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Versatile biobased biocidal nanomaterial for a safer-by-design coated food packaging

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Abstract

The preservation of food products using packaging with antimicrobial properties is a major challenge. Using a simple dipping procedure, we propose to functionalize the surface of commercial polypropylene (PP) films with various hybrid components based on biopolymers with silver nanoparticles (AgNPs) that are known for their bactericidal properties. The chosen biopolymers are polysaccharides in solid form such as cellulose nanocrystals (CNC) and chitin nanocrystals (ChiNC), as well as hydrosoluble molecules such as alginate and chitosan for which some OH groups serve as nucleation points for AgNPs. The successful coating was demonstrated via the presence of biopolymers on the PP film surface using electron and fluorescence microscopies. The effective presence of AgNPs on the PP film was then confirmed by atomic absorption spectroscopy measurements. Migration tests performed over 10 days made it possible to track the kinetic release of Ag^+ in a food simulant. A persistent and increasing release over time was observed, especially for hybrids in solid form (i.e., CNC/AgNP, ChiNC/AgNP), bearing the smaller AgNPs (20 nm). The maximum value of Ag + released was ~0.30 mg Ag/kg, which is significantly lower than the value authorized by international organizations.

KEYWORDS

biopolymers and renewable polymers, hydrophilic polymers, packaging, polysaccharides

1 | INTRODUCTION

Food contact materials are essential to preserve product quality without microbiological hazards to human health and to reduce food waste.[1] In this context, active packaging with antimicrobial properties represents a good solution to extend the shelf life of food without any detriment to product properties caused by bacteria and microbial activity.[1,2] For this purpose, metallic nanoparticles (NPs) have recently been introduced into food packaging solutions[3] because of their resistance to processing conditions (e.g., high temperatures[4]). Silver nanoparticles (AgNPs) are known for their antimicrobial properties due to the release of Ag^+ ions that are able to induce structural and morphological changes in bacteria[5–7] as a result of their interaction with the components of the cell membrane (e.g., thiols[5]), inducing their inactivation.[6] Several studies[2,3,8] have shown that Ag in the packaging acts as a broad-spectrum antimicrobial agent, avoiding the formation of a biofilm at the food-contact surface, indicating that even a small amount of silver makes it possible to achieve a good biocide effect. The biocidal activity of

AgNPs (i.e., Ag^+ release) strongly depends on the morphological characteristics of the AgNPs (i.e., size and shape[9]), with small particles providing the strongest biocidal properties due to a higher specific surface.[6,10,11] Obtaining well-stabilized and well-dispersed AgNPs is crucial to achieving good Ag^+ release and to enhancing the biocidal properties of an active package.

Among the methods for AgNP synthesis, the chemical reduction of Ag^+ ions represents one of the most efficient methods, using a reducing agent such as sodium borohydride[12] or hydrazine.[13] Several polysaccharides and biopolymers have been proposed as excellent stabilizers for AgNPs. These include chitosan,[14,15] alginate,[16] cellulose nanocrystals (CNCs),[17,18] and chitin nanocrystals (ChiNCs),[19] thus forming hybrid NPs. These bio-based polymers are used as carriers of AgNPs since they make it possible to obtain well-stabilized AgNPs that maximize the AgNP specific surface and thus minimize the amount of AgNPs in the system without being detrimental to the biocide effect. Furthermore, the biodegradability of the bio-based substrate at the end-of-life of the material provides an expected eco-friendly biocidal material that combines an extension of the service life of the packaged product and, consequently, a reduction in losses and waste, in addition to a reduced environmental impact in the long term. The resulting material is then highly dispersible in water, leading to transparent, and low viscosity suspensions. This safer-by-design approach in highly controlled conditions is then particularly valuable for environment, non-visible once applicated and easy to use.

To produce an active packaging with biocidal properties, metallic or hybrid NPs can either be incorporated into a synthetic plastic film (polyethylene (PE) or polypropylene (PP))[20] or coated on to the surface of the packaging material,[21] allowing the inhibition of bacterial colony growth. These two strategies obviously differ in the release kinetics of the active agent and can therefore be distinctly adapted to the preservation of different foods. Like any material intended to come into contact with food, active packaging must meet the requirements and inertness criteria defined by the EU/1935/2004 framework regulation. For plastic-based materials, their suitability for food contact must be assessed by migration tests, the standard conditions of which are described in the EU/2016/1416 regulations. It is important to point out that silver is not included among the metallic substances subject to a specific migration limit listed in Annex II. Despite this lack of restriction, the migration of silver in a nano or ionic form has been the subject of numerous investigations, and the release of silver in nanoscale form is not guaranteed even though some studies have clearly established the effective migration of silver in nanoparticulate form.[22,23]

In this work, we first synthetized and characterized hybrid aqueous suspensions formulated with various biopolymers (i.e., CNC, ChiNC, alginate, chitosan) used as substrates to stabilize AgNPs. We then proposed a simple dipping procedure to coat a commercial PP film used for food packaging applications, making it an active packaging material. We tested the migration properties describing the release kinetics of Ag^+ from AgNPs in a food simulant (i.e., 3% acetic acid), evaluating the impact of the biopolymer used.

2 | EXPERIMENTAL SECTION

2.1 | Chemicals

Cellulose nanocrystals (CNCs) were purchased from CelluForce (product num. 2015–009). They were obtained from bleached kraft pulp by acid hydrolysis and then neutralized to sodium form and spray-dried (length = 183 ± 88 nm; cross-section = 6 ± 2 nm; aspect ratio = 31 and 0.6 e/nm^2).[24] Chitin nanocrystals (ChiNCs) were extracted from commercial shrimp shell powder (Sigma Aldrich) by acid hydrolysis according to the experimental protocol proposed by Perrin et al.[25] Briefly, 4 g of shrimp shell powder was dispersed in 80 mL of boiling 3 N HCl solution and stirred for 90 min. The resulting suspension was then neutralized and purified by filtration (1.2 µm pore size cellulose nitrate membranes from Millipore), and finally dialyzed against ultrapure water for several days. The final suspension (i.e., 9 g/L) was dispersed in ultra-purified water at pH 5.5. The ChiNCs had an average length = 150 ± 60 nm, a width of 17 ± 7 nm, and a surface charge of 0.48–1.08 e/nm^2.[26] Low viscosity sodium alginate powder was purchased from Kimica and low molecular weight chitosan was purchased from Sigma Aldrich (ref 448,869).

Silver nitrate ($AgNO_3 \geq 99\%$), sodium borohydride ($NaBH_4 \geq 96\%$), and acetic acid (HAc, 100%) were purchased from Sigma-Aldrich and used without further purification. All of the aqueous suspensions and solutions were prepared using ultra-pure water.

Commercial polypropylene (PP) film for food packaging applications was purchased from Cenpac (40 µm thickness, 150 mm × 500 m, product num. 16,172,056). Poly(allylamine hydrochloride) (PAH, *M*w = 120–200,000 g/mol, Sigma Aldrich) was used as a polycationic polymer and poly(sodium 4-styrenesulfonate) (PSS, *M*w ~ 70,000 g/mol, Sigma Aldrich) as a polyanion polymer. Both of these polymers were dispersed in a 1 M NaCl solution (4 g/L).

2.2 | Synthesis of biopolymer/AgNP hybrid suspensions

All of the four different biopolymers (CNCs, ChiNCs, sodium alginate, and chitosan) were dispersed or dissolved in ultra-pure water, except chitosan powder, which was dispersed in 1%v HAc aqueous solution, at a final concentration of 5 g/L. The biopolymer/AgNP hybrid suspensions were synthesized using the experimental method developed in another study by our group.[27] Briefly, a volume of 100 mL CNC and ChiNC suspension, or alginate and chitosan solution (5 g/L) were mixed with 6 mL of $AgNO_3$ aqueous solution (0.1 M) for 1 min at room temperature. A volume of 20 mL of freshly prepared $NaBH_4$ aqueous solution (0.1 M, 20 mL, $AgNO_3/NaBH_4$ molar ratio of 3) was then added to synthesize AgNPs by chemical reduction. The resulting sample was stirred for 24 h at room temperature and protected from light using aluminum foil in order to avoid AgNP oxidation. Finally, the samples were dialyzed against water for 24 h (dialysis bath volume to sample volume = 10:1). To summarize, four hybrids were prepared with the same nominal AgNP content (i.e., ~ 11 wt %): (i) CNC/AgNP; (ii) ChiNC/AgNP; (iii) alginate/AgNP; (iv) chitosan/AgNP in suspensions in water.

2.3 | Characterization of biopolymer/AgNP hybrid suspensions

The hybrid suspensions were characterized by different techniques. A Mettler-Toledo UV7 spectrophotometer equipped with a 10-mm quartz cell made it possible to record the light-visible absorbance of (1:10) diluted hybrid suspensions in the 300–900 nm range. Ultra-pure water was used as a blank reference.

Atomic absorption spectroscopy, AAS (ICE 3300 AAS, Thermo Fisher, USA) was used to determine the AgNP content in hybrid suspensions. A volume of 1 mL of sample was digested in 40 mL water/aqua regia mixture (i.e., 30% v aqua regia; HCl/HNO_3: 3/1), and then analyzed. A calibration curve was obtained using a silver standard solution (1000 µg/mL, Chem-Lab NV, Belgium) diluted in water/aqua regia mixture at different concentrations ranging from 0.25 to 10 ppm. Two independent measurements were repeated for each sample. The final AgNP content was expressed in mg of AgNP per g of sample (wt%).

Scanning transmission electron microscopy (STEM) images were acquired with a Quattro S field emission gun scanning microscope (Thermo Fisher Scientific, USA) at 10 kV using a STEM detector. The hybrid suspensions were previously diluted with ultra-pure water at 0.5 g/L in biopolymer content and a volume of 10 µL was deposited on glow-discharged carbon coated grids (200 meshes, Delta Microscopies, France) for 2 min and the excess was removed using Whatman filter paper. The grids were dried overnight in air and coated with a 0.5-nm platinum layer by an ion-sputter coater (LEICA AM ACE600, Germany).

The STEM images were finally analyzed by ImageJ software that made it possible to determine the average Feret diameter of the AgNPs (i.e., the largest distance between two tangents to the contour of the measured particle). A total of 100 AgNPs was considered for each sample.

The X-ray diffraction (XRD) diffractogram of each hybrid sample was recorded using a Bruker D8 Discover diffractometer (USA) with which a Cu-Kα1 radiation (Cu Kα1, 1.5405 Å) was produced in a sealed tube at 40 kV and a 40 mA, and parallelized using a Gobël mirror parallel optics system and collimated to produce a 500-mm beam diameter. The acquisition time was fixed at 10 min in the 3°–60° 2θ range.

2.4 | PP films functionalized with biopolymer/AgNP hybrids

The experimental protocol for the surface-functionalization of the PP films (5 cm × 5 cm) with hybrids was adapted from the dipping protocol proposed by Moreau et al.[28] First, the PP film was cleaned using compressed air to remove residual dust. The film was exposed to a plasma cleaner treatment under oxygen (Plasma Cleaner from Harrick Plasma, 20 min, USA) to increase its wettability. For the case of hybrids formulated with CNCs and alginate (i.e., mostly negatively-charged biopolymers), the film was then alternatively immersed in 100 mL of PAH solution and 100 mL of hybrid suspension for 10 min. After each immersion step, the PP film was rinsed in pure water and dried under a nitrogen stream and stocked at room temperature in the dark. For the alginate/AgNP hybrid, the pH was set at around 8. In the case of hybrid suspensions obtained working with ChiNCs and chitosan, which are mostly positively charged, the PP film surface had to be functionalized with an anionic polymer (PSS). After the PAH treatment, the PP film was immersed in 100 mL of PSS solution for 10 min and dried under a nitrogen flow before being immersed in the hybrid suspension. To adjust the amount of AgNP on the film surface, the hybrid suspension was diluted in CNC with a content of 0.25, 0.5, 1.5, or 3 g/L.

A schematic representation of the experimental protocol for the functionalization of the PP films using the

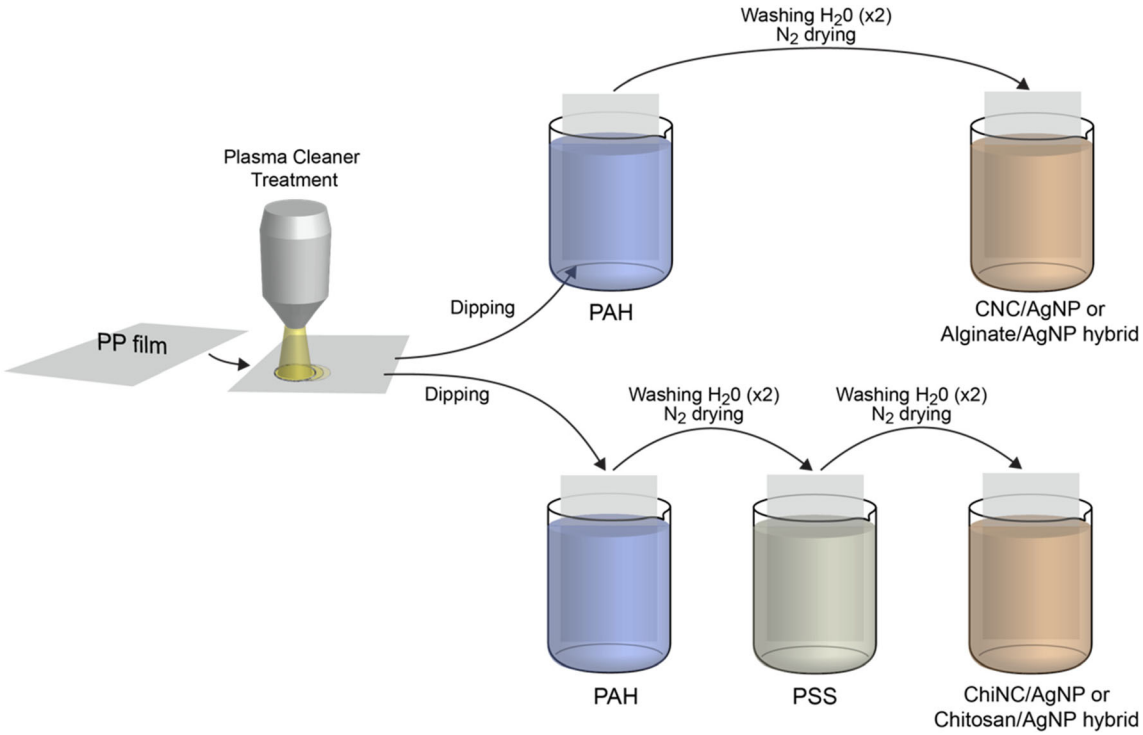

FIGURE 1 Scheme of experiment dipping protocol for polypropylene film coated with biopolymer/AgNP hybrids. AgNP, silver nanoparticle. [Color figure can be viewed at wileyonlinelibrary.com]

biopolymer/AgNP hybrid suspensions is reported in Figure 1.

2.5 | Characterization of PP films functionalized with biopolymer/AgNP hybrids

The transmittance (%) of the films was measured with a UV–vis spectrophotometer described in the previous section. For each sample, five measurements were performed at a 400-nm wavelength.

Scanning electron microscopy in transmission mode (STEM) images were recorded to observe the surface morphology of the functionalized PP films. Circular disks with a diameter of 5 cm were cut and then glued onto a sample holder using a conductive adhesive film. The samples were then sputter-coated with a 0.5-nm platinum layer (LEICA EM ACE600, Germany), and brightfield images were recorded using a field emission gun scanning electron microscope (Quattro S, Thermo Fischer Scientific, USA) operating at 5 kV with a scanning electron microscopy (SEM) detector.

The amount of AgNPs on each film was determined by AAS using the experimental protocol previously described. For each sample, a film of 5 × 5 cm was cut into small pieces and placed in a volume of 10 mL of aqua regia. After 24 h, the film pieces were removed and the residual solution was analyzed. The final AgNP content on the film is expressed in μg of Ag per cm^2, considering the functionalization of both sides of the film.

2.6 | Migration tests

Migration tests were performed in accordance with the recommendations contained in EU regulation N° 10/2011 on plastic materials and articles intended to come into contact with food. Considering the high solubility of silver in acidic media, the only food simulant used was 3% acetic acid (3%v HAc solution), which represents the worst test conditions in terms of quantity of ions released. Ag^+ ion release kinetics from AgNPs in 3% v HAc solution was followed under standardized testing conditions (for 10 days at 20°C). In this case, a functionalized PP film of 5 × 10 cm was cut into small pieces and placed in 10 mL of HAc solution. At each measurement time (i.e., 1, 6, 72, 240 h), the film was removed and the total solution containing the released ions was analyzed by AAS. A calibration curve was obtained using a silver standard solution (1000 μg/mL, Chem-Lab NV, Belgium) diluted in 3%v HAc solution at different concentrations, from 0.25 to 1.5 ppm. Two independent measurements were repeated for each sample. The final AgNP content on the film is expressed in μg of Ag per cm^2.

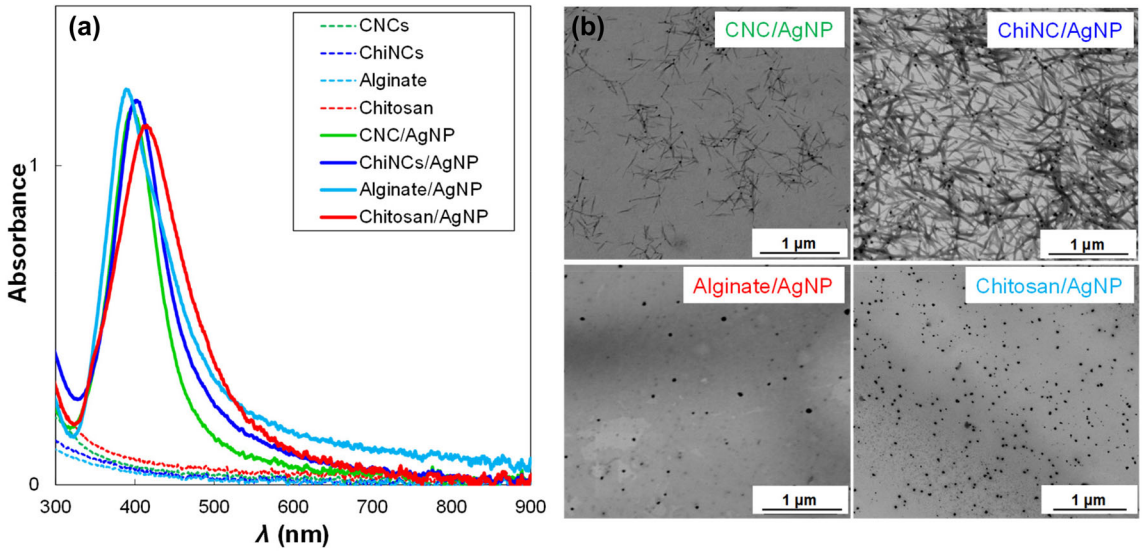

FIGURE 2 (a) UV–vis spectra; and (b) STEM images of CNC/AgNP (silver nanoparticle), ChiNC/AgNP, alginate/AgNP, chitosan/AgNP hybrid suspensions. CNC, cellulose nanocrystals; ChiNC, chitin nanocrystals. [Color figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS AND DISCUSSION

3.1 | Biopolymer/AgNP hybrids

The CNC/AgNP hybrid suspension obtained by the addition of the Ag precursor ($AgNO_3$) and the reducing agent ($NaBH_4$) to the CNC aqueous suspension was deeply characterized in previous studies.[27,29] Using a similar approach, we compared the grafting of AgNPs onto two NPs and two soluble biopolymers leading to CNC, ChiNC, alginate, and chitosan-based hybrid suspensions. As soon as the silver precursor was reduced by $NaBH_4$, the initial translucent aqueous suspension turned light or dark yellow, indicating the formation of AgNPs. Regardless of the biopolymer used, the UV–vis spectra exhibited a narrow dominant in-plane absorption peak at λ_{max} ~400 nm (Figure 2a), which is typical of the synthesis of well-dispersed AgNPs. To confirm this experimental evidence, STEM images were acquired (Figure 2b). For the CNC/AgNP and ChiNC/AgNP case, the AgNPs of about 20 nm were anchored at the CNC and ChiNC surface, whereas AgNPs with an average diameter of 40 nm were detected for hybrids formulated with alginate and chitosan. This suggests that polymer chains are equally able to well-stabilize AgNPs due to the fact that the nucleation of AgNPs takes place on the hydroxyl groups available on the NC surface or on polymer chains.[20] The size distributions of AgNPs of the various systems, reported in, Figure S1 reveals particle size profiles that are significantly dependent on the nature of the biopolymer, whereas AgNP content determined by AAS in the hybrid systems proved to be similar to about 11 wt%: (i) CNC/AgNP at 11.6 wt% ± 0.1; (ii) ChiNC/AgNP at 11.1 wt% ± 0.3; (iii) alginate/AgNP at 10.2 wt% ± 0.02; and (iv) chitosan/AgNP at 11.1 wt% ± 0.3.

Finally, the analysis of the XRD diffractograms of the hybrid samples (Figure S2) indicated the presence of a peak at 38°, which is usually associated with the (111) lattice plane of the face-centered cubic silver structure (JCPDS Card No. 89–3722). Another characteristic peak was detected at 44° (i.e., [200] crystalline plane), thus confirming the isotropic nature of the crystals.[30]

3.2 | PP films coated with biopolymer/AgNP hybrids

These hybrids were deposited on the surface of commercial films using various polymer/particle assemblies that present attractive interactions for the surface. A first positive layer of PAH was deposited before adsorption of negatively-charged biopolymers, rinsed with clear water, and dried. SEM images (Figure 3a) clearly show the PP films covered with CNC/AgNP and ChiNC/AgNP hybrids at various concentrations. This result validated the efficiency of the method. Furthermore, the coating layer was highly organized in a dense network and good coverage of the surface was obtained for CNC or ChiNC at a concentration as low as 0.5 g/L. It could also be observed that the coating of the surface was more homogeneous with CNC compared to ChiNC. This experimental evidence can be explained considering the propensity of ChiNCs to aggregate at neutral pH compared to CNCs,

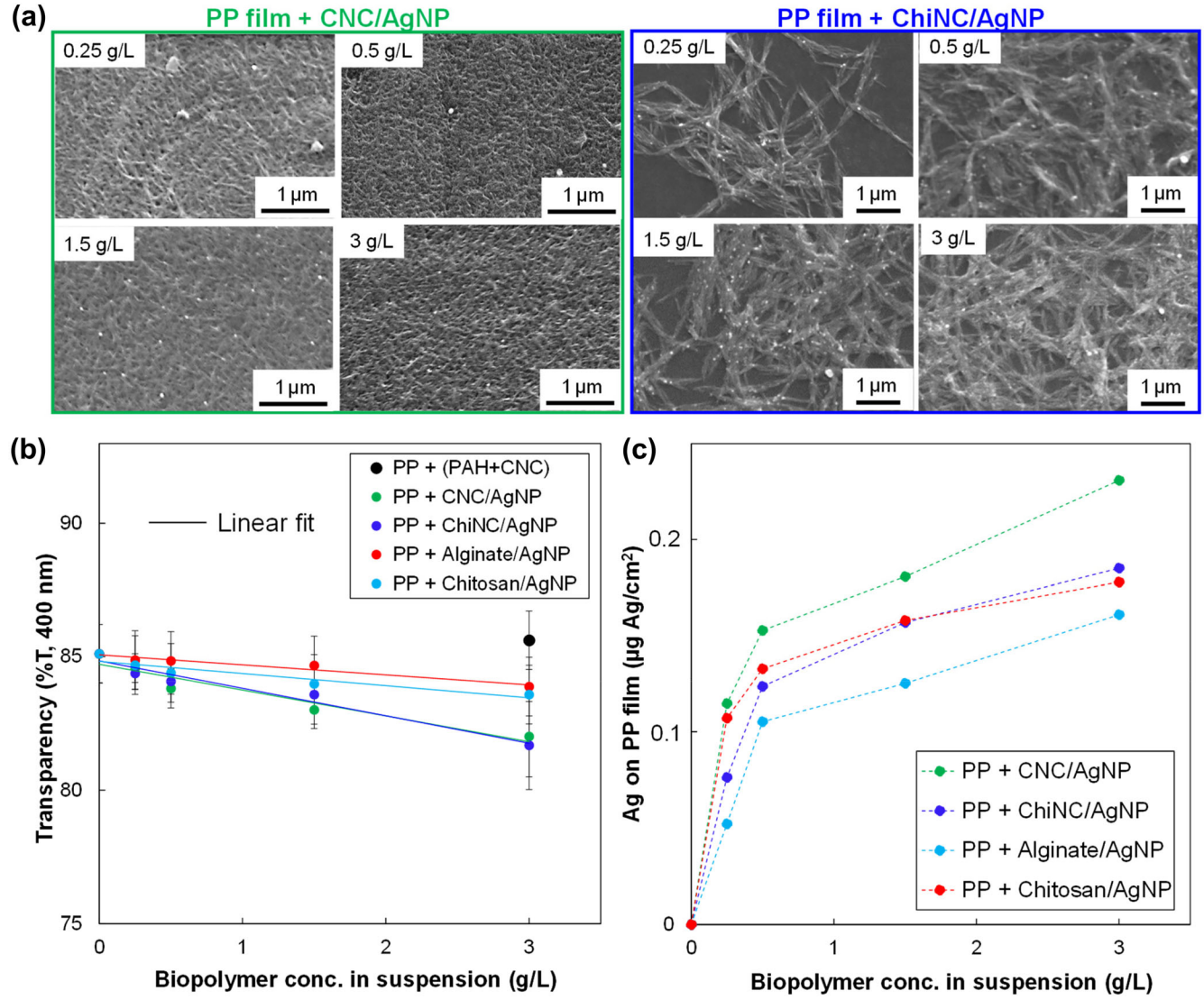

FIGURE 3 (a) SEM images of PP films coated with CNC/AgNP and ChiNC/AgNP at various NC concentrations; (b) transparency; and (c) AgNP content measured by AAS of PP films coated with the four hybrids at various starting suspension concentrations. AAS, atomic absorption spectroscopy; AgNP, silver nanoparticles; CNC, cellulose nanocrystals; ChiNC, chitin nanocrystals; PP, polypropylene. [Color figure can be viewed at wileyonlinelibrary.com]

which are well-dispersed thanks to their negative surface charge. Moreover, a slight improvement of the coating quality was obtained with increasing CNC or ChiNC concentrations (Figure 3a), but some inhomogeneities appeared from the NC aggregation and overlapping, as observed from the slight increase of the error bar on the transparency measurements (Figure 3b).

Whereas the NCs were observable on the film surface for films coated with CNC/AgNP or ChiNC/AgNP, alginate, and chitosan could not be visualized by electron microscopy since they were individual chains, and AgNPs were not clearly identified because they were dispersed. In order to prove that these biopolymer chains were actually fixed on the PP surface, alginate, and chitosan chains were chemically functionalized with a fluorescent dye, rhodamine (see SI for the detailed procedure) and deposited on the PP film surface. As shown in Figure S3, the films exhibit a homogeneous fluorescent surface, proving the effective adsorption of biopolymers in the form of a thin layer that is not visible by electronic microscopy. It can be assumed that the anchoring of the alginate and chitosan chains indirectly proved the presence of the AgNPs at the film surface.

To check this assumption, the AgNP content grafted on the PP surface was measured by AAS and reported in Figure 3c. The presence of the AgNPs was detected for all the samples with an AgNP content that increased with the hybrid concentration, with a higher efficiency when using more diluted hybrid suspensions, below 1 g/L for the four samples. This might signify that above this

concentration, less hybrids are adsorbed since they lose contact with the film.

Finally, the transparency of the various functionalized PP films was examined since silver is known to induce a dark color, which is unattractive for food packaging applications.Figure 3b shows that all the analyzed films maintained good transparency, close to that of the untreated PP film (i.e., $T = 85\%$). It could be observed that the strongest variation of transparency was obtained for CNC/AgNP and ChiNC/AgNP. This also corresponds to the highest amount of AgNPs as measured by AAS. Moreover, the increase of the error bar for the transparency values of PP film at higher biopolymer concentrations suggested more inhomogeneity on the film surface.

3.3 | Migration test; an Ag^+ kinetic release in food simulant

The compliance of such active materials dedicated to food contact application with regard to European requirements falls under regulation 450/2009 relative to active and intelligent materials for preparations that deliberately incorporate substances intended to be released into food. As such, our preparation should only be used under the conditions set out in the relevant community or national provisions. Where the provisions provide for an authorization of the substance, the substance and its use should comply with the requirements for authorization under the specific legislation on food, such as that of food additives. As a consequence, the development of a silver-based active material is the result of a compromise where silver particles must effectively exert their antimicrobial effect without presenting any possible risk to consumer health. On this last point, it is therefore important that the level of release of silver from the packaging to the food does not in any case exceed the authorized limit. Obviously, the state in which the silver is present in the food is a determining factor insofar as its toxicity is different depending on whether it is in ionic or nano form.

The materials were tested for their compliance for use in food contact applications according to regulation EU/2016/1416, using 3% acetic acid (3% HAc) as a food simulating liquid that is known to be the most solubilizing solvent and can be considered as the most "aggressive" solvent to be used in a worst-case scenario. To quantitatively analyze the release in the selected food simulant, Ag^+ ion release kinetics were investigated using PP films coated with different hybrids but with a similar AgNP content fixed at 0.15 µg Ag/cm^2 for a low content that leads to very well-dispersed hybrids and fully transparent films. The biopolymer concentration was chosen accordingly, as reported in Figure 3b (i.e., 0.5 g/L for CNC/AgNP; 1.5 g/L for ChiNC/AgNP and chitosan/AgNP; 3 g/L for alginate/AgNP). The release kinetics followed over 10 days are reported in Figure 4.

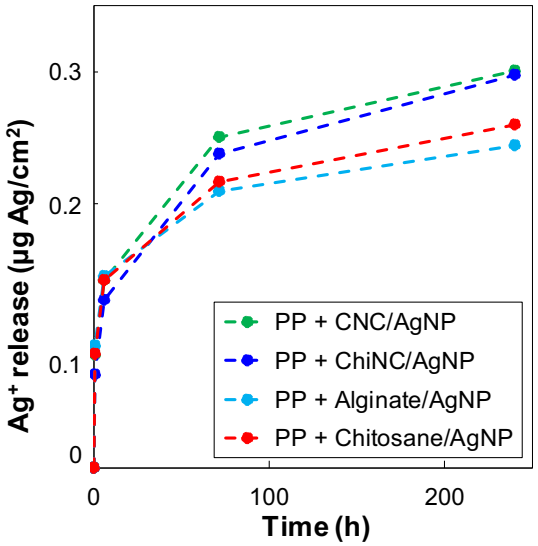

FIGURE 4 Kinetic release of Ag^+ from the surface of PP films coated with CNC/AgNP, ChiNC/AgNP, alginate/AgNP, chitosan/AgNP hybrids at the same initial AgNP content. AgNP, silver nanoparticles; CNC, cellulose nanocrystals; ChiNC, chitin nanocrystals; PP, polypropylene. [Color figure can be viewed at wileyonlinelibrary.com]

The migration kinetics are characterized by curves that reveal a non-Fickian transfer according to a diffusion-desorption mechanism conventionally observed in the case of additive studies. These results seem to indicate a two-phase release mechanism. The first phase describes a rapid release that probably involves the desorption of particles on the surface of the films in contact with the simulant. The second phase after 6 h of contact describes a slower release of silver ions without reaching an equilibrium state at the end of the 10-day experiment. This result demonstrates a more complex mechanism that produces a slow and continuous salting-out of Ag^+ ions, possibly based on a dissolution/solubilization of AgNPs in response to the sorption of acetic acid into the bulk of the material. When this behavior was observed independently of the nature of the support matrix, thus revealing the biocidal potential of all packaging films, significant differences in release rate were observed with the different matrix with, in particular, the highest values obtained for PP films coated with the CNC/AgNP or the ChiNC/AgNP hybrids. Such a result could be explained considering the solubilization process and the size of the AgNPs in the initial hybrid suspensions. Even if the release of AgNPs in the medium is generally considered in the migration process,[31] Ag is mainly present in the food simulant solution in an Ag^+

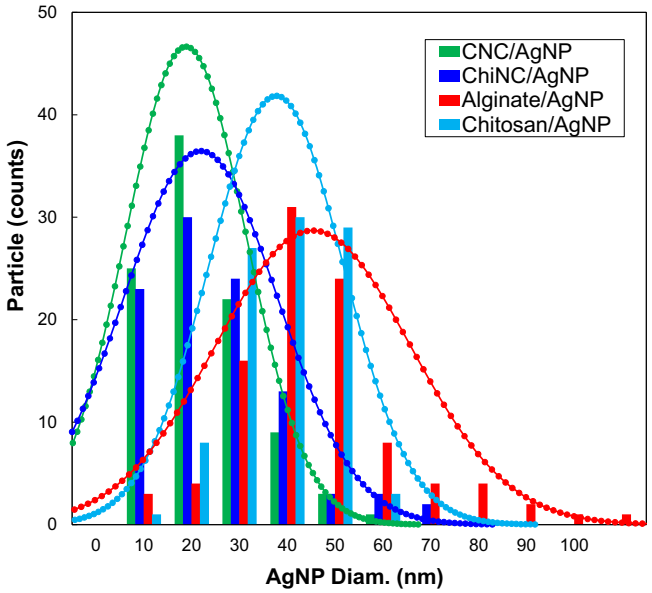

FIGURE 5 AgNP size distributions in CNC/, ChiNC/, alginate/, and chitosan/AgNP hybrids obtained by quantitative analysis of STEM images. AgNP, silver nanoparticles; CNC, cellulose nanocrystals; ChiNC, chitin nanocrystals. [Color figure can be viewed at wileyonlinelibrary.com]

ionic form associated with the oxidative attack of the AgNP surface induced by 3% HAc. Such an AgNP oxidative dissolution strongly depends on the AgNP size, and it is recognized in the literature that the Ag^+ ion release decreases with the increase in the average diameter of the AgNPs.[32,33] Regarding the size distribution of AgNPs in the different hybrid systems reported in Figure 5, the average size of the AgNPs increased from 20 nm for the CNC/AgNP and the ChiNC/AgNP cases, to about 40 nm for the alginate/AgNP and chitosan/AgNP suspensions, thus explaining the reduction of the long-term Ag^+ release for the PP films coated with these last two hybrids.

Due to a differential release rate between the samples, the amounts of silver ions released during the time of contact are significantly different depending on the nature of the hybrid system. As equilibrium is not reached at the end of the 10-day contact period, it is useful to consider the application and functional aspects of the packaging films. Thus, the differentiation of the behavior of the different systems can be considered as a means to best meet the needs of the packaged food, and the choice of the active system must be based on the

TABLE 1 Release and total migration values of PP films coated with CNC/AgNP and ChiNC/AgNP. Alginate/AgNP and chitosan/AgNP hybrids with the same AgNP content.

Hybrid	[biopolymer] (g/L)	Initial [AgNP] on film (μg Ag/cm^2)	Release time (h)	Ag^+ release (μg Ag/cm^2, surf. 100 cm^2)	Ag^+ release (μg Ag)	Daily intake (μg Ag/kg, bw = 100 kg)
CNC/AgNP	0.5	0.152 ± 0.003	1	0.00856	0.00086	0.09
			6	0.01425	0.00142	0.14
			72	0.02509	0.00251	0.25
			240	0.03008	0.00301	0.30
ChiNC/AgNP	1.5	0.157 ± 0.005	1	0.00708	0.00071	0.07
			6	0.01272	0.00127	0.13
			72	0.02383	0.00238	0.24
			240	0.02976	0.00298	0.30
Alginate/AgNP	3	0.161 ± 0.003	1	0.00930	0.00093	0.09
			6	0.01453	0.00145	0.15
			72	0.02095	0.00210	0.21
			240	0.02440	0.00244	0.24
Chitosan/AgNP	1.5	0.158 ± 0.002	1	0.00864	0.00086	0.09
			6	0.01424	0.00142	0.14
			72	0.02170	0.00217	0.22
			240	0.02596	0.00260	0.26

Abbreviations: AgNP, silver nanoparticles; CNC, cellulose nanocrystals; ChiNC, chitin nanocrystals; PP, polypropylene.

targeted biocidal effect and, therefore, on the kinetics of microbial growth likely to occur in the various packaged foods. The analysis of the Ag + migration levels after 10 days of contact is of critical interest from a regulatory point of view. The specific migration values are in good agreement with the literature.[22,31,34] The release is persistent over all the periods and values were always below the permitted limit of 0.05 mg Ag/kg food (EFSA 2006[35]), considering a surface area to weight ratio factor of 12 dm^2/kg,[36] even if no specific legislation is defined for AgNPs.[31] These migration values also respect the acceptable daily intake for insoluble silver substances (e.g., silver metal including nano-forms, silver oxide) fixed at 30 mg Ag/Kgbw/day for oral ingestion by the European Chemicals Agency (ECHA), based on the work of Kim et al.[37] (Table 1). Since the release using HAc, the most solubilizing solvent, is below 0.3 μg Ag/cm^2, the daily intake limits are respected with a safety factor of 100.[38]

4 | CONCLUSION

This study proposes an efficient and easy method in the safer-by-design approach to functionalize PP films using aqueous hybrid biopolymer/AgNP suspensions, providing biocidal properties, and making them suitable for food-packaging applications. First, various hybrids based on polysaccharides (i.e., CNC, ChiNC, alginate, chitosan) were synthesized. It was shown that all the biopolymers used made it possible to stabilize AgNPs of about 20–40 nm. These hybrids dispersed in aqueous solution were then used in a dipping procedure to coat the surface of PP films. For all the hybrids, successful and uniform coverage of the PP films was visualized, and the presence of Ag was detected by AAS (i.e., ∼0.15 μg Ag/cm^2).

The films as prepared are suitable for active food-packaging applications with biocidal properties. Migration kinetics of Ag^+ from AgNP oxidative dissolution were implemented in a 3% HAc food simulant at a comparable AgNP content. These tests clearly showed an increase of the Ag^+ release over time, which becomes more relevant on the long term when using hybrids based on CNC and ChiNC. For these two systems, AgNP size was 20 nm, compared to 40 nm for alginate- and chitosan-based hybrids. This result again confirmed how smaller AgNPs provide the strongest release activity. Moreover, the recorded total migration values (i.e., maximum value ∼0.3 μg Ag/kg) always remain below the value authorized by international authorities.

These results open the way to the design of new packaging with active products in as small amounts as possible, allowing the controlled and long-term release of Ag + for protection that is suitable for food applications. More importantly, this work can be easily adapted to the treatment of various contact surfaces against bacterial growth.

AUTHOR CONTRIBUTIONS

Dafne Musino: Conceptualization (equal); data curation (lead); formal analysis (lead); visualization (equal); writing – original draft (lead); writing – review and editing (equal). **Stephane Peyron:** Conceptualization (equal); supervision (equal); validation (equal); writing – review and editing (equal). **Isabelle Capron:** Conceptualization (equal); funding acquisition (lead); supervision (lead); validation (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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