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

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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 (AgNO₃) was evaluated for inactivating norovirus surrogates, the feline calicivirus (FCV) and the murine norovirus (MNV). These norovirus surrogates were exposed to AgNO₃ 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.

**Keywords:** Noroviruses, Silver nanoparticles, Active packaging, Polyhydroxyalkanoates, Electrospinning.
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,
2016a) have demonstrated that poly (3-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 et al., 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 et al., 2014). 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 & Nandedkare, 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 et al. 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 et al., 1994; Silvestry-Rodriguez et al., 2007; Galdiero et al., 2011; Khandelwal et al., 2014; De Gusseme et al., 2010; Bekele et al., 2016). However, it is known that silver ions are easily inactivated by many different physical or chemical factors (Ilg & Kreyenschmidt, 2011; Castro-Mayorga et al, 2016b). 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 et al., 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 first part of this work, norovirus surrogates were exposed to different concentrations of silver nitrate and the virucidal activity was assessed using cell culture. 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 AgNO₃ solution at 169.9 mg/L was added dropwise to generate in situ stabilized silver nanoparticles. The obtained PHBV18/AgNP suspension was centrifuged at 17387×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 AgNO₃ 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. Feline calicivirus (F9 strain, ATCC VR-782) was cultured in CRFK cells (ATCC CCL-94). Semipurified 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 (TCID₅₀) 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/N_t), where N_0 is the number of infections viruses on the suspension without silver and N_t 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-Trifluoroethanol (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 to the cathode of the power supply and oriented perpendicular to the syringe.
The distance between the needle and the plate was 1.2 cm and the voltage was maintained in the range 10-12 kV. All experiments were carried out at room temperature under a steady flow-rate of 7 mL/h. After electrospinning, the fiber mats were dried at 40°C under vacuum for 24 h to completely remove the solvent.

Finally, the coated structure was assembled placing 250-300 g/kg of fiber mat of about 100 µm of thickness onto PHBV3 films. The resulting coated system was thermally post-processed in a hot press (Carver 4122, USA) at 150°C during 2 min (without pressing) to form a continuous film by fiber coalescence.

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

2.5. Scanning Electron Microscopy (SEM)

The morphology of the PHBV18/AgNP electrospun fibers and bilayer films was analyzed using SEM. The SEM was conducted on a Hitachi microscope (Hitachi S–4800) at an accelerating voltage of 5 kV and a working distance of 8-10 mm before the examination, the films were cryo-fractured using liquid N₂ and sputtered with Au/Pd under vacuum. The microanalysis and elemental mapping were conducted by Energy Dispersive Analysis of X-rays (EDAX) from SEM images of carbon coated samples. Fibers thicknesses were measured by means of the of the Adobe Photoshop CS4 software from 300 fibers at random from SEM images.

2.6. Determination of virucidal activity of silver based films

To test the virucidal activity of silver based films, a modification of the ISO 22196:2011 (Measurement of antibacterial activity on plastics and other non-porous surfaces) was used. Briefly, a suspension of viruses diluted in PBS buffer (4-6 log TCID₅₀/mL) was placed onto...
the test films of 3×3 cm and covered by an inert piece of Low-Density Polyethylene (LDPE) of 2.5×2.5 cm and 10 µm thickness. Samples were incubated at 37 or 25°C overnight at 100% relative humidity (RH). Thereafter, the top film was lifted, and the virus droplet-exposed sides were recovered and 10-fold diluted with PBS. Lastly, the corresponding cell culture assays were performed to determine whether the silver films were effective in inactivating the tested viruses. A control film (without silver) was used as the negative control material.

Virucidal activity was calculated by comparing the number of infectious viruses on control films (without silver) and on the silver films. Each experimental condition was performed in triplicate.

2.7. Determination of silver content

The quantification of total silver content in the developed films was carried out by inductively coupled plasma- optical emission spectroscopy (ICP-OES, Perkin-Elmer, USA) using silver standard solution (traceable to SRM from NIST, AgNO₃ in HNO₃ 2-3 % 1000 mg/L Ag Certipur®, Merck, Germany) for calibration. To this end, 100 mg of sample were subjected to acid digestion with 2 mL of HNO₃ (69% for trace metal analysis, Panreac, Spain) at 80°C for 16 h. The resultant digestant was diluted to a final volume of 5 mL and analyzed. All measurements were done, at least, in triplicate.

2.8. Statistical analysis

The significance of differences among the mean numbers of viruses determined after the control and AgNP films to assess the antiviral effect was determined by Student's t test with a significance level of p<0.05. The post-hoc Tukey's method (p<0.05) was used for
pairwise comparison and to determine differences among silver nitrate and silver 
nanoparticles treatments on viruses (XLSTAT, Addinsoft SARL).

3. Results and discussion

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

As shown in Fig.1 and 2, in all tested aging times, the exposition of norovirus surrogates, 
MNV and FCV, to silver ions or silver nanoparticles, produced a clear reduction in the 
virus titers. The results indicated that the antiviral activity of silver, in any of its forms, is 
dose-dependent, where increasing concentrations of silver showed increased reduction in 
viral titers.

In the case of MNV, the silver nitrate suspension produced a higher reduction of MNV 
infectivity during the first 75 days of aging. However, the antiviral activity was 
significantly reduced after 150 days of storage probably due to the physical and chemical 
instability of silver ions (i.e. reduction and aggregation) as it has been previously reported 
(Castro-Mayorga et al., 2014). Silver ions can be reduced to elemental silver or silver 
nanoparticles by weak reducing treatments, such as many solvents, UV-light, thermal 
treatment, ligands, etc. Since water was used as a solvent, external agents such as UV-light 
could compromise the stability of silver ions and the formation of elemental silver and 
silver nanoparticles in uncontrolled way forming particles with different forms and size 
which are not stabilized and can easily coalesce. In fact, one of the main problems which 
could compromise the final properties of an antimicrobial/antiviral packaging material is 
the stability of silver ions and the chemical environment where the material has to exert its 
effect and even the conditions to which the material will be exposed (Martínez-Abad, 
Lagarón, & Ocio, 2014).
In contrast, the antiviral activity of silver nanoparticles at concentrations higher than 2.1 ppm increased or remained constant during all the time evaluated (150 days) (Fig.1). This effect can be ascribed to the nanosize diameter of AgNP (∼7±3 nm previously reported by Castro-Mayorga et al. (2014)) and the enhanced surface reactivity, making them able to affect more effectively the capsid of the viruses. Indeed, a synergic effect between silver ions release from the AgNP and AgNP themselves might enhance and extend the virucidal activity. The initial increase in the antiviral activity of the AgNP could be attributed to both the action of residual silver ions and the excess of reducing agent which could produce some more nanoparticles in the first days of the storage, increasing the virucidal efficacy. It is worth mentioning that the in situ synthesis of AgNP implied their stabilization in a biopolymer matrix (PHBV18) which could also enhanced their virucidal activity preventing the aggregation of AgNP, as had been demonstrated by Castro-Mayorga et al. (2014) for enhancing the antimicrobial activity. As a result, the AgNP suspensions exhibited a high and prolonged (even after 150 days) virucidal activity against MNV.

On the other hand, the FCV appeared more susceptible to the action of silver nitrate and the reduction in viral titers was higher than for their counterparts obtained for MNV (Fig.2). This appreciation leads to infer that the virucide effect of silver might depend to the differences in capsid structure and capsid composition of the treated virus. Thus, for FCV, the silver nitrate suspension had a highest reduction of its infectivity. This fact could be a consequence of a combined effect between the high activity of soluble silver ions and the higher susceptibility of FCV at Ag\(^0\) particles produced by uncontrolled reduction (having bigger size). Both, silver ions and the Ag\(^0\) formed in the suspension could be able to disrupt
the FCV capsid more easily than in the case of the MNV. AgNP suspensions followed a slight different pattern in FCV than in its counterparts prepared with MNV (Fig. 2).

To sum up, the results revealed that silver nitrate and AgNP were effective in reducing the titers of FCV and MNV. The differences found between the virucide activity of the two different silver forms and the two different viruses evaluated bring to light that might exist different mechanisms of action depending on the virus structure and composition (Galdiero et al., 2011). In this respect, the efficacy of a micrometer-sized magnetic hybrid colloid (MHC) decorated with AgNP has recently been assessed on MNV. Park, et al. (2014) reported that a suspension of AgNP with a size of 30 nm and a concentration of 400 ppm (Ag30-MHCs) had the highest antiviral activity, reporting about $6 \log_{10}$ reduction of MNV after exposure at 25°C for 6 h while Ag7-MHCs (corresponding to 57.5 mg/L and 7 nm) did not reduce the MNV infectivity. More recently, Bekele and collaborators (Bekele et al., 2016) have reported the effect of the size (10, 75 and 110 nm) and dose (25, 50 and 100 mg/L) of AgNP on FCV, showing that only the smallest AgNP (10 nm) were effective in reducing the FCV titers. Therefore, comparing these results with those obtained in the present study (where the highest antiviral effect was achieved with AgNP of 7±3 nm at 21 mg/L, it could be stated that the virucidal activity of AgNP is strongly dependent on their stabilization degree, size and concentration.

3.2. Fibers and films morphology

The morphology of the PHBV18 and PHBV18/AgNP fibers obtained from electrospinning was studied by SEM and representative micrographs are shown in Fig.3a and 3b, respectively. As it can be observed, smooth and continuous fibers without beads were attained in both cases. The electrospun fibers presented a diameter of 0.92±0.36 and 1.1±0.40 μm for PHBV18 and PHBV18/AgNP respectively. Interestingly, the addition of
AgNP did not result in a significant change in fiber diameter as it can be deduced from the SEM image and size distribution (Fig. 3c). However, it has been previously reported that the addition of salts usually increases the charge density in the ejected jets and, thus, stronger elongation forces are imposed due to the self-repulsion of the excess charges under the electrical field, resulting in electrospun fibers having straighter shape and smaller diameter (Jeon et al., 2008; Martínez-Abad et al., 2012). In the present work, the low silver loading, the appropriate stabilization of AgNP into the polymer matrix and the electrospinning solution minimize the reduction of residual silver ion and the aggregation of AgNP or any significant impact on the fiber diameter.

The surface and cross-section of the coated systems prepared with PHBV3 and PHBV18/AgNP was also analyzed by SEM. The coated system presented a uniform and smooth surface (Fig. 4a) formed by the continuous layer of annealed active fibers whose thickness was not easily discerned, but it was measured to have a thickness of about 60 µm (Fig. 4b). The morphology of the coating layer suggests that a partial melting and contraction of fibers could take place during the annealing step, favoring the adhesion between the two layers. Furthermore, the presence of silver was confirmed by EDAX analysis (Fig. 4c) and the AgNP distribution assessed by mapping from the SEM images. The elemental mapping image of the Fig. 4d shows matched spatial distribution of silver, indicating that the AgNP are homogeneously distributed into the coating and onto the PHBV3/PHBV18 layer.

Fig. 5 shows the overall appearance images of the neat PHBV3 film and the coated systems containing or not AgNP. The first clear observation is that coated systems prepared without AgNP showed a darker yellowish coloration as compared to the neat PHBV3. This effect could be ascribed to the presence of some impurities in the PHBV18 due to the
fermentation process, which resulted in Maillard reactions during the thermal treatment (Castro-Mayorga, Fabra & Lagaron, 2016). In contrast, when the AgNP were added to the coating, the yellowish coloration disappeared and it turned light grey, thus indicating that both the thermal stability of the polymer matrix and the dispersion of nanoparticles were enhanced by means of this procedure.

3.3. Antiviral effects of AgNP films

Taking into account the good performance of AgNP obtained in the first part of this work, PHBV3/PHBV18/AgNP coated systems were fabricated as described above and their antiviral activity was evaluated. The AgNP-films were inoculated with norovirus surrogates adapting the ISO 22196:2011 and incubated at 25°C and 100% RH. Table 1 shows that FCV and MNV titers decreased by 1.42 and 0.14 log TCID<sub>50</sub>/mL respectively. However, the results were not found statistically significant (p>0.05). The effectiveness of AgNP-films was also evaluated at 37°C and 100% RH. After 24 h exposure, no infectious FCV were recovered when in contact with the AgNP films while MNV titers decreased by 0.86 log TCID<sub>50</sub>/mL (Table 1). As for other natural compounds AgNP-films exerted the strongest antiviral effect at 37°C (Sánchez & Aznar, 2015).

In a similar work, an active renewable packaging material with virucide properties was synthesized by the incorporation of silver ions into PLA films by solvent casting technique. These films also showed antiviral activity on FCV. When FCV was exposed to PLA-silver films for 24 h at 25°C, FCV titers decreased by 2 log TCID<sub>50</sub>/mL when treated with PLA films at concentrations of 1 g/kg of silver, while in films containing 10 g/kg of silver, FCV infectivity was completely eliminated (Martínez-Abad et al., 2013). Likewise, Silvestry-Rodriguez et al. (2007) evaluated the antiviral activity of active packaging, reporting that...
FCV titers were reduced by 5 log when in contact with plastic coupons impregnated with 100 g/kg silver–copper zeolites. In the present study, PHBV3/PHBV18/AgNP films containing a total silver concentration of 270 ± 10 mg/kg (as it was quantified by ICP-OES) demonstrated to have a higher antiviral activity against FCV than the above-mentioned publications. The highest antiviral activity observed for FCV as compared to MNV, could be due to the release of silver ions from the immobilized AgNP resulting in a final increased antiviral effect. Even if this assumption is in line with the higher sensitivity of FCV than MNV to silver ions as reported in the suspension antiviral assay additional research on the migration of silver ions or silver nanoparticles are required to confirm this hypothesis.

Conclusions

The effect of silver nitrate and silver nanoparticles on norovirus surrogates was investigated. It was found that both chemical forms (i.e. metallic and ionic silver) significantly decreased the MNV and FCV infectivity in a dose-dependent manner. Meanwhile, its effect depends on other factors, such as the aging time, the type of virus and the stabilization degree. Furthermore, biopolymeric materials consisting of a matrix of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and AgNP-based coating obtained by means of electrospinning were also developed. Interestingly, the addition of very low loadings of stabilized AgNP into the electrospun coating provided a virucidal activity against norovirus surrogates and did not significantly modify the optical properties of films. The technology here proposed allows the design of custom made active adapted to the final intended use of packaging and contact surface industries.
Acknowledgments

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Table 1. Antiviral effect of AgNP films on norovirus surrogates (MNV and FCV) after 24 h contact following the ISO 22196:2011 at different temperatures

<table>
<thead>
<tr>
<th>Virus</th>
<th>Type of films</th>
<th>Temperature</th>
<th>Recovered titer (log₁₀ TCID₅₀/mL)</th>
<th>Reduction</th>
<th>Recovered titer (log₁₀ TCID₅₀/mL)</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNV</td>
<td>Control</td>
<td>25 °C</td>
<td>6.22 ± 0.15</td>
<td></td>
<td>4.45 ±0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AgNP films</td>
<td>25 °C</td>
<td>6.08 ± 0.17</td>
<td>0.14</td>
<td>3.59 ±0.08</td>
<td>0.86</td>
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<tr>
<td></td>
<td></td>
<td>37 °C</td>
<td>0.1725</td>
<td></td>
<td>0.0023</td>
<td></td>
</tr>
<tr>
<td>FCV</td>
<td>Control</td>
<td>25 °C</td>
<td>7.12 ± 0.19</td>
<td></td>
<td>3.41 ± 0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AgNP films</td>
<td>25 °C</td>
<td>5.70 ± 1.59</td>
<td>1.42</td>
<td>&lt;1.15</td>
<td>&gt;2.26</td>
</tr>
<tr>
<td></td>
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<td>37 °C</td>
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</tr>
</tbody>
</table>

*a p<0.05 means that the differences between control and AgNP films are statistically significant
FIGURE CAPTIONS

**Figure 1.** Effect of silver nitrate (A) and silver nanoparticles (B) on murine norovirus (MNV) over the storage time. Different letters denote significant differences between treatments (p<0.05). *One negative samples out of three. **Two negative samples out of three. Lines indicate the detection limit for the TCID$_{50}$ assay.

**Figure 2.** Effect of silver nitrate (A) and silver nanoparticles (B) on feline calicivirus (FCV) over the storage time. Different letters denote significant differences between treatments (p<0.05). *One negative samples out of three. **Two negative samples out of three. Lines indicate the detection limit for the TCID$_{50}$ assay.

**Figure 3.** SEM images of electrospun fibers, A) without AgNP (PHBV18), B) with AgNP (PHVB18/AgNP), and C) size distribution of fibers.

**Figure 4.** SEM micrographs and EDAX analysis of PHBV3/PHBV18/AgNP films. A) film surface, B) film cross-section, C) EDAX spectrum confirming the silver presence, D) Elemental mapping of coated system by EDAX from SEM image (silver in red).

**Figure 5.** Contact transparency pictures of PHBV3, PHBV3/PHBV18 and PHBV3/PHBV18/AgNPs.
• Antiviral activity of AgNPs and AgNO₃ was evaluated on MNV and FCV

• Both significantly decrease FCV and MNV infectivity in a dose-dependent manner

• A PHBV18/AgNPs electrospun coating was produced

• The AgNP electrospun coating completely inactivated FCV based on ISO 22196:2011