LWT - Food Science and Technology 79 (2017) 503–510

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Antiviral properties of silver nanoparticles against norovirus surrogates and their efficacy in coated polyhydroxyalkanoates systems

J.L. Castro-Mayorga [a], W. Randazzo [a, b], M.J. Fabra [a], J.M. Lagaron [a], R. Aznar [a, b], G. Sánchez [a, b, *]

[a] Food Safety and Preservation Department, Institute of Agrochemistry and Food Technology (IATA-CSIC), Avda. Agustin Escardino 7, 46980 Paterna, Valencia, Spain
[b] Microbiology and Ecology Department, University of Valencia, Av. Dr. Moliner, 50, 46100 Burjassot, Valencia, Spain

ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form
20 January 2017
Accepted 23 January 2017
Available online 27 January 2017

Keywords:
Noroviruses
Silver nanoparticles
Active packaging
Polyhydroxyalkanoates
Electrospinning

ABSTRACT

Silver nanoparticles (AgNP) have strong broad-spectrum antimicrobial activity and gained increased attention for the development of AgNP based products, including medical and food applications. Initially, the efficacy of AgNP and silver nitrate ($AgNO_3$) was evaluated for inactivating norovirus surrogates, the feline calicivirus (FCV) and the murine norovirus (MNV). These norovirus surrogates were exposed to $AgNO_3$ and AgNP solutions for 24 h at 25 °C and then analyzed by cell-culture assays. Both AgNP and silver ions significantly decreased FCV and MNV infectivity in a dose-dependent manner between concentrations of 2.1 and 21 mg/L. Furthermore, poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) films were prepared by depositing a coating of thermally post-processed electrospun PHBV18/AgNP fiber mats over compression moulded PHBV3 films. After 24 h exposure at 37 °C and 100% RH, no infectious FCV were recovered when in contact with the AgNP films while MNV titers decreased by 0.86 log. The morphology of the PHBV18 and PHBV18/AgNP fibers studied by SEM showed smooth and continuous fibers in both cases and the EDAX analysis confirmed the homogeneously distribution of AgNP into the coating and onto the PHBV3/PHBV18 layer. This study showed, for the first time, the suitability of the PHBV18/AgNP electrospun coating for antiviral surfaces.

1. Introduction

Human norovirus (family *Caliciviridae*) are reported as the leading causes of viral gastroenteritis in industrialized countries, and worldwide constituting a high public health concern. Norovirus gastroenteritis is self-limiting but extremely infectious with a low infectious dose (10–100 particles). This non-enveloped, single-stranded, positive-sense RNA virus is responsible for over 90% cases of non-bacterial and approximately half of all cases of gastroenteritis. Recently, the World Health Organization has estimated the global burden of foodborne diseases, reporting that infectious agents that cause diarrhoeal diseases accounted for the vast majority (550 million cases per year), in particular human norovirus (120 million cases per year) (WHO, 2015).

Moreover human norovirus is responsible for many outbreaks, especially in closed environments e.g. health-care facilities and cruise ships, whereas the contribution of contaminated surfaces in the spread of infection has a key role (Lopman et al., 2012). To effectively prevent norovirus outbreaks, the scientific community has been working to develop strategies for treating and preventing norovirus infection. The use of antimicrobial surfaces in food, clinical and community environments may help to reduce the spread of norovirus infection. Among them, the use of silver has emerged as a very efficient technology to prevent microbial proliferation on medical and food-contact surfaces (Kuorwel, Cran, Orbell, Buddhadasa, & Bigger, 2015) and, more concretely, silver nanoparticles (AgNP) have received considerable attention due to their attractive physico-chemical and antimicrobial properties (Moritz & Geszke-Moritz, 2013; Rai, Yadav, & Gade, 2009) such as the high surface-to-volume ratio, nanosize diameter and enhanced surface reactivity, making them able to inactivate microorganisms more effectively than their micro- or macro-scale counterparts. For instance, Castro-Mayorga and collaborators (Castro-Mayorga, Fabra, & Lagaron, 2016a) have demonstrated that poly (3-

* Corresponding author. Institute of Agrochemistry and Food Technology (IATA-CSIC). Avda. Agustín Escardino, 7, Paterna, Valencia, Spain.
E-mail address: gloriasanchez@iata.csic.es (G. Sánchez).

http://dx.doi.org/10.1016/j.lwt.2017.01.065
0023-6438/

hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)-AgNP packaging materials exhibited a strong and prolonged (even after seven months) antibacterial activity against *Listeria monocytogenes* and *Salmonella enterica* at very low AgNP loadings (0.4 g/kg). On the other hand, Martínez-Abad and collaborators (Martínez-Abad, Ocio, Lagarón, & Sánchez, 2013) developed active renewable food packaging materials based on polylactic acid (PLA) and silver ions (from 0.1 to 10 g/kg) to control feline calicivirus (FCV) in vegetables. These packaging materials showed a remarkable potential for food-contact applications as well as active packaging to maintain or extend food quality and safety. However, the maximal antimicrobial potential can hardly be achieved in most cases because silver has low solubility or compatibility with the polymers matrices, leading to the agglomeration and blackening of the films, or simply because the amount of silver available in the film surface is insufficient to exert antimicrobial effect.

As an alternative, metal nanoparticles can be incorporated into sub-micro or nano fibers by means of electrospinning technique in order to generate masterbatches which are subsequently melt, mixed with polymers pellets, or even better, used as active coating over polymer surfaces (Amna, Yang, Ryu, & Hwang, 2015). The electrospun fibres lead the development of novel materials with useful features for antibacterial applications such as fibrous membranes for water filtration (Botes & Cloete, 2010), wound dressings, implant materials or tissue engineering (Navalakhe & Nandedkar, 2007). Concretely, in the area of active food packaging, the electrospinning technique successfully avoids the agglomerations of zinc oxide nanoparticles and greatly increases their antimicrobial activity (Castro-Mayorga, Fabra, Pourrahimi, Olsson, & Lagarón, 2016c).

Since human noroviruses cannot routinely be propagated by using cell-culture systems, cultivable surrogates such as FCV and murine norovirus (MNV) are commonly used as experimental models to study human norovirus infectivity and the efficacy of inactivation technologies (D'Souza, 2014). Pioneering studies demonstrated the potential of silver ions and silver nanoparticles for enteric virus inactivation (Abad, Pinto, Diez, & Bosch, 1994; Silvestry-Rodriguez at al., 2007; Bekele, Gokulan, Williams, & Khare, 2016; De Gusseme et al., 2010; Galdiero et al., 2011; Khandelwal at al., 2014). However, it is known that silver ions are easily inactivated by many different physical or chemical factors (Castro-Mayorga et al., 2016b; Ilg & Kreyenschmidt, 2011). For instance, thermal treatments or exposure to light or UV can prompt the formation of sulphides or other silver complexes without antimicrobial properties and usually producing a strong brownish or blackish coloration of the materials (Kasuga, Yoshikawa, Sakai, & Nomiya, 2012). Accordingly, the use of stabilized AgNP could not only improve the thermal stability, the visual appearance and optical properties of the active films but also enhance their antimicrobial performance. However, there is lack of information about the influence of storage time on their antiviral activity and its efficacy when incorporated into composites. Thus, silver nitrate and silver nanoparticles at different concentrations and with different aging time were investigated for their effect on norovirus surrogates. In the second part, PHBV18/AgNP fiber mats were fabricated by electrospinning and used to coat PHBV3 films in order to develop virucidal biopolymers that may be suitable as active material, particularly in food and medical contact surfaces.

2. Material and methods

2.1. Silver nitrate and silver nanoparticles

Stabilized AgNP were synthesized by chemical reduction into unpurified poly (3-hydroxybutyrate-*co*-18 mol%- 3-hydroxyvalerate) (PHBV18) suspension according to a previously reported method (Castro-Mayorga et al., 2014). To this end, 500 mg/kg of PHBV18 was suspended in ultrapure Milli-Q® water (Millipore Corporation Co., USA) and then sodium borohydride was added to get 75.7 mg/L concentration. Thereafter, 10 mL of an aqueous $AgNO_3$ solution at 169.9 mg/L was added dropwise to generate *in situ* stabilized silver nanoparticles. The obtained PHBV18/AgNP suspension was centrifuged at 17 387 × g for 15 min and the precipitate was dried at 40 °C under vacuum for 24 h. The dried material was used as stock to evaluate the antiviral activity at three different concentrations (21, 10.5 and 2.1 mg/L). Analogous $AgNO_3$ solution (without PHBV18 and without sodium borohydride) was prepared to compare the antiviral activity of silver ions to AgNP.

2.2. Viral strains, cell lines and infections

Murine norovirus (MNV-1 strain) was propagated and assayed in RAW 264.7 cells (kindly provided by Prof. H. W. Virgin Washington University School of Medicine, USA). Feline calicivirus (F9 strain, ATCC VR-782) was cultured in CRFK cells (ATCC CCL-94). Semi-purified viruses were obtained following three cycles of freeze-thawing infected cells and centrifugation at 660 × *g* for 30 min. The supernatant was stored at −80 °C until use. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$) with eight wells per dilution and 20 μL of inoculum per well using the Spearman-Karber method (Abad et al., 1994).

2.3. Determination of antiviral activity

Each silver solution was mixed with an equal volume of each virus suspension and further incubated at 25 °C in a water-bath shaker at 150 rpm for 16 h (overnight). Then, infectious viruses were enumerated by cell culture assays as described above. Positive controls were virus suspensions added with water. Antiviral activity of silver was estimated by comparing the number of infectious viruses on suspensions without silver and on the silver-treated virus suspensions. Each treatment was performed in triplicate. The value of antiviral activity (Reduction, R) was calculated by determining $\log_{10}(N_0/Nt)$, where N_0 is the number of infections viruses on the suspension without silver and Nt is the number of infections viruses on the suspension added with silver.

2.4. Preparation of AgNP based films

A coated structure was fabricated by coating the poly(3-hydroxybutyrate-*co*-3 mol%- 3-hydroxyvalerate) (PHBV3) films with PHBV18/AgNP fibers mat produced by means of the electrospinning technique. PHBV3 films used as matrix were compression molded using hot plates hydraulic press (Carver 4122, USA) at 180 °C, 1.8 MPa during 5 min. The so-obtained films had a thickness of 246 ± 22 μm as measured with a digital micrometer (Mitutoyo, Spain, ± 0.001 mm) by averaging four measurements on each sample.

To prepare the active coating, AgNP were firstly synthesized by chemical reduction into polymer suspensions on the bases of a previously reported method (Castro-Mayorga et al., 2014). Then, PHBV18/AgNP masterbatch was dispersed in 2,2,2-Trifuoroethanol (TFE, ≥ 99%, Sigma Aldrich) having a total solids content of 60 g/kg The biopolymer solution was transferred to a 5 mL glass syringes, connected through polytetrafluoroethylene (PTFE) tubes to a stainless steel needle (0.9 mm of inner diameter) and processed using a Fluidnatek® LE-10 electrospinning equipment, trademark of the engineering division of Bioinicia S.L. (Valencia, Spain). Processed samples were collected on a stainless-steel plate connected