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

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Abstract

23   Silver nanoparticles (AgNP) have strong broad-spectrum antimicrobial activity and gained

24   increased attention for the development of AgNP based products, including medical and

25   food applications. Initially, the efficacy of AgNP and silver nitrate (AgNO$_3$) was evaluated

26   for inactivating norovirus surrogates, the feline calicivirus (FCV) and the murine norovirus

27   (MNV). These norovirus surrogates were exposed to AgNO$_3$ and AgNP solutions for 24 h

28   at 25°C and then analyzed by cell-culture assays. Both AgNP and silver ions significantly

29   decreased FCV and MNV infectivity in a dose-dependent manner between concentrations

30   of 2.1 and 21 mg/L. Furthermore, poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)

31   films were prepared by depositing a coating of thermally post-processed electrospun

32   PHBV18 /AgNP fiber mats over compression moulded PHBV3 films. After 24 h exposure

33   at 37°C and 100% RH, no infectious FCV were recovered when in contact with the AgNP

34   films while MNV titers decreased by 0.86 log. The morphology of the PHBV18 and

35   PHBV18/AgNP fibers studied by SEM showed smooth and continuous fibers in both cases

36   and the EDAX analysis confirmed the homogeneously distribution of AgNP into the

37   coating and onto the PHBV3/PHBV18 layer. This study showed, for the first time, the

38   suitability of the PHBV18/AgNP electrospun coating for antiviral surfaces.

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40   **Keywords**: Noroviruses, Silver nanoparticles, Active packaging, Polyhydroxyalkanoates,

41   Electrospinning.

42

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 et al., 2015) and, more concretely, silver nanoparticles (AgNP) have received considerable attention due to their attractive physico-chemical and antimicrobial properties (Rai, Yadav, & Gade, 2009; Moritz & Geszke-Moritz, 2013) 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,

67 2016a) have demonstrated that poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)-

68 AgNP packaging materials exhibited a strong and prolonged (even after seven months)

69 antibacterial activity against *Listeria monocytogenes* and *Salmonella enterica* at very low

70 AgNP loadings (0.4 g/kg). On the other hand, Martínez-Abad and collaborators (Martínez-

71 Abad et al., 2013) developed active renewable food packaging materials based on

72 polylactic acid (PLA) and silver ions (from 0.1 to 10 g/kg) to control feline calicivirus

73 (FCV) in vegetables. These packaging materials showed a remarkable potential for food-

74 contact applications as well as active packaging to maintain or extend food quality and

75 safety. However, the maximal antimicrobial potential can hardly be achieved in most cases

76 because silver has low solubility or compatibility with the polymers matrices, leading to the

77 agglomeration and blackening of the films, or simply because the amount of silver available

78 in the film surface is insufficient to exert antimicrobial effect.

79 As an alternative, metal nanoparticles can be incorporated into sub-micro or nano fibers by

80 means of electrospinning technique in order to generate masterbatches which are

81 subsequently melt, mixed with polymers pellets, or even better, used as active coating over

82 polymer surfaces (Amna et al., 2014). The electrospun fibres lead the development of novel

83 materials with useful features for antibacterial applications such as fibrous membranes for

84 water filtration (Botes & Cloete, 2010), wound dressings, implant materials or tissue

85 engineering (Navalakhe & Nandedkare, 2007). Concretely, in the area of active food

86 packaging, the electrospinning technique successfully avoids the agglomerations of zinc

87 oxide nanoparticles and greatly increases their antimicrobial activity (Castro-Mayorga et al.

88 2016c).

89 Since human noroviruses cannot routinely be propagated by using cell-culture systems,

90 cultivable surrogates such as FCV and murine norovirus (MNV) are commonly used as

91   experimental models to study human norovirus infectivity and the efficacy of inactivation

92   technologies (D'Souza, 2014). Pioneering studies demonstrated the potential of silver ions

93   and silver nanoparticles for enteric virus inactivation (Abad et al., 1994; Silvestry-

94   Rodriguez at al., 2007; Galdiero et al., 2011; Khandelwal at al., 2014; De Gusseme et al.,

95   2010; Bekele et al., 2016). However, it is known that silver ions are easily inactivated by

96   many different physical or chemical factors (Ilg & Kreyenschmidt, 2011; Castro-Mayorga

97   et al, 2016b). For instance, thermal treatments or exposure to light or UV can prompt the

98   formation of sulphides or other silver complexes without antimicrobial properties and

99   usually producing a strong brownish or blackish coloration of the materials (Kasuga et al.,

100 2012). Accordingly, the use of stabilized AgNP could not only improve the thermal

101 stability, the visual appearance and optical properties of the active films but also enhance

102 their antimicrobial performance. However, there is lack of information about the influence

103 of storage time on their antiviral activity and its efficacy when incorporated into

104 composites. Thus, silver nitrate and silver nanoparticles at different concentrations and with

105 different aging time were investigated for their effect on norovirus surrogates. In the first

106 part of this work, norovirus surrogates were exposed to different concentrations of silver

107 nitrate and the virucidal activity was assessed using cell culture. In the second part,

108 PHBV18/AgNP fiber mats were fabricated by electrospinning and used to coat PHBV3

109 films in order to develop virucidal biopolymers that may be suitable as active material,

110 particularly in food and medical contact surfaces.

111

112 **2. Material and methods**

113 *2.1. Silver nitrate and silver nanoparticles*

114   Stabilized AgNP were synthesized by chemical reduction into unpurified poly (3-

115   hydroxybutyrate-co-18 mol%- 3-hydroxyvalerate) (PHBV18) suspension according to a

116   previously reported method (Castro-Mayorga et al., 2014). To this end, 500 mg/kg of

117   PHBV18 was suspended in ultrapure Milli-Q® water (Millipore Corporation Co., USA)

118   and then sodium borohydride was added to get 75.7 mg/L concentration. Thereafter, 10 mL

119   of an aqueous $AgNO_3$ solution at 169.9 mg/L was added dropwise to generate *in situ*

120   stabilized silver nanoparticles. The obtained PHBV18/AgNP suspension was centrifuged at

121   17387×g for 15 min and the precipitate was dried at 40°C under vacuum for 24 h. The dried

122   material was used as stock to evaluate the antiviral activity at three different concentrations

123   (21, 10.5 and 2.1 mg/L). Analogous $AgNO_3$ solution (without PHBV18 and without

124   sodium borohydride) was prepared to compare the antiviral activity of silver ions to AgNP.

125

126   *2.2. Viral strains, cell lines and infections*

127   Murine norovirus (MNV-1 strain) was propagated and assayed in RAW 264.7 cells. Feline

128   calicivirus (F9 strain, ATCC VR-782) was cultured in CRFK cells (ATCC CCL-94). Semi-

129   purified viruses were obtained following three cycles of freeze-thawing infected cells and

130   centrifugation at 660×*g* for 30 min. The supernatant was stored at -80°C until use.

131   Infectious viruses were enumerated by determining the 50% tissue culture infectious dose

132   ($TCID_{50}$) with eight wells per dilution and 20 μL of inoculum per well using the Spearman-

133   Karber method (Abad et al., 1994).

134

135   *2.3. Determination of antiviral activity*

136   Each silver solution was mixed with an equal volume of each virus suspension and further

137   incubated at 25°C in a water-bath shaker at 150 rpm for 16 h (overnight). Then, infectious

138 viruses were enumerated by cell culture assays as described above. Positive controls were

139 virus suspensions added with water. Antiviral activity of silver was estimated by comparing

140 the number of infectious viruses on suspensions without silver and on the silver-treated

141 virus suspensions. Each treatment was performed in triplicate. The value of antiviral

142 activity (Reduction, R) was calculated by determining $\log_{10}$ (N_0/Nt), where N_0 is the

143 number of infections viruses on the suspension without silver and Nt is the number of

144 infections viruses on the suspension added with silver.

145

146 *2.4. Preparation of AgNP based films*

147 A coated structure was fabricated by coating the poly(3-hydroxybutyrate-co-3 mol%- 3-

148 hydroxyvalerate) (PHBV3) films with PHBV18/AgNP fibers mat produced by means of the

149 electrospinning technique. PHBV3 films used as matrix were compression molded using

150 hot plates hydraulic press (Carver 4122, USA) at 180°C, 1.8 MPa during 5 min. The so-

151 obtained films had a thickness of 246±22 μm as measured with a digital micrometer

152 (Mitutoyo, Spain, ± 0.001 mm) by averaging four measurements on each sample.

153 To prepare the active coating, AgNP were firstly synthesized by chemical reduction into

154 polymer suspensions on the bases of a previously reported method (Castro-Mayorga et al.,

155 2014). Then, PHBV18/AgNP masterbatch was dispersed in 2,2,2-Trifuoroethanol (TFE, ≥

156 99 %, Sigma Aldrich) having a total solids content of 60 g/kg The biopolymer solution was

157 transferred to a 5 mL glass syringes, connected through polytetrafluoroethylene (PTFE)

158 tubes to a stainless steel needle (0.9 mm of inner diameter) and processed using a

159 Fluidnatek® LE-10 electrospinning equipment, trademark of the engineering division of

160 Bioinicia S.L. (Valencia, Spain). Processed samples were collected on a stainless-steel plate

161 connected to the cathode of the power supply and oriented perpendicular to the syringe.

162   The distance between the needle and the plate was 12 cm and the voltage was maintained in

163   the range 10-12 kV. All experiments were carried out at room temperature under a steady

164   flow-rate of 7 mL/h. After electrospinning, the fiber mats were dried at 40°C under vacuum

165   for 24 h to completely remove the solvent.

166   Finally, the coated structure was assembled placing 250-300 g/kg of fiber mat of about 100

167   µm of thickness onto PHBV3 films. The resulting coated system was thermally post-

168   processed in a hot press (Carver 4122, USA) at 150°C during 2 min (without pressing) to

169   form a continuous film by fiber coalescence.

170   Neat PHBV3/PHBV18 films without silver were used as control for comparative purposes.

171

172   *2.5. Scanning Electron Microscopy (SEM)*

173   The morphology of the PHBV18/AgNP electrospun fibers and bilayer films was analyzed

174   using SEM. The SEM was conducted on a Hitachi microscope (Hitachi S–4800) at an

175   accelerating voltage of 5 kV and a working distance of 8-10 mm before the examination,

176   the films were cryo-fractured using liquid N_2 and sputtered with Au/Pd under vacuum. The

177   microanalysis and elemental mapping were conducted by Energy Dispersive Analysis of X-

178   rays (EDAX) from SEM images of carbon coated samples. Fibers thicknesses were

179   measured by means of the of the Adobe Photoshop CS4 software from 300 fibers at random

180   from SEM images.

181

182   *2.6. Determination of virucidal activity of silver based films*

183   To test the virucidal activity of silver based films, a modification of the ISO 22196:2011

184   (Measurement of antibacterial activity on plastics and other non-porous surfaces) was used.

185   Briefly, a suspension of viruses diluted in PBS buffer (4-6 log $TCID_{50}$/mL) was placed onto

186   the test films of 3×3 cm and covered by an inert piece of Low-Density Polyethylene

187   (LDPE) of 2.5×2.5 cm and 10 μm thickness. Samples were incubated at 37 or 25°C

188   overnight at 100% relative humidity (RH). Thereafter, the top film was lifted, and the virus

189   droplet-exposed sides were recovered and 10-fold diluted with PBS. Lastly, the

190   corresponding cell culture assays were performed to determine whether the silver films

191   were effective in inactivating the tested viruses. A control film (without silver) was used as

192   the negative control material.

193   Virucidal activity was calculated by comparing the number of infectious viruses on control

194   films (without silver) and on the silver films. Each experimental condition was performed

195   in triplicate.

196

197   *2.7. Determination of silver content*

198   The quantification of total silver content in the developed films was carried out by

199   inductively coupled plasma- optical emission spectroscopy (ICP-OES, Perkin-Elmer, USA)

200   using silver standard solution (traceable to SRM from NIST, $AgNO_3$ in HNO_3 2-3 % 1000

201   mg/L Ag Certipur®, Merck, Germany) for calibration. To this end, 100 mg of sample were

202   subjected to acid digestion with 2 mL of HNO_3 (69% for trace metal analysis, Panreac,

203   Spain) at 80°C for 16 h. The resultant digestant was diluted to a final volume of 5 mL and

204   analyzed. All measurements were done, at least, in triplicate.

205

206   *2.8. Statistical analysis*

207   The significance of differences among the mean numbers of viruses determined after the

208   control and AgNP films to assess the antiviral effect was determined by Student's t test with

209   a significance level of $p < 0.05$. The post-hoc Tukey's method ($p < 0.05$) was used for

9

210 pairwise comparison and to determine differences among silver nitrate and silver

211 nanoparticles treatments on viruses (XLSTAT, Addinsoft SARL).

212

213 **3. Results and discussion**

214 *3.1. The effect of silver nitrate and silver nanoparticles on MNV and FCV*

215 As shown in Fig.1 and 2, in all tested aging times, the exposition of norovirus surrogates,

216 MNV and FCV, to silver ions or silver nanoparticles, produced a clear reduction in the

217 virus titers. The results indicated that the antiviral activity of silver, in any of its forms, is

218 dose-dependent, where increasing concentrations of silver showed increased reduction in

219 viral titers.

220 In the case of MNV, the silver nitrate suspension produced a higher reduction of MNV

221 infectivity during the first 75 days of aging. However, the antiviral activity was

222 significantly reduced after 150 days of storage probably due to the physical and chemical

223 instability of silver ions (i.e. reduction and aggregation) as it has been previously reported

224 (Castro-Mayorga et al., 2014). Silver ions can be reduced to elemental silver or silver

225 nanoparticles by weak reducing treatments, such as many solvents, UV-light, thermal

226 treatment, ligands, etc. Since water was used as a solvent, external agents such as UV-light

227 could compromise the stability of silver ions and the formation of elemental silver and

228 silver nanoparticles in uncontrolled way forming particles with different forms and size

229 which are not stabilized and can easily coalesce. In fact, one of the main problems which

230 could compromise the final properties of an antimicrobial/antiviral packaging material is

231 the stability of silver ions and the chemical environment where the material has to exert its

232 effect and even the conditions to which the material will be exposed (Martínez-Abad,

233 Lagarón, & Ocio, 2014).

234   In contrast, the antiviral activity of silver nanoparticles at concentrations higher than 2.1

235   ppm increased or remained constant during all the time evaluated (150 days) (Fig.1). This

236   effect can be ascribed to the nanosize diameter of AgNP ($\sim 7 \pm 3$ nm previously reported by

237   Castro-Mayorga et al. (2014)) and the enhanced surface reactivity, making them able to

238   affect more effectively the capsid of the viruses. Indeed, a synergic effect between silver

239   ions release from the AgNP and AgNP themselves might enhance and extend the virucidal

240   activity. The initial increase in the antiviral activity of the AgNP could be attributed to both

241   the action of residual silver ions and the excess of reducing agent which could produce

242   some more nanoparticles in the first days of the storage, increasing the virucidal efficacy. It

243   is worth mentioning that the *in situ* synthesis of AgNP implied their stabilization in a

244   biopolymer matrix (PHBV18) which could also enhanced their virucidal activity preventing

245   the aggregation of AgNP, as had been demonstrated by Castro-Mayorga et al. (2014) for

246   enhancing the antimicrobial activity. As a result, the AgNP suspensions exhibited a high

247   and prolonged (even after 150 days) virucidal activity against MNV.

248   On the other hand, the FCV appeared more susceptible to the action of silver nitrate and the

249   reduction in viral titers was higher than for their counterparts obtained for MNV (Fig.2).

250   This appreciation leads to infer that the virucide effect of silver might depend to the

251   differences in capsid structure and capsid composition of the treated virus. Thus, for FCV,

252   the silver nitrate suspension had a highest reduction of its infectivity. This fact could be a

253   consequence of a combined effect between the high activity of soluble silver ions and the

254   higher susceptibility of FCV at Ag^0 particles produced by uncontrolled reduction (having

255   bigger size). Both, silver ions and the Ag^0 formed in the suspension could be able to disrupt

11

256   the FCV capsid more easily than in the case of the MNV. AgNP suspensions followed a

257   slight different pattern in FCV than in its counterparts prepared with MNV (Fig. 2).

258   To sum up, the results revealed that silver nitrate and AgNP were effective in reducing the

259   titers of FCV and MNV. The differences found between the virucide activity of the two

260   different silver forms and the two different viruses evaluated bring to light that might exist

261   different mechanisms of action depending on the virus structure and composition (Galdiero

262   et al., 2011). In this respect, the efficacy of a micrometer-sized magnetic hybrid colloid

263   (MHC) decorated with AgNP has recently been assessed on MNV. Park, et al. (2014)

264   reported that a suspension of AgNP with a size of 30 nm and a concentration of 400 ppm

265   (Ag30-MHCs) had the highest antiviral activity, reporting about 6 $\log_{10}$ reduction of MNV

266   after exposure at 25°C for 6 h while Ag7-MHCs (corresponding to 57.5 mg/L and 7 nm)

267   did not reduce the MNV infectivity. More recently, Bekele and collaborators (Bekele et al.,

268   2016) have reported the effect of the size (10, 75 and 110 nm) and dose (25, 50 and 100

269   mg/L) of AgNP on FCV, showing that only the smallest AgNP (10 nm) were effective in

270   reducing the FCV titers. Therefore, comparing these results with those obtained in the

271   present study (where the highest antiviral effect was achieved with AgNP of 7±3 nm at 21

272   mg/L, it could be stated that the virucidal activity of AgNP is strongly dependent on their

273   stabilization degree, size and concentration.

274   *3.2. Fibers and films morphology*

275   The morphology of the PHBV18 and PHBV18/AgNP fibers obtained from electrospinning

276   was studied by SEM and representative micrographs are shown in Fig.3a and 3b,

277   respectively. As it can be observed, smooth and continuous fibers without beads were

278   attained in both cases. The electrospun fibers presented a diameter of 0.92±0.36 and

279   1.1±0.40 μm for PHBV18 and PHBV18/AgNP respectively. Interestingly, the addition of

280 AgNP did not result in a significant change in fiber diameter as it can be deduced from the

281 SEM image and size distribution (Fig.3c). However it has been previously reported that the

282 addition of salts usually increases the charge density in the ejected jets and, thus, stronger

283 elongation forces are imposed due to the self-repulsion of the excess charges under the

284 electrical field, resulting in electrospun fibers having straighter shape and smaller diameter

285 (Jeon et al., 2008; Martínez-Abad et al., 2012). In the present work, the low silver loading,

286 the appropriate stabilization of AgNP into the polymer matrix and the electrospinning

287 solution minimize the reduction of residual silver ion and the aggregation of AgNP or any

288 significant impact on the fiber diameter.

289 The surface and cross-section of the coated systems prepared with PHBV3 and

290 PHBV18/AgNP was also analyzed by SEM. The coated system presented a uniform and

291 smooth surface (Fig.4a) formed by the continuous layer of annealed active fibers whose

292 thickness was not easily discerned, but it was measured to have a thickness of about 60μm

293 (Fig.4b). The morphology of the coating layer suggests that a partial melting and

294 contraction of fibers could take place during the annealing step, favoring the adhesion

295 between the two layers. Furthermore, the presence of silver was confirmed by EDAX

296 analysis (Fig.4c) and the AgNP distribution assessed by mapping from the SEM images.

297 The elemental mapping image of the Fig.4d shows matched spatial distribution of silver,

298 indicating that the AgNP are homogeneously distributed into the coating and onto the

299 PHBV3/PHBV18 layer.

300 Fig.5 shows the overall appearance images of the neat PHBV3 film and the coated systems

301 containing or not AgNP. The first clear observation is that coated systems prepared without

302 AgNP showed a darker yellowish coloration as compared to the neat PHBV3. This effect

303 could be ascribed to the presence of some impurities in the PHBV18 due to the

304 fermentation process, which resulted in Maillard reactions during the thermal treatment

305 (Castro-Mayorga, Fabra & Lagaron, 2016). In contrast, when the AgNP were added to the

306 coating, the yellowish coloration disappeared and it turned light grey, thus indicating that

307 both the thermal stability of the polymer matrix and the dispersion of nanoparticles were

308 enhanced by means of this procedure.

309

310 *3.3. Antiviral effects of AgNP films*

311 Taking into account the good performance of AgNP obtained in the first part of this work,

312 PHBV3/PHBV18/AgNP coated systems were fabricated as described above and their

313 antiviral activity was evaluated. The AgNP-films were inoculated with norovirus surrogates

314 adapting the ISO 22196:2011 and incubated at 25°C and 100% RH. Table 1 shows that

315 FCV and MNV titers decreased by 1.42 and 0.14 log $TCID_{50}$/mL respectively. However,

316 the results were not found statistically significant (p>0.05). The effectiveness of AgNP-

317 films was also evaluated at 37°C and 100% RH. After 24 h exposure, no infectious FCV

318 were recovered when in contact with the AgNP films while MNV titers decreased by 0.86

319 log $TCID_{50}$/mL (Table 1). As for other natural compounds AgNP-films exerted the

320 strongest antiviral effect at 37°C (Sánchez & Aznar, 2015).

321 In a similar work, an active renewable packaging material with virucide properties was

322 synthesized by the incorporation of silver ions into PLA films by solvent casting technique.

323 These films also showed antiviral activity on FCV. When FCV was exposed to PLA-silver

324 films for 24 h at 25°C, FCV titers decreased by 2 log $TCID_{50}$/mL when treated with PLA

325 films at concentrations of 1 g/kg of silver, while in films containing 10 g/kg of silver, FCV

326 infectivity was completely eliminated (Martínez-Abad et al., 2013). Likewise, Silvestry-

327 Rodriguez et al. (2007) evaluated the antiviral activity of active packaging, reporting that

14

328   FCV titers were reduced by 5 log when in contact with plastic coupons impregnated with

329   100 g/kg silver–copper zeolites. In the present study, PHBV3/PHBV18/AgNP films

330   containing a total silver concentration of 270 ± 10 mg/kg (as it was quantified by ICP-OES)

331   demonstrated to have a higher antiviral activity against FCV than the above-mentioned

332   publications. The highest antiviral activity observed for FCV as compared to MNV, could

333   be due to the release of silver ions from the immobilized AgNP resulting in a final

334   increased antiviral effect. Even if this assumption is in line with the higher sensitivity of

335   FCV than MNV to silver ions as reported in the suspension antiviral assay additional

336   research on the migration of silver ions or silver nanoparticles are required to confirm this

337   hypothesis.

338

339   **Conclusions**

340   The effect of silver nitrate and silver nanoparticles on norovirus surrogates was

341   investigated. It was found that both chemical forms (i.e. metallic and ionic silver)

342   significantly decreased the MNV and FCV infectivity in a dose-dependent manner.

343   Meanwhile, its effect depends on other factors, such as the aging time, the type of virus and

344   the stabilization degree.

345   Furthermore, biopolymeric materials consisting of a matrix of poly (3-hydroxybutyrate-co-

346   3-hydroxyvalerate) and AgNP-based coating obtained by means of electrospinning were

347   also developed. Interestingly, the addition of very low loadings of stabilized AgNP into the

348   electrospun coating provided a virucidal activity against norovirus surrogates and did not

349   significantly modify the optical properties of films. The technology here proposed allows

350   the design of custom made active adapted to the final intended use of packaging and

351   contact surface industries.

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