Carbohydrate Polymers 92 (2013) 1177–1187

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Antibacterial activity and cell viability of hyaluronan fiber with silver nanoparticles

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ARTICLE INFO

Article history:
Received 6 August 2012
Received in revised form 23 August 2012
Accepted 25 August 2012
Available online 12 September 2012

Keywords:
Green chemistry
Hyaluronan fiber
Silver nanoparticles
Cell viability
Antibacterial activity
Medical applications

ABSTRACT

Silver has been used since time immemorial in different chemical form to treat burns, wounds and several different infections caused by pathogenic bacteria, advancement of biological process of nanoparticles synthesis is evolving into a key area of nanotechnology. The current study deals with the green synthesis, characterization, and evaluation of the biological activity and cell viability of hyaluronan fibers with incorporated silver nanoparticles (HA-Ag NPs). Hyaluronan fiber was prepared by the dissolving of sodium hyaluronate (HA) in aqueous alkaline solution to prepare a transparent solution, which was used for the preparation of fibers by a wet-spinning technique. Consequently, hyaluronan fiber was used as capping and stabilizing agent for the preparation of fibers with silver nanoparticles. HA-Ag NPs were confirmed by transmission electron microscopy, dynamic light scattering, UV/VIS spectroscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy, thermal analysis, nuclear magnetic resonance, Fourier transform infrared spectroscopy, and X-ray photoelectron spectroscopy. HA-Ag NPs showed high antibacterial activity of against *Staphylococcus aureus* and *Escherichia coli*. Cell viability tests indicated that hyaluronan, hyaluronan fibers and hyaluronan fibers with silver nanoparticles were non-toxic on the cell growth. Two different particles size of Ag NPs (10, 40 nm) had not any toxicity till the concentration limit. These tests were performed using mouse fibroblast cell line 3T3.

1. Introduction

Nanotechnology is the newest and one of the most promising areas of research in modern medical science. Nanoparticles exhibit new and improved properties based on size, distribution and morphology than the larger particles of the bulk materials from which the particles are made (Jae & Beom, 2009). The surface to volume ratio of the nanoparticles is inversely proportional with to their size. The biological effectiveness of nanoparticles can increase proportionally with an increase in the specific surface area due to the increase in their surface energy and catalytic reactivity (Singh, Jain, Upadhyay, Khandelwal, & Verma, 2010). Although there are many routes available for the synthesis of nanoparticles, there is an increasing need to develop high-yield, low cost, non-toxic and environmentally friendly procedures (Emilio et al., 2012; Panacek et al., 2009; Robert & Zboril, 2011). Silver has long been recognized as an effective antimicrobial agent that exhibit low toxicity in human and has diverse in vitro and in vivo applications (Farooqui, Chauhan, Krishnamoorthy, & Shaik, 2010). Currently, silver-based optical dressing and widely used to treat infections in open wound and chronic ulcers. These dressing also protect the host materials from oxidation and discoloration (Upendra, Preeti, & Anchal, 2009).

Wound dressings are usually used to encourage the various stages of wound healing and create better healing conditions. They often cover the wound surface to accelerate its healing. A desirable wound dressing should (a) create and keep the moist environment, (b) protect the wound from secondary infections, (c) absorb the wound fluids and exudates, (d) reduce the wound surface necrosis, (e) prevent the wound desiccation, (f) stimulate the growth factors and also be (g) elastic, non-antigenic and biocompatible (Lin, Chen, & Run, 2001; Purna & Babu, 2000). Based on the types of wounds and modes of healings, numerous materials are developed for use

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0144-8617/$ – see front matter.
http://dx.doi.org/10.1016/j.carbpol.2012.08.098

as wound dressing. These materials include synthetic polymers like polyurethane, poly (lactic acids), silicon rubber and natural polymers such as alginates, chitosan, gelatin and collagen (Dagalakis, Flink, Stasikelis, Burke, & Yannas, 1980).

With the increasing awareness of environmental protection, people are inclined to focus on the green chemistry. For this purpose, the natural compounds like D-glucose (Raveendran, Fu, & Wallen, 2003) and chitosan (Abdel-Mohsen, Abdel-Rahman, et al., 2012; Abdel-Mohsen, Aly, Hrdina, & El-Aref, 2012; Dash, Chiellini, Ottenbrite, & Chiellini, 2011; Wan, Sun, Li, & Li, 2009) was used to stabilize the Ag nanoparticles with other reducing agents. In addition, the soluble starch has been used as both the reducing and stabilizing agents to synthesize the Ag nanospheres via a one-pot green method (Vigneshwaran, Nachane, Balasubramanya, & Varadarajan, 2006).

Green synthesis of Ag-NPs involve three main steps, which must be evaluated based on green chemistry perspectives, including selection of solvent medium, reducing agent, and nontoxic substance for Ag NPs stability (Raveendran, Fu, & Wallen, 2006). Three main steps in the preparation of nanoparticles that should be evaluated from a green chemistry perspective are the choice of the solvent medium used for the synthesis, the choice of an environmentally benign reducing agent, and the choice of a nontoxic material for the stabilization of the nanoparticles. Most of the synthetic methods reported to date rely heavily on organic solvents. This is mainly due to the hydrophobicity of the capping agents used. There have been approaches reported (Templeton, Chen, Cross, & Murray, 1999) for the synthesis of water-soluble metal nanoparticles. However, to date a unified green chemistry approach to the overall process of nanoparticle production has not been reported.

Hyaluronan (HA) is a high molecular weight glycosaminoglycan in extracellular matrix (ECM) and plays a vital role in maintaining tissue integrity, as well as in facilitating adhesion and differentiation of cells during inflammation, wound repair, and embryonic development. HA has been implicated in the formation of vessels for years (Slevin et al., 2007; Slevin, Kumar, & Gaffney, 2002; West, Hampson, Arnold, & Kumar, 1985). In animal models, topically applied HA accelerated dermal wound healing and decreased fibrosis and scar formation in rats and hamsters (Lees, Fan, & West, 1995; Proctor et al., 2006; Rajapaksa, Cowin, Adams, & Wormald, 2005; Schimizzi et al., 2006; Takahashi et al., 2005). HA is also used as a carrier for other wound healing agents and in cosmetic formulations (Price, Berry, & Navsaria, 2007; Tezel & Fredrickson, 2008). These biological effects of HA are complex and one mechanism, the regulation of angiogenesis, has been reported to be involved, which depends on HA concentration and molecular size (David-Raoudi et al., 2008). It is generally accepted that high molecular weight HA (HMW-HA) inhibits endothelial cell (EC) proliferation, a phenomena supporting by the findings that avascular regions are rich in HMW-HA and that expression of this form of HA in normally vascular areas results in decreased vascularity (Marsano et al., 2007; Smith et al., 2008). In contrast, low molecular weight HA or oligosaccharides of hyaluronan (o-HA) stimulates EC proliferation, induces in vitro endothelial tube formation, and stimulates neovascularization in chick chorioallantoic membranes and collagen production by endothelial cells (Rooney, Wang, Kumar, & Kumar, 1993; Slevin et al., 2002; West et al., 1985).

In the present approach, methanol, ethanol, and propan-2-ol were utilized as the environmentally benign solvent and reducing agent throughout the preparation. The second concern in a green nanoparticle preparation method is the choice of the reducing agent. The majority of methods reported to date use reducing agents for preparation of Ag-NPs. The use of a strong reducing agent, such as $NaBH_4$, results in tiny particles that are well-dispersed (Ji, Chen, Wai, & Fulton, 1999; Shah, Holmes, Doty, Johnston, & Korgel, 2000). Nowadays, a succession of chemical reductants used for synthesis of noble metal nanoparticles which contain $NaBH_4$ (Shameli, Ahmad, & Yunus, 2010; Shen, Shi, & Li, 2010), ethylene glycol (Wang, Ren, & Deng, 2000), citrate (Pathak, Greci, & Kwong, 2000), or ascorbic acid (Lim, Jiang, & Yu, 2010). The final and perhaps most important issue in the preparation of nanoparticles is the choice of the capping material used to protect or passivity the nanoparticle surface. There are several issues that should guide the choice of the capping agent, and these vary significantly from the required size ranges and morphologies of the nanoparticles to the targeted application. In the presented preparation method, a hyaluronic fiber (a hyaluronan molecule) serves as the protecting and stabilizing agent.

Thus, in this work the hyaluronan fiber was prepared and used it as the capping and stabilizing agent to prepare the Ag nanoparticles, where the effect of solvents medium on the particle size of silver nanoparticles were studied. HA-Ag NPs were confirmed by TEM, DLS, UV/VIS spectroscopy, SEM, EDX, TGA, DTG, DSC NMR, FTIR, and XPS. HA-Ag NPs showed high antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Cell viability tests indicated that hyaluronan, hyaluronan fibers and hyaluronan fibers with silver nanoparticles were non-toxic on the cell growth. Two different particles size of Ag NPs (10, 40 nm) had not any toxicity. These tests were performed using mouse fibroblast cell line 3T3.

2. Experimental

2.1. Materials

Sodium hyaluronate high molecular weight (1.75 MDa, determined by SEC-MAALS) was purchased from CPN Ltd., Dolni Dobrouč, Czech Republic, sodium hydroxide, formic acid, acetic acid, silver nitrate, ethanol, propan-2-ol, and methanol were obtained from Sigma–Aldrich, Germany. Demineralized water was used for all experiments.

2.2. Methods

2.2.1. Preparation of hyaluronan fibers

Hyaluronan fiber was successfully prepared via previous work (Abdel-Mohsen, Hrdina, et al., 2012; Burgert, Hrdina, Masek, & Velebny, 2012). Briefly, a sodium hyaluronate (1.75 MDa, 6 g) was dissolved under stirring in 94 g of water with the addition of NaOH (0.64 g) to obtain homogenous, well-flowing viscous solution suitable for spinning. This solution was pressed (wet-spinning technique) through a nozzle with the diameter of 0.4 mm to the coagulation bath having the composition: 600 ml of methanol and 400 ml of acetic acid (98%). The prepared fibers were left in the coagulation bath for 15 h, then washed with absolute methanol and dried.

2.2.2. Preparation of silver nanoparticles incorporated in hyaluronic fibers

Silver nanoparticles were prepared by means of a simple chemical reduction of silver nitrate by hyaluronic fibers (Abdel-Mohsen, Hrdina, et al., 2012). A certain weight of the so-prepared (HA fiber) was dispersed in a certain volume of methanol, ethanol and propan-2-ol using a heating magnetic stirrer. After dispersion of HA in different dispersing agents, the pH of the solution was adjusted within the range (4–11.5), followed by the raising of temperature (20–80 °C). A certain amount of silver nitrate solution was then added drop-wise. The reaction mixture was kept under continuous stirring for different time (15–180 min). Short time after the addition of silver nitrate, the HA fiber acquires a clear yellow color indicating the formation of silver nanoparticles. The progression of the reaction was controlled by UV/Vis absorption; aliquots from