Carbohydrate Polymers 151 (2016) 452–457

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Formation and properties of hyaluronan/nano Ag and hyaluronan-lecithin/nano Ag films

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ARTICLE INFO

Article history:
Received 22 November 2015
Received in revised form 27 May 2016
Accepted 28 May 2016
Available online 29 May 2016

Keywords:
Biocomposites
Nanometals
Nanosilver
Hyaluronic acid
Lecithin

ABSTRACT

A facile and environmentally friendly method of the preparation of silver nanoparticles embedded in hyaluronan (Hyal/Ag) and hyaluronan-lecithin (Hyal-L/Ag) matrix was developed. Thin, elastic foils were prepared from gels by an *in situ* synthesis of Ag in an aqueous solution of sodium hyaluronate (Hyal), using aq. D-(+)-xylose solution as a reducing agent. The gels were applied to a clean, smooth, defatted Teflon surface and left for drying in the air. The dry foils were stored in a closed container.

UV–vis spectroscopy, transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) spectra confirmed formation of about 10 nm ball-shaped Ag nanoparticles situated within the polysaccharide template. Thermal properties of the composites were characterized involving differential scanning calorimetry (DSC) and thermogravimetric (TGA) analyses, whereas molecular weights of polysaccharide chains of the matrix were estimated with the size exclusion chromatography coupled with multiangle laser light scattering and refractometric detectors (HPSEC-MALLS-RI). An increase in the molecular weight of the hyaluronate after generation of Ag nanoparticles was observed. The foils showed specific properties.

The study confirmed that silver nanoparticles can be successfully prepared with environmentally friendly method, using hyaluronan as a stabilizing template. Hyaluronan and hyaluronan-lecithin matrices provide nanocrystals uniform in size and shape. The composites demonstrated a bacteriostatic activity.

1. Introduction

Over the past few decades, inorganic nanoparticles have induced considerable interest as their nanoscale size structures exhibit unique physical, chemical, biological and functional properties (Jeon & Baek, 2010; Kango et al., 2013).

Considerable interest is paid to polymer-metal nanoparticle composites for their application in optoelectronics, nonlinear optical devices and color filters. Nanophase and nanostructured materials have potential biological and pharmaceutical applications (Hanemann & Szabo, 2010). Specifically formulated metal nanoparticles provided considerable antibacterial activity (Morones et al., 2005).

Metal nanoparticles are usually prepared by reduction of metal ions in solution. The approach meets some limits as induced by high surface energy aggregation of nanoparticles, surface passivation and capping reagents obscure their manufacture (Adhyapak, Singh, Vijayan, Aiyer, & Khanna, 2007; Fu, Yang, Li, Yu, & Long, 2006; Khachatryan, Khachatryan, Fiedorowicz, Para, & Tomasik, 2013). Biodegradable polymers as matrices for the formation of nanostructures provide stable nanometal structures embedded in polymer matrix and, at the same time, eliminate the use of harmful solvents and reducing agents. Such materials facilitate an integration of nanometals with biologically relevant systems. In order to operate within area of so-called green chemistry, the reagents including the reducing reagent and reaction medium should be environmentally benign (Huang & Yang, 2004; Khachatryan et al., 2013; Raveendran, Fu, & Wallen, 2003).

This paper presents an environmentally benign "green" method for the preparation of silver nanoparticles in hyaluronan (Hyal) and Hyal-lecithin matrices. Hyal acts as an aggregation protecting,

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http://dx.doi.org/10.1016/j.carbpol.2016.05.104
0144-8617/

lecithin as a complexing agent and xylose is a reducer of the silver ions.

Hyal has already been used (Khachatryan et al., 2009) as a matrix for the synthesis of quantum dots (QDs). Nanometer-sized semiconducting ZnS and CdS particles have been successfully prepared within the hyaluronic acid (HyalH) film matrix. This polysaccharide was proven to be efficient in preventing aggregation and controlling growth for ZnS and CdS nanocrystals because of involvement of its coordination centers.

Hyal is a polysaccharide consisting of up to 10 000 units of D-glucuronic acid and *N*-acetyl-D-glucosamine moieties linked with alternating β-1,4- and β-1,3-glycosidic bonds (Necas, Bartosikova, Brauner, & Kolar, 2008). Its molecular weight depends on biological origin and reaches 4×10^6 Da (Kobayashi, Okamoto, & Nishinari, 1994). In all vertebrates Hyal is as a major constituent of extracellular matrices in which the majority of cells differentiate (Lee & Spicer, 2000). A high level of Hyal can be also found in extracellular matrices of matured tissues. Because of its biological properties, Hyal offers promising medical and pharmaceuticals applications (Karbownik & Nowak, 2013). Hyal might be considered as a building block for new biocompatible and biodegradable polymers, however, they exhibit poor biomechanical properties. For that sake, several chemical modifications have been checked to meet the goal. Such novel biomaterials were developed for the tissue engineering, wound healing, surgical adhesions and drug delivery (Allison & Grande-Allen, 2006; Burdick & Prestwich, 2011; Jia, Colombo, Padera, Langer, & Kohane, 2004; Prestwich, 2011; Prestwich, 2008). For instance, Hyal bound to liposomes via reaction with carbodiimide formed bioadhesive liposomes useful as site adherent and sustained release carriers of drugs for the topical therapy of wounds and burns (Maroda et al., 2011).

Lecithin is a significant constituent of nervous tissue and brain substance. It is used as edible and digestible, lipotropic surfactant and emulsifier (Scholfield, 1981).

Recently, a novel preparation method of stable Hyal-lecethin and lecithin foils without using any cross-linking agent was patented (Tomasik, Fiedorowicz, Khachatryan, Białopiotrowicz, & Bakoš, 2009). In contrast to other chemically modified Hyal-based preparations that solution offered material composed entirely of natural components (Bialopiotrowicz et al., 2006; Bakoš et al., 2007; Tomasik et al., 2009). In this paper a new method for the generation of nanosilver in such foils is presented as an alternative way of increasing potential applicability of such biomaterials.

2. Experimental

2.1. Materials

Sodium hyaluronate (Sigma-Aldrich, Poland, PubChem CID: 3084049), $AgNO_3$ (Aldrich, Poland, 99.99%, PubChem CID: 24470), NH_3 (Sigma-Aldrich, Poland, PubChem CID: 18944693), Lecithin (Sigma, PubChem CID: 16213884) and D-(+)-xylose (Sigma-Aldrich, BioXtra, PubChem CID: 135191) and deionized water were applied for the preparation of composites with nanoAg.

Bacteriostatic acitivity assays involved strains derived from *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14028), *Escherichia coli* (ATCC 25922), all from LGC Standards, Łomianki, Poland).

For all the tested microorganisms the TSA medium (BIOCORP, POLAND) and, additionally, for staphylococci the Chapman medium (BIOCORP, POLAND) were applied.

2.2. Synthesis of metal nanoparticle composites

2.2.1. Hyal/Ag composite

Aq. sodium hyaluronate (0.4 g in 40 mL water) was maintained at 30 °C on continuous stirring. Aq. $AgNO_3$ solution (1.5 g in 100 mL water) (0.3 mL) was introduced to it followed by addition of 4% w/w aq. ammonia (1 mL) and 4% w/w aq. xylose (4 mL). That reaction mixture was then 30 min agitated with magnetic stirrer at 60 °C in a thermostated water bath. The resulting suspension of nanoAg in the Hyal matrix was brought to room temperature and then centrifuged (5000 rpm). The deposit was applied to a clean polypropylene surface and dried at 50 °C to constant weight. The dry foils (Fig. 1) were collected and stored in closed vessels.

2.2.2. Hyal-L/Ag composite

The procedure described under 2.2.1. was repeated followed by addition of the aq. lecithin suspension (0.1 g in 10 mL water) (10 mL) magnetically agitated for 24 h at 37 °C in a thermostated water bath. The resulting suspension of nanoAg in Hyaluronate-Lecithin (Hyal-L) matrix was brought to room temperature and then centrifuged (5000 rpm). The deposit was applied to a clean polypropylene surface and dried at 50 °C to constant weight. The dry foils (Fig. 1) were collected and stored in closed vessels.

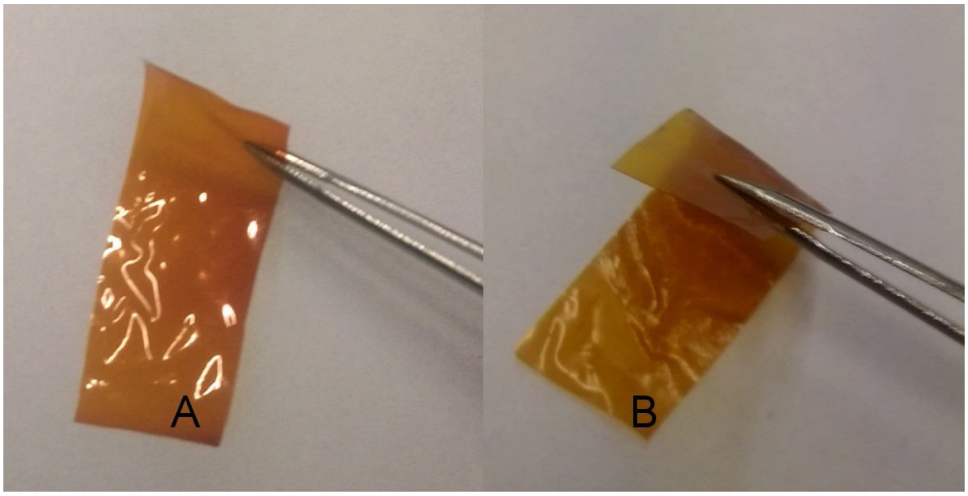

Fig. 1. Hyal/Ag (A) and Hyal-L/Ag (B) foils.