RC–Ch–Ag and RC–Ch–Ag–G composites compared to untreated control. This study revealed that RC–Ch–Ag composite might be a potential, economical wound dressing material and may be tried on the clinical wounds of animals before being applied on humans.

1. Introduction

Wound healing is the body's natural process of regenerating dermal and epidermal tissue. It is the process whereby the body restores the injured part to as near its normal condition as possible. Though wound healing takes place naturally on its own, some of complications like sepsis, disruption of tissue and skin layer, maggot's formation, extension of infection to adjacent and interior organs occur in major cases. It is the primary response to any tissue injury [1] and complex dynamic process that involves many cascades of events like hemostasis, inflammation, proliferation and remodeling of tissues in order to fill the damage area and re-establish the skin barrier [2,3]. A good wound dressing should maintain a moist environment upon absorption of the wound exudates, protect the wound from secondary infection, provide adequate gaseous exchange, regulate and/or mediate the release of certain growth factors and cytokines, and also be elastic, biocompatible, non-toxic and non-antigenic [4–7]. Recently many researchers are entailed to produce new and improved wound dressing materials by synthesizing and modifying biomaterials that are eco-friendly and sustainable.

Chitosan is nontoxic, biocompatible, biodegradable polymer [8–12]. It is used in drug delivery, cell delivery systems, orthopedics, wound healing, ophthalmology and bone healing [13]; it enhances the function of polymorphonuclear cells, macrophages [14] and fibroblastic proliferation and migration [15]. Chitosan exhibits antimicrobial activity against bacteria [16] fungi, and yeast. It is hypoallergenic, has rapid blood clotting property, haemostatic and acts as fat attractor by binding to dietary lipids [17]. The presence of active groups in chitosan molecules allows for easy chemical modification and therefore is used in many fields, including medicine. Extensive investigations are being aimed at the wider use of chitosan in wound dressings [18]. The increasing interest in the material is caused by its biological activity resulting from its susceptibility to degradation under the influence of enzymes present in body fluids such as lysozyme and N-acetylglucosoamidase. The degradation products, being chitooligomers, are able to stimulate macrophages and positively influence collagen sedimentation, thus accelerating the wound healing process [19].

Silver metal and its compounds have been known for their antimicrobial activities. Silver ions work against bacteria in a number of ways, they interact with the thiol groups of enzyme and proteins that are important for bacterial respiration and transport of important substance across the cell membrane and within the cell [20,21]. Silver ions also bound to the bacterial cell wall, altering the function of the bacterial cell membrane [22]. Thus sil-

* Corresponding author.
E-mail address: iniyasahamed@gmail.com (M.I.N. Ahamed).

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ver metal and its compounds are effective in preventing infection of the wound [23].

Cellulose is a natural hydrogel whose properties are better than the hydrogel produced from synthetic polymers; for example, it displays high water content (98–99%), good adsorption of liquids, high wet strength, and high chemical purity and can be safely sterilized without any change to its structure and properties [24]. Being similar to human skin, bacterial cellulose can be applied as skin substitute in treating extensive burns [25]. It is an interesting material for using as a wound dressing since it can control wound exudates and can provide moist environment to a wound resulting in better wound healing. However, cellulose itself has no antimicrobial activity to prevent wound infection. To achieve an antimicrobial activity, in this work, silver nanoparticles were impregnated into regenerated cellulose-chitosan composite (RC–Ch–Ag). As per a study, documented that bacterial cellulose-silver nanoparticle nanofiber had good antibacterial activity and excellent healing effects in a second-degree rat wound model [26,27].

These RC–Ch–Ag composites were characterized for their various physico-chemical characterization studies like tensile strength, thermo gravimetric analysis (TGA), differential scanning colorimetric analysis (DSC), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). It was used as wound dressing material in experimental rats. The progress of the wound healing in both experimental and control groups was evaluated by planimetric studies, macroscopic observations, biochemical studies and mechanical properties.

2. Materials and methods

2.1. Materials

Crab shells were collected from nearby fish market (Chennai, India). Silver nitrate (Analytical grade) was purchased from Sigma–Aldrich Chemical Co., USA. All other reagents used were of analytical grade.

2.2. Preparation of chitosan (Ch) solution

Chitosan was obtained by alkaline deacetylation of chitin from crab shells. The chitosan solution was prepared by dissolving 2 g of chitosan in 100 ml of 0.1 N HCl solutions.

2.3. Preparation of regenerated cellulose–chitosan biocomposite (RC–Ch)

In 90 ml distilled water 9.5 g of sodium hydroxide and 4.5 g of Thiourea were dissolved and then the resultant solution was pre-cooled to −5 °C to prepare a new solvent of cellulose according to a modified method of Dong et al. [28]. Various amounts of cellulose were dissolved separately in this solution; 2% chitosan solution was added to each of this solution (Table 1). Finally, the pH of the solutions was brought to 7.0 and sieved to get the regenerated cellulose–chitosan (RC–Ch) composites. The resultant materials were dried at 30 °C. The stoichiometric ratio which gave better tensile strength to the RC–Ch biocomposite was used for the incorporation of AgNP solutions.

2.4. Preparation of silver nanoparticles (AgNP) solution

Silver nanoparticles were prepared using tea grains (*Camellia Sinensis*) as a reducing as well as capping agent for $AgNO_3$ as per earlier method [29].

2.5. Incorporation of silver nanoparticles on to the RC–Ch biocomposites (RC–Ch–Ag)

The prepared RC-Ch biocomposite was soaked in solution containing 0.01% of silver nanoparticles for about 24 h. Silver nanoparticles incorporated RC-Ch was taken out of the solution and dried at 30 °C. The dried material was denoted as RC–Ch–Ag.

2.6. Coupling of gentamicin drug into RC–Ch–Ag biocomposites (RC–Ch–Ag–G)

The drug gentamicin (G) 0.04% was coupled by soaking RC–Ch–Ag composites into the gentamicin containing solution and dried at 30 °C. The dried material was denoted as RC–Ch–Ag–G and sterilized by UV irradiation under sterile conditions.

2.7. Characterization

The mechanical properties of the composites were measured using universal testing machine (Instron model 4501). Thermo gravimetric analysis was carried out with a PerkinElmer TGA over a temperature range of 30–800 °C at a heating rate of 20 °C/min under nitrogen atmosphere. Differential scanning calorimetry was carried out using DSC Q200 V23.10 Build 79 at a heating rate of 5 °C/min under nitrogen atmosphere (flow rate is 40 ml/min). The morphological studies were carried out on a Leica stereo scan-440 Scanning electron microscope equipped with phoenix EDX attachment.

2.8. Animal experiments

All experiments were performed according to the Institutional Animal Ethical Committee approval and guidelines [1142/ab/07/CPCSEA]. Male Albino Wister rats, weighing 150–200 g, were divided into four groups: control, standard soframycin ointment, RC–Ch–Ag and RC–Ch–Ag–G dressings. Throughout the experiment, rats were maintained in an air-conditioned room at 25 ± 1 °C with a lighting schedule of 12 h light and 12 h dark and were fed with commercial balanced diet and water *ad libitum*.

2.9. Surgical procedure and treatment

Each animal was given a dose of sodium pentobarbital 40 mg/kg body weight intra peritoneal and the dorsal surface of the rat below the cervical region was shaved on its back under aseptic conditions. An open excision wound of 2 cm × 2 cm was created on the shaved dorsal side of rats using sterile surgical blade. For the control animals (group I) sterile cotton gauze dipped with gentamicin was applied on the wound. The group II was applied with standard soframycin ointment, the group III was applied with wound dressing material of RC–Ch–Ag and for the group IV; the RC–Ch–Ag–G was applied. The dressings were periodically changed at an interval of 5 days with the respective materials. Three rats were sacrificed periodically on 5th, 10th, 15th, 20th and 25th day of post wound creation and the granulation tissues formed were removed and stored at −70 °C until analysis. The progress of wound healing in the four groups was evaluated visually, planimetrically and biomechanically by periodical monitoring of wound surface.

2.10. Biochemical parameters

In the present study total protein, collagen, hexosamine and uronic acid levels were estimated in the granulation tissue of control and experimental wounds on days 5, 10, 15, 20 and 25. The granulation tissue was collected after sacrificing the animals on the respective days. Estimation of protein was determined by Lowry's