Microbiological and cytotoxic perspectives of active PCL/ZnO film for food packaging

Hermano de Vasconcelos Pina¹, Andreza Josiary Aires de Farias¹, Francivandi Coelho Barbosa¹, José William de Lima Souza¹, Ana Beatriz de Sousa Barros¹, Márcio José Batista Cardoso¹, Marcus Vinicius Lia Fook¹ and Renate Maria Ramos Wellen

¹ Federal University of Campina Grande, Department of Materials Engineering, Campina Grande, PB. 58249-140, Brazil
² State University of Paraíba, Department of Collective Health, Campina Grande, PB. 58429-900, Brazil
³ Federal University of Paraíba, Department of Materials Engineering, João Pessoa, PB. 58051-085, Brazil

E-mail: wellen.renate@gmail.com

Keywords: PCL/ZnO films, antimicrobial analysis, cytotoxicity, biodegradable polymers, food packaging

Abstract

Nanoparticles of zinc oxide (ZnO) were added to poly(ε-caprolactone) (PCL), and PCL/ZnO casting films were produced, afterwards films were characterized using Fourier Transform Infrared Spectroscopy (FTIR), x-ray Diffraction (XRD), Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM). Additionally, antimicrobial and cytotoxic parameters were determined using microbial adhesion testing according to JIS Z 2801: 2000 (E) and agar diffusion method according to ISO 10993-5 2009, respectively. From collected data, the chemical identity of individual components was kept and the surface control could be achieved changing the composition. According to FTIR spectra and using the Lambert-Beer law higher interaction ratios were met for higher ZnO content which is linked to antimicrobial action. As revealed by AFM analysis, at 5% of ZnO, nanoparticles were well dispersed in PCL matrix with uniform surface film. Analyses from antibacterial activity and cytotoxicity suggested pathogenic Staphylococcus aureus growth was hindered in ZnO films; specifically for PCL/ZnO 5% optimal antibacterial activity and toxicity absent were reached. Summing up, PCL/ZnO5% nanocomposites films offer great potential for commercial applications as active food packaging.

Introduction

Currently, the sophisticated food industry needs modern packaging able to protect food, extending their shelf life and keeping them fresh and safe, allowing to be transported to long distances for consumer market supplying. On this way, traditional packaging is in accelerating development to fulfill the customer’s wishes. Improvements offering smart solutions are necessary since development of ‘active packaging’, besides acting as barrier between external and internal environments, must provide food preservation, which is an innovative approach to keep and extent the food’s shelf life while ensuring its quality, safety and integrity (Han et al 2018).

Scientific and technological trends suggest the development of functional packaging with antimicrobial character able to inhibit, or even quenching, spoilage and/or pathogenic microorganisms, at same time being environmentally friendly. Biodegradable films development with active additives arises to the food industry not only as prosperous option but also as urgent necessity. To attain these purposes, films based on ZnO₅nano with antimicrobial activity and biodegradable PCL are proper alternatives (Yildirim et al 2018).

PCL is biodegradable aliphatic thermoplastic polyester with high thermal stability, great potential for technological applications; good toughness and flexibility (Sogut and Seydim 2019). PCL presents superior viscoelastic properties related to other polyesters, being compatible with polymers from renewable and nonrenewable sources, it is properly used to process multilayer films with increased mechanical and barrier properties, which are essential for the packaging industry (Figueroa-Lopez et al 2018, Li et al 2018).
Nevertheless, up to now, most of commercial packaging have high production costs and environmental impact, therefore, the development of monolayer packaging with improved properties, lightweight, transparency and biodegradability is desired and nanotechnology advances can make this task affordable (Bumbudsanpharako et al 2015, Garcia et al 2018). Additionally, current properties found in PCL are thermal and chemical stabilities; biocompatibility; low toxicity and immunogenicity. As result, it appears as an ideal biodegradable matrix for nanostructured metal oxide with antimicrobial properties, such as ZnO (Capelezzo et al 2018).

Metallic nanoparticles have been incorporated into polymeric films, including ZnO for packaging production with antimicrobial activity, mainly due to their small size and large specific surface area (Espitia et al 2013, Kanmani and Rhim 2014). Researches have reported addition of nanostructured ZnO with antimicrobial properties to films based on biodegradable polymers can improve their barrier (water and gas), mechanical properties and increase UV light blockage (Pantani et al 2013, Venkatesan and Rajeswari 2017). Nevertheless, to the best of our knowledge, there are unclear and contradictory information in this area and more studies must be conducted. Even though ‘nanomaterial’ definition is not homogeneous, the Food and Drug Administration (FDA), on the ‘Guidance for Industry’, granted GRAS status to ZnO which approves its food safety (FDA 2017), as well as the European Union, where ZnO is also allowed for food packaging (EC 2017). However, despite the existence of specific ZnO legislation, the health risks of exposure to food packaging with nanoparticles remain poorly evaluated and further studies to assess cytotoxicity are required (Maisanaba et al 2014, Maisanaba et al 2015, Garcia et al 2018).

As optimal antibacterial efficacy and reduced toxicity remain a challenge to be reached; and nanoparticles dispersion inside the polymeric matrix directly influences the mechanical and barrier properties, and possibly the biological ones, the surface investigation associated to biological properties becomes an interesting strategy for PCL/ZnO films viability (Wiesenthal et al 2011, Zhang et al 2014, El Yamani et al 2016, Hu et al 2018). Based on the aforementioned, in the present work, PCL/ZnO nanocomposites films, with ZnO at 5, 10 and 15 wt%, were processed through solution casting. Antimicrobial properties against S. aureus on films surface were evaluated by microbial adhesion method, and the cytotoxicity was evaluated using the agar diffusion method. Chemical and topographic characterization were performed by FTIR, AFM and XRD and associated with biological properties in order to measure films potentiality as active food packaging. The aim of this work was to produce nanocomposites films for food packaging with optimal antibacterial activity and reduced toxicity.

Materials and methods

Materials
PCL (trade name CAPA 6500) with average molecular weight 50000 was purchased from Perstorp Winning Formulas (Sweden) and was used without any further treatment. Zinc oxide (ZnO) was purchased from Acros Organics as bulk-powder with 99.5% purity and specific area of 28 m² g⁻¹. In this work, ZnO was added to PCL matrix without any dispersion agent. Acetone was purchased from Química Moderna with 99.5% purity.

Processing of PCL and PCL/ZnO films
PCL films were produced dissolving PCL pellets in a concentration of 5% (mass/volume) into acetone (50 ml). The mixture was heated at 40 °C under constant magnetic stirring for 30 min; afterwards, PCL was completely solubilized. The solution was poured out in Petri dishes with 15 cm diameter, followed by oven drying at 40 °C for 18 h.

PCL/ZnO films followed the same methodology as neat PCL, and ZnO was added to PCL solution. PCL/ZnO films were produced with 5%, 10% and 15% ZnO (weight).

Films characterization
Fourier transform infrared spectroscopy (FTIR)
This technique was used to verify chemical groups changes on films. FTIR spectra were collected in a Perkin Elmer Spectrum 400 Series spectrometer (USA), in the mid-infrared range with wavelengths ranging from 4000-450 cm⁻¹ using the attenuated total reflectance (ATR) at 2 cm⁻¹ resolution and 16 scans. Tests were performed according to ASTM F2778-09.

X-ray diffraction (XRD)
X-ray diffraction (XRD) measurements were performed to investigate crystalline phases of PCL films and ZnO addition effect. The XRD analyses carried out in a DRX-7000 device from Shimadzu (Japan) using Ka copper
Films' surface topography was investigated in an Atomic Force Microscope from Park NX-Bio, (Suwon, Korea), with an XY scan range: 100 μm × 100 μm. Micrographs were obtained from different surface regions in order to verify the surface topography and film roughness, afterwards, verifying ZnO effects on them.

Antimicrobial activity
Antimicrobial activity of PCL/ZnO films was evaluated by microbial adhesion testing according to JIS Z 2801: 2000 (E). Gram-positive bacteria Staphylococcus Aureus (ATCC 25923) was selected as test organism. The test strain was cultured in BRAIN-HEART INFUSION AGAR (BHI) (accumulated) in a bacteriological oven at 35 °C for 24 h. The bacterial suspension was prepared in 0.5 McFarlands' scale. Films were sized at 1 cm², UV sterilized for 30 min and placed in 24-well culture dish with 900 μl culture medium MUELLER HINTON broth (KASVI) and 100 μl of bacterial suspension. Then the 24-well culture dish was incubated in a bacteriological oven at 35 °C for 24 h. The samples were washed with 0.9% saline solution and fixed with 2.5% glutaraldehyde and sequentially dehydrated using 15, 30, 50, 70, 85 and 99.6% ethanol after incubation at 35 °C for 24 h in a bacteriological oven.

After fixation and dehydration, films were investigated to elucidate the antibacterial activity by Scanning Electron Microscopy (SEM) in a Pro - X 800-07334 World Phenom (Eindhoven, The Netherlands). Using the public domain software Fiji-ImageJ version 1.53i, the bacteria average size was quantified and the percentage of occupied area related to total analyzed area was measured. At 3000X magnification, total sample area of approximately 10 μm² was analyzed. The surfaces were evaluated using several images with proper magnifications to reveal greater structural details, as also interactions in the interface between PCL and bacteria fixed in the samples. To ensure reliability, the assays were performed in triplicate.

Cytotoxicity assay

The cytotoxic activity was evaluated using the agar diffusion method according to ISO 10993-5 2009 and ISO 7405 2008 and adapted from the method used by Torabinejad et al 1995 and Kim et al (2005).

L929 Mouse Fibroblast Cell Line (ATCC NCTC clone 929, Rio de Janeiro Cell Bank, Brazil) were cultured in RPMI 1640 medium (Gibco - Invitrogen Corporation, Grand Island, USA) to confluence and trypsinized using trypsin at 0.25% (Gibco®, Life Technologies). Cell density was determined using an automatic cell counter (Interwoven - Thermo Fisher, Waltham, Massachusetts, USA); the concentration was adjusted to 1.0 × 10⁵ cells ml⁻¹. Cell suspensions were distributed in 6 well plates (4 ml/well) and incubated for 48 h. Those with uniform cell monolayer and confluence greater than 80%, the culture medium was replaced with 1 ml freshly prepared 2X Eagle MEM agar medium (Gibco®-Invitrogen Corporation, Grad Island, USA) and 0.01% neutral red solution (Sigma-Aldrich, USA). Plates with agar media remained for 10 min in the dark at room temperature for solidification. Sample tests with 1 cm² were placed in the center of agar surfaces, along with the positive triplicate (latex sheet) and negative (high-density polyethylene - HDPE) controls. The plates were incubated in the inverted position, wrapped in foil to prevent cell damage by neutral red photo activation (ISO 10993-5) for at least 24 h in humidified 35 °C (± 1 °C) greenhouse with 5% ± 1% CO₂. Cytotoxicity was examined to measure discoloration zone and evaluate cell lyses under a Nikon Eclipse TS100 inverted digital microscope (Minato, Tokyo, Japan) using established criteria (ISO 10993-5, 2009 and ISO 7405 2008) after 24 h as incubation time.

Results and discussion

Fourier transform infrared spectroscopy (FTIR)

Based on PCL and ZnO chemical structures, as presented in figure 1(a), chemical interactions between these two components were suggested in figure 1(b). There are dipole-dipole and hydrogen bonding interactions in which oxygen atoms being more electronegative are charged with free electrons resulted from the double bond breakdown, and this mechanism provides an electromagnetic resonance allowing linkage reordering, their charges are rebalanced after breaking down the interactions with ZnO nanoparticles, which agree with Augustine et al 2014 and Mallakpour and Nouruzi 2016. According to Augustine et al 2014 and Hu et al 2018, the absorption bands observed on FTIR spectra of figure 2 for PCL and PLC/ZnO films are: 2942 and 2870 cm⁻¹ attributed to the asymmetric and symmetrical CH₂ bond stretching, 1722 cm⁻¹ PCL ester carboxylic group (C=O) stretching, 1243 and 1166 cm⁻¹ corresponding to asymmetric and symmetrical stretching of C–O–C, respectively.
In figure 2, an unfinished band is observed with maximum intensity around 450 cm \(^{-1}\) due to ZnO band formed by compounds with 10 and 15%, in which their intensities increased upon nanoparticle content. For PCL/ZnO5% this band was not clearly observed due to the lower ZnO content, as well as its low vibration intensity, these arguments are in line with Mallakpour and Nouruzi \textsuperscript{2016} and Hu \textit{et al} \textsuperscript{2018} reports.

In order to understand ZnO interaction with PCL functional groups, which takes place in C=O group characterized by changes in 1722 cm \(^{-1}\) band, additional measurements were performed using Lambert-Beer law and the relative area of these bands was computed. Related to PCL, the PCL/ZnO5% increased at 0.8 ratio in the band area, while compounds with 10% and 15% ZnO increased at ratios of 14 and 20, respectively. These mean the higher the ratio value (as seen for 10 and 15% of ZnO) the greater the ZnO interaction intensity. The bacterial adhesion phenomenon depends on physicochemical interactions between cell surface and substrate. As the bacterium approaches to the substrate surface, interaction development resulting from the ionic atmosphere...
surrounding bacteria and substrate begins; therefore, increasing of chemical groups interaction intensity will decrease their antimicrobial activity (Augustine et al 2014, Hu et al 2018).

**X-Ray diffraction (XRD)**

X-ray diffractograms of PCL and PCL/ZnO films are presented in figure 3. PCL is semi-crystalline and two peaks are observed, at $2\theta = 21^\circ$ and $23^\circ$, similarly found by Correa et al (2017).

PCL/ZnO films displayed diffraction peaks which intensity was accentuated upon higher ZnO content. Nanocomposite films showed $2\theta$ diffraction peaks at $31.6^\circ; 34.6^\circ; 36.5^\circ; 47.7^\circ; 56.7^\circ; 62.9^\circ; 67.9^\circ$ and $69.2^\circ$, due to zinc oxide diffraction planes $(100), (002), (101), (102), (110), (110), (200)$ and $(112)$, respectively (Zak et al 2011). Suggesting the hexagonal wurtzite structure of ZnO nanoparticles was not altered after its addition to PCL matrix.

These diffraction patterns are in agreement with literature where synthesized or commercial ZnO$_{nano}$ were added to biopolymer matrices (Trandafilović et al 2012, Thirumavalavan et al 2013, Mohandas et al 2015).

ZnO addition increased PCL crystallinity, and the degree of crystallinity evaluated from the diffractograms are 25.7%, 34.4%, 36.0% and 71.54%, for PCL and nanocomposites with 5, 10 and 15% of ZnO respectively, similar results were found by Li et al 2010 in their researches with ZnO$_{nano}$ added to chitosan films. It is suggested, this trend related to ZnO content increase and consequent increase of PCL crystallinity, is possibly due to the ZnO nanoparticles are improving PCL crystallization producing new crystalline centers.

**Atomic force microscopy (AFM)**

AFM has been successfully introduced to surface morphology investigations, concise and effectively, AFM has been described as an analytical tool to elucidate surface characteristics of synthetic and biological materials (Jones 2016). AFM analysis is particularly suitable for active food packaging (films) due to their topographic modification as result of active substance dispersion on their surface (Marinello et al 2018). Aiming to evaluate topography and roughness on PCL/ZnO films, AFM analyses were performed, as shown in figure 4.

Analyzing AFM images of PCL and PCL/ZnO films it was observed increase in the films average and quadratic roughness upon ZnO addition, especially at 10% and 15% ZnO, as shown in figure 4. However, changes on roughness may influence material adherence, wettability, chemical and biological functionalities and therefore are relevant in the field of active packaging (Barish and Goddard 2011, Marinello et al 2018).

Films maximum height did not significantly change, related to the material guidelines. However, there was clear increase in the film morphology roughness upon ZnO addition especially in films with 10% and 15% ZnO, where significant increase in the mean and quadratic roughness was verified as shown in figure 4. This find...
indicates topography modification compared to PCL and PCL/ZnO5% films, and possible properties change as above mentioned.

Contrarily to Sadeghi and Shahedi 2016 who tested ZnO effect in morphology of polymeric films, Sadeghnejad et al 2014 who studied the surface modification of PE films obtaining films with increased antimicrobial activity and roughness. Presented results in this work suggest through microbial adhesion test as seen in SEM images from films surface (figure 5), formation of protrusions, i.e., roughness increase upon ZnO nanoparticles addition, which drives to cavities and facilitates microbial proliferation reducing ZnO antimicrobial activity, as also shown in acquired data from Lambert-Beer law.

Antimicrobial activity
To investigate the potentiality of PCL films, with and without ZnO addition, to be used as active food packaging and the susceptibility to microbial action that favors its degradation, the antibacterial activity effect must be investigated. Among the relevant pathogens for food application are gram (+), such as Staphylococcus aureus, bacteria known to cause food poisoning (Argudín et al 2010).

Nanoparticles dispersion inside matrix influences the mechanical and barrier properties as well as the biological ones. The surface analysis associated to biological properties showed ZnO content is not unique determining factor for antibacterial activity, since the antimicrobial activity increases with decrease of ZnO content. Possibly, as presented by the Lambert-Beer Law relative area ratio, the higher the ratio value (as it was for compounds with 10% and 15% ZnO) the stronger should be the interactions between ZnO and PCL, consequently their antimicrobial activity will be lower (Augustine et al 2014, Hu et al 2018). Corroborating with
AFM results where the film surface led to protrusions, i.e., roughness increase upon higher nanoparticles addition producing cavities which facilitate microbial proliferation thus reducing ZnO antimicrobial activity on film surface.

In the present work, the control film showed no antimicrobial activity against tested bacteria, with bacteria covered area of 59.2%, as shown in table 1. In contrast, PCL/ZnO films significantly reduced bacteriostatic S. aureus cell count, microbial growth inhibition was mainly by contact, having as major inhibition PCL/ZnO5% film with 0.056% of occupied area.

These results are quite satisfactory considering ZnO antimicrobial power related to high load of evaluated gram (+) microorganisms. Produced PCL/ZnO films controlled the contact bacterial population, which supports its use as antimicrobial food packaging, as it is able to inhibit microorganisms’ growth on the film packaging surface, as also possibly, to prevent contaminating microorganisms’ invasion into food.

### Cytotoxicity

*In vitro* tests for cytotoxicity assessment are required in order to ensure the use of these films for food packaging. The health risks when exposed to nanoparticles remain poorly assessed and further deeper evaluation into cytotoxicity is required (Wiesenthal *et al* 2011, Maisanaba *et al* 2014, Zhang *et al* 2014, Maisanaba *et al* 2015, Garcia *et al* 2018, Hu *et al* 2018).

To evaluate films cytotoxicity, the qualitative agar diffusion method, proper for high-density devices was chosen. This method evaluates the produced effects by the sample through the agar layer, which protects mouse connective tissue cells (lineage fibroblasts L-929) from mechanical damage during sample placement and allows chemicals diffusion from polymeric specimens.

Table 2 presents evaluated results from agar diffusion test, which were computed as the mean value of 4 different sample quadrants (done in triplicate). As negative control, HDPE mold was used, which did not promote cell lyses (figure 6(a)); while in toxic latex, positive control, a clear halo was observed (figure 6(b)), evidencing cell lyses, resulted from cytotoxicity grade 4 (severely cytotoxic) and the cell lysed index was rated as maximum, i.e., 5 for cytotoxicity interpretation (severely cytotoxic). Neat PCL and PCL/ZnO5% films were similarly rated to negative control (figures 6(c) and (d), respectively), presenting cytototoxicity absence, i.e., 0 (zero) grade and cell lysed 0 (zero) indexes. For PCL/ZnO10% (figure 6(e)) and PCL/ZnO15% (figure 6(f)) films, clear halo was observed under and around the sample, qualifying the sample cytotoxicity at grade 3 (moderate) and grade 3 and/or 4 of cell lysed indexes, respectively, thus considered unsatisfactory according to ISO 10993-5 2009.

Figure 6 shows, microscopically, discoloration zones on PCL/ZnO10%, PCL/ZnO15% and positive control (toxic latex) films that showed complete discoloration and cell lysed under and around the sample. PCL, PCL/ZnO5%, and negative control (HDPE) films did not show any discoloration zone under or around the sample. Cells were visualized at 100x magnification. Absorption of neutral red dye fibroblast cells after 24 h, the presence of red dye and absence of lysed cells show these cells are vital, enhancing samples qualification as lacking cytotoxicity, as seen in table 2.

### Table 1. Collected surface area quantification of tested films by SEM.

| Compound       | Bacteria average size ($\mu$m$^2$) | %Occupied Area by bacteria (%) |
|----------------|-------------------------------------|-------------------------------|
| PCL            | 0.673                               | 59.210                        |
| PCL/ZnO5%      | 0.133                               | 0.056                         |
| PCL/ZnO10%     | 0.193                               | 2.317                         |
| PCL/ZnO15%     | 0.253                               | 6.689                         |

* Evaluated percentage for an area with 10 $\mu$m.

### Table 2. Results of agar diffusion test.

| Test material       | Discoloration Index | Lyses index | Interpretation         |
|---------------------|---------------------|-------------|------------------------|
| Positive control    | 4                   | 5           | Severely cytotoxic     |
| Negative control    | 0                   | 0           | Non cytotoxic          |
| PCL/ZnO5%           | 0                   | 0           | Non cytotoxic          |
| PCL/ZnO10%          | 3                   | 3           | Moderate cytotoxic     |
| PCL/ZnO15%          | 3                   | 4           | Moderate cytotoxic     |
In the present study, no cytotoxic effects on PCL/ZnO5% were found; however, significant increase in cell cytotoxicity was observed on PCL/ZnO10% and PCL/ZnO15% films for cell line L929. Nanoparticle cytotoxicity is highly related to its surface charges (Yu et al 2019). Thus, negatively charged metal nanoparticles may promote less mitochondrial damage and disruption of mammalian cell membrane integrity than positively charged nanoparticles (Fröhlich 2012).

It is believed that greater dispersion and less interaction between PCL/ZnO5% are the main factors for the changes between surface loads, consequently for the sample cytotoxicity absence.

**Conclusion**

PCL/ZnO nanocomposites films were successfully produced by solvent casting method. FTIR spectra indicated interactions between PCL and ZnO groups and the higher the concentration of ZnO the stronger and/or greater the interaction between ZnO and PCL. The crystalline and chemical characters of PCL and ZnO were not significantly modified, as shown by XRD diffractograms. Surface control could be achieved changing composition; in addition, AFM topographic images from PCL/ZnO5% showed ZnO is well distributed along with PCL matrix. Surface study associated to biological analysis showed ZnO content is not the single determining factor for antibacterial activity. Increased roughness and greater interaction between PCL and ZnO chemical groups, due to higher content of nanoparticles, facilitated microbial proliferation reducing ZnO antimicrobial activity on film surface. These finds were specifically observed on film with 5%ZnO, showing uniform surface throughout the film, obtained the optimum antibacterial efficacy and toxicity absent. Summing up, PCL/ZnO5% film is promising for using as active food packaging.

**Acknowledgments**

Authors thank to Laboratory of Evaluation and Development of Biomaterials of the Northeast (CERTBIO) for laboratory infrastructure, and to MCTIC/CNPq, and CAPES/PNPD for financial support. Prof Renate Wellen and Prof Marcus Fook are CNPq fellows.

**Conflicts of interest**

There are no conflicts of interest.

**Ethics approval and consent to participate**

Not applicable.
Consent for publication

Authors agree with the current paper publication.

Authors’ contributions

All authors have contributed with the paper’s writing, analysis and discussion.

ORCID iDs

Renate Maria Ramos Wellen https://orcid.org/0000-0002-3565-7366

References

Argudin M A, Mendoza M C and Rodicio M R 2010 Food poisoning and Staphylococcus aureus enterotoxins Toxins 2 1751–73
Augustine R, Malik H N, Singh D K, Mukherjee A, Malakar D, Kalarikkal N and Thomas S 2014 Electrosprun polycaprolactone/ZnO nanocomposite membranes as biomaterials with antibacterial and cell adhesion properties J. Polym. Res. 21 347
Barish J A and Goddard J M 2011 Topographical and chemical characterization of polymer surfaces modified by physical and chemical processes J. Appl. Polym. Sci. 120 2863–71
Bumbudsanpharoke N, Choi J and Ko S 2015 Applications of nanomaterials in food packaging J. Nanosci. Nanotechnol. 15 6357–72
Capelezzo A P, Mohr L C, Dalcanton F, Barreta C R, Martins M A, Fiori M A and de Mello J M 2018 Antimicrobial biodegradable polymer through adlulation with zinc based compounds Quant. Nova 41 367–74
Correa E, Moncada M E and Zapata V H 2017 Electrical characterization of an ionic conductivity polymer electrolyte based on polycaprolactone and silver nitrate for medical applications Mater. Lett. 205 135–7
EC 2017 Commission regulation (EU) 2017/752 of 28 April 2017 amending and correcting Regulation (EU) No 10/2010 on plastic materials and articles intended to come into contact with food (Text with EEA relevance) Official Journal of the European Union L113 18–23 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0752
Espitia P J P, Soares N D F F, Teó Correa E, Moncada M E and Zapata V H 2017 Electrical characterization of an ionic conductivity polymer electrolyte based on polycaprolactone and silver nitrate for medical applications Mater. Lett. 205 135–7
FDA 2017 Substances Generally Recognized As Safe. 1–28 https://ecfr.gov/cgi-bin/textidx?SID=956de415ab4e63e4e45d1b6902098&mce=1&node=pt21.3.182&rng=div5#/se21.3.182_18991, https://www.fda.gov/media/109117/download
Figueredo-Lopez K, Castro-Mayorga J, Andrade-Mahecha M, Cabedo L and Lagaron J 2018 Antibacterial and barrier properties of gelatin coated by electrosprun polycaprolactone ultrathin fibers containing black pepper oleoresin of interest in active food biopackaging applications Nanomaterials 8 199
Fröhlich F 2012 The role of surface charge in cellular uptake and cytotoxicity of medical nanoparticles Int. J. Nanomed. 7 5577
Garcia C V, Shin G H and Kim J T 2018 Metal oxide–based nanocomposites in food packaging: Applications, migration, and regulations Trends in Food Science & Technology. (https://doi.org/10.1016/j.tifs.2018.09.021)
Han J W, Ruiz-Garcia L, Qian J P and Yang X T 2018 Food packaging: a comprehensive review and future trends Comprehensive Reviews in Food Science and Food Safety 17 66–70
Hu M, Li C, Li X, Zhou M, Sun J, Sheng F and Lu L 2018 Zinc oxide/silver bimetallic nanoencapsulated in PVP/PCL nanofibres for improved antibacterial activity Artificial cells, nanomedicine, and biotechnology 46 1248–57
‘ISO 10993-5 2009 biological compatibility of medical devices—Part 5: Tests for cytotoxicity In Vitro Methods (Geneve, Switzerland: International Organization of Standardization) https://www.iso.org/standard/36406.html
‘ISO 7405 2008 Evaluation of Biocompatibility of Medical Devices Used in Dentistry (Geneve, Switzerland: International Organization for Standardization-ISO) https://www.iso.org/standard/38059.html
Jones O G 2016 Developments in dynamic atomic force microscopy techniques to characterize viscoelastic behaviors of food materials at the nanoscale Current Opinion in Food Science 17 76–80
Kanmani P and Rhim J W 2014 Properties and characterization of bionanocomposite films prepared with various biopolymers and ZnO nanoparticles Carbohydrate Polym. 106 190–9
Kim D H, Lee S H, Kim K N, Kim K M, Shin J B and Lee Y K 2005 Cytotoxicity of ferrite particles by MTT and agar diffusion methods for hyperthermic application J. Magn. Magn. Mater. 293 267–92
Li W, Shi L, Zhang X, Liu K, Ullah I and Cheng P 2018 Electrospinning of polycaprolactone nanofibers using H2O as benign additive in polycaprolactone/glacial acetic acid solution J. Appl. Polym. Sci. 135 45578
Li L H, Deng J C, Deng H R, Liu Z L and Xin L 2010 Synthesis and characterization of chitosan/ZnO nanoparticle composite membranes Carbohydr. Res. 345 994–8
Maisanaba S, Pichardo S, Jordá-Beneyto M, Aucejo S, Carneán A M and Jos À 2014 Cytotoxicity and mutagenicity studies on migration extracts from nanocomposites with potential use in food packaging Food Chem. Toxicol. 66 356–72
Maisanaba S, Pichardo S, Puerto M, Gutierrez-Praena D, Carneán A M and Jos À 2015 Toxicological evaluation of clay minerals and derived nanocomposites: a review Environ. Res. 138 233–54
Malakpour S and Nouruzi N 2016 Effect of modified ZnO nanoparticles with biosafe molecule on the morphology and physiochemical properties of novel polycaprolactone nanocomposites Polymer 89 94–101
Marinello F, La Storia A, Maurillo G and Passeri D 2018 Atomic Force Microscopy Techniques to Investigate Activated Food Packaging Materials. (https://doi.org/10.1016/j.jifs.2018.05.028)
Mohandas A, Sudheesh Kumar P T, Raja B, Lakshmanan V K and Jayakumar R 2015 Exploration of alginate hydrogel/nano zinc oxide composite bandages for infected wounds Int. J. Nanomed. 10 53
Pantani R, Gorrasi G, Vigliotta G, Murariu M and Dubois P 2013 PLA-ZnO nanocomposite films: Water vapor barrier properties and specific end-use characteristics Eur. Polym. J. 49 3471–82
Sadeghi K and Shahedi M 2016 Physical, mechanical, and antimicrobial properties of ethylene vinyl alcohol copolymer/chitosan/nano-ZnO (ECNZn) nanocomposite films incorporating glycerol plasticizer Journal of Food Measurement and Characterization 10 137–47
Sadeghnejad A, Aroujalian A, Raisi A and Fazel S 2014 Antibacterial nano silver coating on the surface of polyethylene films using corona discharge Surf. Coat. Technol. 245 1–8
Sogut E and Seydim A C 2019 The effects of chitosan-and polycaprolactone-based bilayer films incorporated with grape seed extract and nanocellulose on the quality of chicken breast fillets LWT 101 799–805
Thirumavalavan M, Huang K L and Lee J F 2013 Synthesis and properties of nano ZnO using polysaccharides as chelating agents: effects of various parameters on surface modification of polysaccharides Colloids Surf., A 417 154–60
Torabinejad M, Hong C U, Ford T P and Kettering J D 1995 Cytotoxicity of four root end filling materials J. Endodontics 21 489–92
Trandafilović L V, Božanić D K, Dimitrijević-Branković S, Luyt A S and Djoković V 2012 Fabrication and antibacterial properties of ZnO–alginate nanocomposites Carbohydrate Polym. 88 263–9
Venkatesan R and Rajeswari N 2017 ZnO/PBAT nanocomposite films: Investigation on the mechanical and biological activity for food packaging Polym. Adv. Technol. 28 247–54
Wiesenthal A, Hunter L, Wang S, Wickliffe J and Wilkerson M 2011 Nanoparticles: small and mighty International journal of dermatology 50 247–54
Yıldırım S, Röcker B, Pettersen M K, Nilsen‐Nygaard J, Ayhan Z, Rutkaite R and Coma V 2018 Active packaging applications for food Comprehensive Reviews in Food Science and Food Safety 17 165–99
Yu Z, Wang W, Kong F, Lin M and Mustapha A 2019 Cellulose nanofibril/silver nanoparticle composite as an active food packaging system and its toxicity to human colon cells Int. J. Biol. Macromol. 129 887–94
Zak A K, Majid W A, Abrishami M E and Yousefi R 2011 X-ray analysis of ZnO nanoparticles by Williamson–Hall and size–strain plot methods Solid State Sci. 13 251–6
Zhang T, Wang L, Chen Q and Chen C 2014 Cytotoxic potential of silver nanoparticles Yonsei Medical Journal 55 283–91