Evaluation of antimicrobial activity of magnesium oxide nanocomposite film in combination with ε-poly-L-lysine against Foodborne pathogens in vacuum packaged beef

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Research

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Abstract

Background: The aim of this study was to evaluate the antimicrobial activity of magnesium oxide nanocomposite (MgO NC) film based on Low-density polyethylene (LDPE) alone or in combination with three concentrations of ε-poly-L-lysine (500, 1000 and 2000 µg/ml) on *Escherichia coli* O157:H7 and *Listeria monocytogenes* in culture media and fresh beef.

Methods: MgO NC film were prepared by melt mixing LDPE and MgO nanoparticle in the extruder. For in vitro antibacterial analysis, the MgO NC film alone or in combination with polylysine were evaluated in tryptic soy broth for 5 days at 37 °C. For in vivo analysis, beef samples were inoculated with the selected bacteria and packaged in MgO NC film under vacuum and stored at 4 °C and evaluated for up to 20 days.

Results: Polylysine had an antibacterial effect against *E. coli* and *L. monocytogenes* in TSB. But MgO NC film had a bacteriostatic effect only against *E. coli*. MgO NC film inhibited the growth of *E. coli* on the surface of beef samples. Pollysine at concentrations of 500 µg/ml or more showed inhibitory activity against *E. coli* and *L. monocytogenes* in beef samples. No additional reduction was observed by combining the different concentrations of polylysine with MgO NC film.

Conclusions: Polylysine at all concentrations had an inhibitory effect on *E. coli* and *L. monocytogenes* in the culture medium and beef. Although the migration of MgO nanoparticle from the film to beef was very low, but as it has little antimicrobial effect, it is not recommended as a suitable package for improving the safety of raw beef.

Introduction

In the context of a constantly growing population and globalization of markets, prevention of food contamination by microorganisms or pathogens, is becoming increasingly important [1]. In order to reduce the number of foodborne infections and prevent the drug resistant bacterial contamination, new strategies are therefore needed to identify and use for its control [2, 3]. Antimicrobial packaging is a form of active food packaging, which is beneficial to the consumers as well as to the food industry since it can extend product shelf-life and/or maintain food safety by killing or reducing the growth rate of target microorganisms [4]. Antimicrobial packaging provides an innovative alternative to some of the traditional meat preservation methods and can reduce the addition of large quantities of antimicrobials that are usually incorporated directly into the bulk of the food [5]. ‘Nano food packaging’ with antimicrobial properties represents a new generation of active packaging based on metal nanocomposites (NCs). Inorganic materials such as metals and metal oxides have been the focus of nanotechnology research [6].

Metal oxide nanoparticles (NPs) are a new class of antimicrobial agents that have been increasingly studied for their antibacterial properties and potential applications in the food, the environment, and healthcare [7]. Among these, magnesium oxide nanoparticles (MgO NPs) is particularly interesting due to its strong antibacterial activity, but high thermal stability and low cost [8]. MgO is one of the five Mg

compounds, which is listed as generally regarded as safe (GRAS) by the United States Food and Drug Administration (USFDA). MgO has been used as a mineral supplement for magnesium, an essential nutrient for the human body [8]. As a medicine, it is used for the relief of cardiovascular disease and stomach problems. The exact mechanism of MgO antimicrobial action is unclear. It has been reported that the considerable antibacterial activity of MgO NPs is attributed to the generation of reactive oxygen species (ROS) on the oxide surface such as superoxide anion (O_2) [9, 10] which induces oxidative stress in cells. It was suggested that the MgO NPs relied on the presence of defects or oxygen vacancy at the surface of the NPs which led to the lipid peroxidation and reactive oxygen species generation [11]. In contrast, non-ROS mediated bacterial toxicity was also found in MgO NPs suggesting an electrostatic interaction between NPs and cell surface might be the mechanism of cell death [8, 12]. The alkaline effect has been considered as another primary factor in the antibacterial action of MgO NPs [10].

Recently, from a safety perspective, there has been an increasing demand for natural antimicrobial compounds rather than synthetic ones [13]. ε-Polylysine, (S)-poly (imino (2-amino-1-oxo-1,6-hexanediyl)), is a naturally occurring homo-polymer of L-lysine with a degree of polymerization of 25–35, molecular weight of approximately 5000 g/mol, and characterized by the peptide bonds between the carboxyl and ε- amino groups of L-lysine [14] and isolated from *Streptomyces albulus* sp. *Lysinopolymerus* strain 346 [15, 16]. Polylysine is widely used to preserve packaged food in certain countries for its broad antimicrobial activity against Gram-negative and Gram-positive bacteria, yeasts, and molds [17, 18]. It is characterized as being edible, nontoxic to humans, water soluble, stable at high temperatures, and of low environmental impact due to its biodegradability [19, 20]. The presence of primary amine groups along the backbone of polylysine means that it has a relatively high isoelectric point, therefore it is strongly cationic at pH values < 9 [19]. Polylysine can be completely digested by the body [21] and decomposed into lysine without any side effects on the human body, and serve as a kind of lysine sources. Chronic toxicity and carcinogenicity joint test showed that a person taking polylysine 6500 μg/kg on diet every day was safe [22].

Polylysine has been approved as generally recognized as safe (GRAS) within the United States for certain food applications (GRAS No. 000336 and 000135) [23, 24]. According to the results of Ye et al. (2013) the potential antimicrobial mechanism of polylysine was expressed when a positive charge from polylysine came in contact with the bacteria, which bounds to the membrane surface by electrostatic attraction [25]. The accumulation of such interaction resulted in the disturbance of the cell membrane and led to fractures of the membrane structure. Thus, polylysine entered the cytoplasm through the membrane fractures. Additionally, polylysine induced the generation of ROS and SOS response, which also affected cell death and regulated the expression level of related genes. The synergy, or buildup of these factors affected the cells and finally resulted in the death of the bacteria.
Raw red beef has a high water activity and plenty of nutrients [26]. It can be easily contaminated by microorganisms and support the growth of pathogens, leading to serious foodborne illnesses [27]. *E. coli* O157:H7 and *L. monocytogenes* are two main pathogenic bacteria in meat products. Microbial contamination of beef usually occurs at the surface [26] due to post-processing handling, processing or

cutting. Antimicrobial packaging is gaining interest from researchers and industries due to its potential to prevent the surface growth of pathogenic bacteria in the beef industry. The incorporation of the antimicrobial agents in a film or as a coating has many advantages due to the high exposure areas for the antimicrobial and a reduction of the loss of the antimicrobial into the bulk of the food [28–30]. Since the use of MgO NPs is acceptable in the food industry, therefore, its use based on pathogen control can provide innovation in antimicrobial active packaging technologies, especially in the meat industry. The aim of this work was to prepare MgO NC film based on low density polyethylene (LDPE) and evaluate their antimicrobial effect against *E. coli* O157:H7 and *L. monocytogenes*. The effect of the combination of MgO NC film and polylysine on the growth of bacteria was also investigated.

Materials And Methods

Materials

MgO NPs (average particle size 20 nm and 99% purity) was purchased from US Research Nanomaterials, Inc. Film grade LDPE resin pellets (LF0200, MFI 2 g/10 min, density 0.92 g/ml and softening point 94 ºC) were obtained from Bandar-Imam Petrochemical Co., Iran. Commercial ε-poly-L-lysine powder (Epolyly TM, Belgium, 50% w/w) was purchased from Hendry Co, Belgium and prepared at concentrations of (500, 1000, and 2000 µg/ml) by dissolving it in distilled water. The solutions were sterilized through a 0.22 µm filter and stored at 4°C.

Bacterial Culture

E. coli O157:H7 (ATCC 135218) and *L. monocytogenes* (ATCC 191181) were obtained from the Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Iran. Stock cultures of *E. coli* O157:H7 and *L. monocytogenes* were prepared by inoculating in tryptic soy broth (TSB, Merck, Germany) and incubating at 37°C for 24 h. Then, they sub-cultured individually on MacConkey agar (Merck, Germany) and Palcam agar (Merck, Germany), respectively. A single colony from plates of bacteria transferred into TSB and incubated at 37 ºC for 24 h. The optical density (OD) of the bacterial suspensions was adjusted to the standard of McFarland No. 0.5 (1.5×10^8 CFU/ml) by adding sterilized normal saline.

Preparation of MgO NC film

The combination of MgO NPs powder and LDPE resin pellets (10: 90) were directly blended for 1 h. The mixture was introduced to a twin screw extruder (Brabender, Germany) to be cut into masterbatch nano-granules. The heating profile was set to six zones of the extruder including 160, 160, 175, 150, 150, and 140 ºC. For production of MgO NC film proper amounts of masterbatch were added to pure LDPE resin pellets into a single-screw blowing machine (Brabender, Germany) at 190 ºC in the two barrel zones. MgO NC films were prepared in concentrations of 5 and 10% W/W MgO NPs with a thickness of 60 µm [6, 31–33]. Pure LDPE resin pellets without MgO NPs were analogously prepared and used as a control film.

Characterization Of MgO NC Film

The **X-ray diffraction** (XRD) pattern of the MgO NC film was obtained with X-ray diffractometer (D8 Advance, Bruker AXS, US) in the range of 2 values from 2 º-40 º and λ = 1.54 ºA. The morphology of MgO NC films was characterized by a scanning electronic microscope (SEM, Cambridge Stereo Scan, Leica, Germany). For this work, films were protected with a thin gold layer using a sputter coater. SEM images were taken at an acceleration voltage of 15 kV. [32].

Enumeration of E. coli O157:H7 and L. monocytogenes in the culture media (In vitro)

Effect of MgO NC film (10 % w MgO NPs) and LDPE film (control) with and without polylysine on survival of *E. coli* O157:H7 and *L. monocytogenes* were evaluated [34–37]. MgO NC and LDPE films were cut 2×2 mm^2 cubes and sterilized with 70% ethanol and dried up in air. 200 mg of sterilized film cubes were immersed in 4.5 ml filter sterilized TSB and/or TSB containing 2.5, 5.0 and 10.0 mg polylysine for alone and combined treatments, respectively. Then, 0.5 ml of *E. coli* O157:H7 or *L. monocytogenes* suspensions (10^5 CFU/ml) was inoculated to each flask. Final concentration of polylysine was 500, 1000 and 2000 µg/ml.

The flasks were incubated at 37°C in shaking incubator at 100 rpm for 5 days. Bacteria were enumerated on days 0, 1, 2, 3, 4, and 5 of incubation. Ten fold serial dilutions of samples were made and the surface plated on MacConkey agar and/ or Palcam agar, respectively. The plates were incubated at 37°C for 24–48 h. The bacterial colonies were enumerated and expressed as log CFU/ ml, and their mean value was recorded. All treatments were done in three independent replicate.

Enumeration of E. coli O157:H7 and L. monocytogenes in fresh beef (Invivo)

Freshly cattle muscles (Longissimus dorsi) were prepared from a slaughterhouse and stored in 4°C for overnight. To sterilize surface of the cylindrical beef, it was immersed in boiling water

for 10 seconds, and after removing the cooked sections with a sterile knife under aseptic conditions, they were cut in a transverse direction in size 2×5×5 cm. Two slices were evaluated for the likelihood of *E. coli* O157:H7 and *L. monocytogenes* [38]. The other slices were placed on a sterile tray in a biological safety cabinet, and the upper surface of each beef slice was inoculated with 100 µl of selected bacterial suspensions (10^6 CFU/ml) to obtain an initial bacterial count about 1× 10^5 CFU/sample, and spread evenly over the surface of beef using sterile spreaders. Samples were held for 10 min to allow sorption of the tested bacteria. For polylysine treatments, 1 ml of polylysine solutions (500, 1000 and/ or 2000 µg/ml) was poured to the surface of the treated beef and spreaded. Distilled water was used for control samples instead of polylysine. Afterward, 10×10 cm of ethanol sterilized MgO NC film and/ or LDPE film (as control) was put on the top of each inoculated beef slice. Then they were inserted into polyethylene bag (thickness: 85 micron, density: 1.1 g/ ml) and vacuum packed using a table vacuum machines (Webomatic D-44866, Germany) at 99 % vacuum. The vacuum packed samples were stored at 4 ºC for 20 days and were evaluated on days 0, 5, 10, 15, and 20 of storage.

At each selected time, three samples of each treatment were weighted and homogenized by a stomacher with 9 times normal saline for 2 min. Serial decimal dilution was prepared and appropriate dilutions were plated on the selective media. The plates were incubated at 37 ºC for 24–48 h. The bacterial colonies were enumerated and expressed as log CFU/sample [34, 39, 40].

Measurement of MgO migration from MgO NC film to beef

For migration study, the 10×10 cm slice of beef samples were weighted and vacuum packaged with the MgO NC film and/ or LDPE film in size 10×10 cm and stored for 20 days at 4°C. For digestion, 0.4 g of homogenized beef samples with 3 ml of HNO_3 (65%) and 1 ml of H_2O_2 (30%) poured into the to vessels of microwave oven (Panasonic NN-ST757W, China). After cooling down the vessels, the digested solution was diluted with deionized water and analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (Vista PRO, US). The control was prepared under the same conditions, containing all reagents except the beef. The calibration of ICP-MS was performed using an external Mg standard solution[37, 41].

The amount of migration (µg/ cm^2 of MgO NC film) was calculated from the difference in MgO content of the beef samples covered by MgO NC film from the beef samples covered by LDPE film.

Statestical Analysis

The results were analyzed by variance analysis (ANOVA) using the SPSS software (version 16). The differences between means were compared through the Duncan test at a level of 5% of significance.

Results

SEM and XRD of MgO NC film

Figure 1 shows the SEM images of MgO NC and LDPE films. In MgO NC films, the NPs were almost uniformly distributed in the polymer matrix. However, a slight agglomeration was observed by increasing the concentration of MgO NPs from 5 to 10%, due to the polarity and electrostatic attraction [42]. The Fig. 2 shows XRD patterns of MgO films. The Highly intense peaks were found in 14.2º, 17.05º, 21.6º, and 25.7º were assigned to (111), (200), (220), and (331) planes of MgO NPs respectively.

The peaks obtained at different crystal planes for MgO NPs in the film were found in significant agreement with the Joint Committee on Powder Diffraction Standards (JCPDS NO.39-7746) indicating the cubic structure of MgO [42, 43].

Antibacterial effects of MgO NC film and polylysine in the culture media (In vitro)

Table 1 reflects the survival of *E. coli* O157:H7 cells after treatment with 10% MgO NC film and different concentration of polylysine. After 24 hours of incubation at 37 ºC, the bacterial population in the control film without MgO achieved to 9.42 ± 0.11 log CFU/ml which reduced to 8.34 ± 0.01 log CFU/ml in the presence of MgO NC film. Approximate 1.1 log reduction in *E. coli* O157:H7 cells were produced. By

increasing the incubation time, the damaged bacteria cells could grow and proliferate to a certain extent, so it can be admitted that MgO NC film had a bacteriostatic effect against *E. coli* O157:H7 in TSB.

Table 1
Effect of 10% Magnesium Oxide nanocomposite (MgO NC) film and different concentration of polylysine on the viability of *E. coli* O157:H7 in the culture media during 5 days storage at 37 ºC.

Treatments	Viable counts (Log CFU/ml)					
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
LDPE (control)	4.00 ± 0.02aA	9.42 ± 0.11aA	8.84 ± 0.02 bA	8.75 ± 0.04 bA	8.75 ± 0.10 bA	8.34 ± 0.39 cA
MgO NC	4.00 ± 0.02aA	7.34 ± 0.01 aC	7.04 ± 0.05 bD	7.41 ± 0.15 cE	7.62 ± 0.15 cE	7.46 ± 0.09 cE
LDPE + Polylysine (500 µg/ml)	4.00 ± 0.02aA	0 aB	3.50 ± 0.08 bB	4.23 ± 0.06 cB	4.30 ± 0.09 cB	4.47 ± 0.22 cB
LDPE + Polylysine (1000 µg/ml)	4.00 ± 0.02aA	0 aB	3.45 ± 0.03 bB	3.55 ± 0.06 bC	3.60 ± 0.12 bC	3.60 ± 0.08 bC
LDPE + Polylysine (2000 µg/ml)	4.00 ± 0.02aA	0 aB	0 aC	0 aD	0 aD	0 aD
MgO NC + Polylysine (500 µg/ml)	4.00 ± 0.02aA	0 aB	0 aC	0 aD	0 aD	0 aD
MgO NC + Polylysine (1000 µg/ml)	4.00 ± 0.02aA	0 aB	0 aC	0 aD	0 aD	0 aD
MgO NC + Polylysine (2000 µg/ml)	4.00 ± 0.02aA	0 aB	0 aC	0 aD	0 aD	0 aD

Values are mean ± standard deviation (n = 3). LDPE: Low-density polyethylene. MgO NC: Magnesium Oxide nanocomposite film. Different letters within the same column (uppercase letters) and row (lowercase letters), show the statistically difference ($P < 0.05$).

The number of *E. coli* O157:H7 decreased significantly for all concentrations of polylysine compared to the control ($P < 0.05$), but no differences were detectable from day 3 by the end of the incubation time. After 24 hours, the polylysine (500 and 1000 µg/ml) inhibited the growth of *E. coli* O157:H7, but after 48 hours the damaged cells began to repair and increase their populations. Polylysine showed the bacteriostatic effect against bacteria. The production of injured cells and the continuous reduction observed at 2000 µg/ml polylysine by the end of the incubation time. By increasing the concentration of polylysin to 2000 µg/ml, bacterial inhibition was observed throughout the entire period of 5 days, which express the bactericidal effect. Depending on the experimental conditions, concentration and bacterial strain, polylysine may have a bacteriostatic or bactericidal effect. Although the MgO film alone led to a reduction in the *E. coli* O157:H7 cells, but synergism was observed when used in combination with

polylysine. No colonies of *E. coli* O157:H7 was observed in the combined of MgO film with each polylysine concentration compared to MgO and polylysine alone.

Table 2 reflects the survival of *L. monocytogenes* after treatments with 10% MgO NC film and different concentration of polylysine. After 24 hours (day 1) of incubation at 37 ºC, the MgO NC film reduced the population of *L. monocytogenes* compared to the control (from 8.34 ± 0.02 log CFU/ml to 4.27 ± 0.01 log CFU/ml, $P < 0.05$). In fact, 4.07 log reduction in *L. monocytogenes* cells were observed. The antimicrobial activity of the MgO NC film was time dependent. From the 3rd day of incubation, the antibacterial activity of the MgO NC film decreased. Based on the results of the study, the antimicrobial effects of the MgO NC film were against Gram-positive bacteria (*L. monocytogenes)* higher than Gram-negative bacteria (*E. coli* O157:H7).

Table 2
Effect of 10% Magnesium Oxide nanocomposite (MgO NC) film and different concentration of polylysine on the viability of *L. monocytogenes* in the culture media during 5 days storage at 37 ºC.

Treatments	**Viable counts (Log CFU/ml)**					
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
LDPE (control)	4.11 ± 0.03aA	8.31 ± 0.14 aA	7.32 ± 0.26 bA	8.86 ± 0.14 cA	7.81 ± 0.08 cA	7.904 ± 0.07 cA
MgO NC	4.10 ± 0.04aA	4.27 ± 0.10 aB	6.27 ± 0.14 bB	7.90 ± 0.06 cA	7.96 ± 0.16 cA	7.81 ± 0.05 cA
LDPE + Polylysine (500 µg/ml)	4.14 ± 0.03aA	0 aC	0 aC	0 aB	0 aB	0 aB
LDPE + Polylysine (1000 µg/ml)	4.13 ± 0.02aA	0 aC	0 aC	0 aB	0 aB	0 aB
LDPE + Polylysine (2000 µg/ml)	4.10 ± 0.02aA	0 aC	0 aC	0 aB	0 aB	0 aB
MgO NC + Polylysine (500 µg/ml)	4.10 ± 0.04aA	0 aC	0 aC	0 aB	0 aB	0 aB
MgO NC + Polylysine (1000 µg/ml)	4.15 ± 0.03aA	0 aC	0 aC	0 aB	0 aB	0 aB
MgO NC + Polylysine (2000 µg/ml)	4.09 ± 0.07aA	0 aC	0 aC	0 aB	0 aB	0 aB

Values are mean ± standard deviation (n = 3). LDPE: Low-density polyethylene. MgO NC: Magnesium Oxide nanocomposite film. Different letters within the same column (uppercase letters) and row (lowercase letters), show the statistically difference ($P < 0.05$).

Polylysine at all concentrations (500, 1000 and 2000 µg/ml) completely inhibited *L. monocytogenes* cells growth. The bactericidal effect of polylysine was exhibited at lower concentrations 500 µg/ml against *L. monocytogenes* than *E. coli* O157:H7. It was observed that Gram-positive bacteria were more sensitive

against polylysine than Gram-negative bacteria. The antimicrobial activity of polylysine depends on the concentration and the pathogen type.

Antibacterial effects of MgO NC film and polylysine in beef (In vivo)

The beef samples was free from any primary contamination of *E. coli* O157:H7 and *L. monocytogenes*. Table 3 shows the effect of different film treatment on the viable count of *E. coli* O157:H7 in beef packed under vacuum at 4°C for 20 days. On day 5, the population of *E. coli* O157:H7 in beef packaged with LDPE film reached to 5.55 ± 0.11 log CFU/sample from the initial population of 5.03 ± 0.06 log CFU/sample. In beef packaged with MgO NC film, the bacterial population reached to 5.10 ± 0.09 log CFU/sample, as a result, MgO NC film inhibited the growth of *E. coli* O157:H7 on the surface of beef samples by 0.4 log CFU/sample and from day 5 onwards, the number of bacteria in this group was significantly lower than the control ($P < 0.05$). Polylysine at all concentrations in beef samples showed a weak inhibitory activity against *E. coli* O157:H7 compared to the control ($P < 0.05$). No additional reduction in the growth of *E. coli* O157:H7 cells in beef were seen by combining the different concentrations of polylysin with MgO NC ($P > 0.05$).

Table 3
Effect of 10% Magnesium Oxide nanocomposite (MgO NC) film and different concentration of polylysine on the viability of *E. coli* O157:H7 on beef during 20 days storage at 4 ºC.

Treatments	Viable counts (Log CFU/sample)				
	Day 0	Day 5	Day 10	Day 15	Day 20
LDPE (control)	5.03 ± 0.06 aA	5.55 ± 0.11 bA	6.03 ± 0.03 cA	5.82 ± 0.02 dA	5.41 ± 0.91 bA
MgO NC	5.02 ± 0.02 aA	5.10 ± 0.09 acC	5.49 ± 0.43 bD	5.44 ± 0.07 bC	5.17 ± 0.05 cB
LDPE + Polylysine (500 µg/ml)	5.06 ± 0.07 aA	4.91 ± 0.04 bB	5.17 ± 0.09 aB	4.68 ± 0.10 cB	5.05 ± 0.04 aB
LDPE + Polylysine (1000 µg/ml)	5.01 ± 0.02 aA	5.07 ± 0.06 aC	4.80 ± 0.05 bC	4.79 ± 0.05 bB	4.88 ± 0.08 bC
LDPE + Polylysine (2000 µg/ml)	5.00 ± 0.06 aA	4.75 ± 0.08 bD	4.77 ± 0.04 bC	4.67 ± 0.04 bB	4.68 ± 0.04 bD
MgO NC + Polylysine (500 µg/ml)	5.00 ± 0.02 aA	4.90 ± 0.07 aB	4.75 ± 0.01 bC	5.01 ± 0.08 aD	5.11 ± 0.05 aB
MgO NC + Polylysine (1000 µg/ml)	5.02 ± 0.08 aA	4.84 ± 0.12 bB	4.78 ± 0.09 bC	4.71 ± 0.03 bB	5.05 ± 0.06 aB
MgO NC + Polylysine (2000 µg/ml)	5.01 ± 0.02 aA	4.89 ± 0.08 bB	4.79 ± 0.07 bC	4.91 ± 0.04 bB	5.00 ± 0.06 aB

Values are mean ± standard deviation (n = 3). LDPE: Low-density polyethylene. MgO NC: Magnesium Oxide nanocomposite film. Different letters within the same column (uppercase letters) and row (lowercase letters), show the statistically difference ($P < 0.05$).

Table 4 shows the viable count of *L. monocytogenes* cells after different film treatments in beef samples packaged under vacuum at 4°C for 20 days. On day 5, the population of *L. monocytogenes* decreased in all beef samples similar to control due to low storage temperature. While *L. monocytogenes* cells in control grew to 4.60 ± 0.10 log CFU/sample after 5 days, the number of *L. monocytogenes* cells in beef packaged with MgO NC film were decreased to 4.43 ± 0.0 log CFU/sample. MgO NC film inhibited the growth of bacterial cells in beef, significantly ($P < 0.05$). The MgO NC film showed the least inhibiting effect on beef. On day 20, the populations of *L. monocytogenes* were similar in the presence of MgO NC film and the control ($P > 0.05$).

Table 4
effect of 10% Magnesium Oxide nanocomposite (MgO NC) film and different concentration of polylysine on the viability of *L. monocytogenes* on beef during 20 days storage at 4 ºC

Treatments	Viable counts (log CFU/sample)				
	Day 0	Day 5	Day 10	Day 15	Day 20
LDPE (control)	5.00 ± 0.04 aA	4.60 ± 0.10 bA	4.89 ± 0.22 aA	5.33 ± 0.08 cA	5.41 ± 0.91 dA
MgO NC	5.00 ± 0.11 aA	4.43 ± 0.00 bB	4.65 ± 0.09 bB	4.98 ± 0.02 aD	5.30 ± 0.03 cA
LDPE + Polylysine (500 µg/ml)	5.00 ± 0.04 aA	4.69 ± 0.04 bA	4.67 ± 0.07 bB	4.76 ± 0.05 bB	5.05 ± 0.04 cB
LDPE + Polylysine (1000 µg/ml)	5.00 ± 0.03 aA	4.54 ± 0.03 bAB	4.43 ± 0.09 bBD	4.54 ± 0.20 bBE	4.88 ± 0.08 aC
LDPE + Polylysine (2000 µg/ml)	5.00 ± 0.01 aA	4.54 ± 0.02 bAB	4.16 ± 0.05 cC	3.95 ± 0.06 dC	3.73 ± 0.03 eD
MgO NC + Polylysine (500 µg/ml)	5.00 ± 0.12 aA	4.69 ± 0.08 bA	4.67 ± 0.06 bB	4.80 ± 0.07 bBF	5.46 ± 0.25 cA
MgO NC + Polylysine (1000 µg/ml)	5.00 ± 0.07 aA	4.44 ± 0.08 bB	4.33 ± 0.20 bD	4.44 ± 0.08 bE	4.75 ± 0.02 cE
MgO NC + Polylysine (2000 µg/ml)	5.01 ± 0.08 aA	4.37 ± 0.0 bB	3.96 ± 0.00 cE	3.83 ± 0.03 cF	3.63 ± 0.17 dD

Values are mean ± standard deviation (n = 3). LDPE: Low-density polyethylene. MgO NC: Magnesium Oxide nanocomposite film. Different letters within the same column (uppercase letters) and row (lowercase letters), show the statistically difference ($P < 0.05$).

The population of *L. monocytogenes* in beef samples treated with all concentrations of polylysine decreased compared to the control ($P < 0.05$). The highest reduction of bacterial cells was observed at the end of storage time in 2000 µg/ml polylysine. The antimicrobial activity of polylysine, increased with concentration. The combination of polylysine with MgO NC film did not show the synergistic inhibitory

effect against *L. monocytogenes* on the beef except at concentration 2000 µg/ml which show the synergistic effect.

MgO Migration From MgO NC Film To Beef

The results of the magnesium release of the MgO NC film (10% w MgO) revealed that 9.8 ± 3.4 µg/cm^2 Mg ions migrated from film to beef samples.

Discussion

The antimicrobial activities of MgO NPs well documented [8, 11, 44, 45]. The results of this study have demonstrated that LDPE-MgO NC film had the antimicrobial efficiency against major foodborne pathogens, *E. coli* O157:H7 and *L. monocytogenes*. Gram-positive bacteria had a more antimicrobial sensitivity to MgO NC film when compared with Gram-negative bacteria. More susceptibility of Gram-positive bacteria (*Campylobacter jejuni*) to MgO NPs compare to Gram-negative bacteria (*E. coli* O157:H7 and *Salmonella* Enteritidis*)* also confirmed by He et al. (2016) [8].

The difference in sensitivity of Gram-positive and Gram-negative bacteria to NPs is mostly attributable to the differences in the cell wall structure and surface charge [7, 8, 46]. Gram-positive bacteria have a thick peptidoglycan layer that contains teichoic acids. Presence of phosphodiester bonds between teichoic acid monomers gives an overall negative charge to the Gram-positive bacteria, while Gram-negative bacteria have a thin peptidoglycan layer surrounded by phospholipids and lipopolysaccharides, therefore positively charged ions have more tendency to attach to Gram-positive bacteria as compared with Gram-negative bacteria [7].

In polymer/metal NCs, the main toxic mechanism relates to the NPs meaning, two possible routes depending on the species considered as the active agent: the metal NPs and the metal ions released from the particles [47]. The mechanism of antimicrobial behavior of polymer/metal NCs based on thermoplastic matrices, including the absorption of bacteria on the polymer surface which trigger the diffusion of water through the polymer matrix and after that,water with dissolved oxygen reaches the surface of embedded metal NPs allowing dissolution or corrosion processes and in this way metal ions are realized. These ions reach the composite surface damaging the bacterial membrane and Afterward, metal ions can diffuse into the bacteria [29, 48].

This study indicated that the MgO NC film had a less antimicrobial effect against the foodborne pathogens on the beef when compared to culture media. Reduced activity of antimicrobial agents in real food media has also been reported by others [26, 49]. Since vacuum packaging allows direct contact between the film coating and beef surfaces, inefficiency of the antimicrobial film could be due to properties of beef [26]. It has been reported that MgO has a good bactericidal performance in aqueous environments due to the formation of superoxide (O_2) anions on its surface [50]. It could be explained that, the beef surface has less moisture available as compared with broth culture media, which reduces the release of metal ions from the matrix to the beef surface. This could be related to inactivation of

antimicrobial agents by various components in the food, additionally, the beef matrix provides a protective environment to the pathogen as compared with the culture media, [36].

In the present study, polylysine could reduce the number of *E. coli* O157:H7 and *L. monocytogenes* in the culture media and on the beef surface. The availability of polylysine as an antimicrobial agent against *E. coli* O157:H7 and *L. monocytogenes* has been reported previously [17, 22, 46, 51]. Geornaras et al. (2007) compared the antimicrobial effects of polylysine against pathogens in 6 food extracts. The food extracts were, skim and whole fat milk, beef, bologna, rice, and vegetables.The antimicrobial effects of polylysine were more pronounced in the food extracts that did not contain high protein levels (rice and vegetables) [17]. Solomakos et al. (2008) investigated that the inhibitory activity of nisin against *L. monocytogenes* in minced meat. The efficacy of niacin decreased in the food model, in comparison with the in vitro control media. This might be due to nisin binding to proteins and fat or to react with meat proteases [27]. Numerous studies have reported that higher concentrations of antimicrobial agents are required in food systems to inhibit microorganisms than in growth media [26, 52, 53].

The use of antimicrobial agents can be limited by cost, restrictions on concentrations in foods, and potential changes to organoleptic properties. Combinatorial approaches that produce additive or synergistic effects allow for reductions in individual antimicrobial concentrations while achieving the same level of control [54]. When the combined effect of substances is higher than the sum of the individual effects, this is synergy [55]. In the synergistic inhibitory effect, it is reasonable to suggest that one component may facilitate the uptake or activity of another [56]. In culture media, a synergistic interaction between MgO film and polylysine has identified which was assumed that was due to the membrane destabilizing action which could facilitate enhanced diffusion of antimicrobials through cell membranes. No synergistic effects were observed between MgO NC film with polylysine against mentioned pathogens on the beef.

The studies evaluated the antimicrobial efficacy of MgO NPs in combination have been published. Jin and He (2011) were observed a synergistic effect of MgO NPs with nisin against *E. coli* O157:H7 and *Salmonella* Stanley. It might be explained that the large pores in the membrane caused by nisin, facilitate either MgO molecules penetration into microbial cells to express their antibacterial activity or release the intracellular components [45]. Some studies have been conducted on the synergistic effects of polylysine and other antimicrobial agents. Liu et al. (2015) investigated the combined effects of polylysine and nisin compared with nisin alone. This combination showed total synergism against bacteria, Thus, the following synergistic antibacterial mechanism of polylysine and nisin has been proposed: nisin rapidly destroys the cell wall and membranes of bacteria, and subsequently, polylysine penetrate into the cells to cause DNA damage [57]. Furthermore, there are some generally accepted mechanisms of antimicrobial interaction for synergy such as sequential inhibition of a common biochemical pathway, inhibition of protective enzymes, combinations of cell wall active agents, and use of cell wall active agents enhance the uptake of other antimicrobials [58].

The application of metal oxide NPs as new antibacterial agents strikingly depends on their cytotoxic nature [59]. However, there is limited toxicology information available regarding the effects of MgO NPs [60]. It doesn't seem that MgO NPs at low concentrations can be considered as a health hazard. In vitro toxicological assessment reported that low concentrations (0.3 mg/ml) of MgO NPs were not being toxic to human cells [61, 62]. In another study the toxicity effects of ZnMgO nano and MgO and ZnO nano were evaluated in mammalian cells. In this study pointed out that ZnO nano was toxic when applied to human HeLa cells, while MgO nano and the mixed oxide did not induce any cell damage [9]. The amount of migration of Mg ions was negligible and Mg has proven to be a GRAS compound, so MgO NC film can be considered as safe for active food packaging.

Conclusion

In this study, MgO NC film based on LDPE as a novel antimicrobial film could slightly inhibit the growth of *E. coli* O157:H7 and *L. monocytogenes* in culture media and in raw beef. The MgO NC film was less effective on beef compared to culture media. Polylysine at concentrations of 500 µg/ml or more had an inhibitory effect on *E. coli* and *L. monocytogenes* in the culture medium and beef. This natural preservative had a synergistic inhibitory effect on MgO NC film in the culture medium but not in raw beef. Although the migration of MgO nanoparticle from the NC film to beef was very low, but as it has little antimicrobial effect, it is not recommended as a suitable package for improving the safety of raw beef.

Declarations

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Author Contributions

MS, SSS and HRG designed the experiments. SM and SSS performed the experiments. SM wrote the manuscript. SSS reviewed the article.

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Availability of data and materials

The datas are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

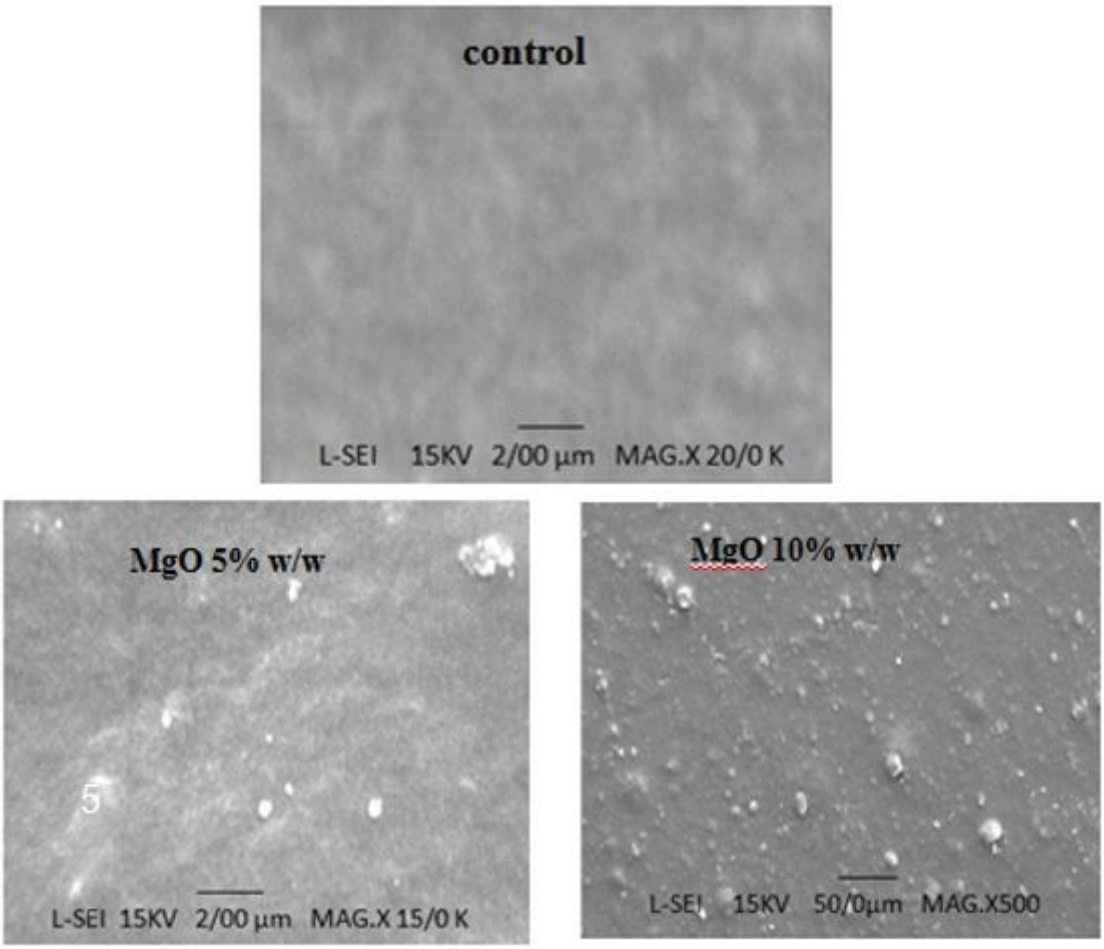

Figure 1

SEM images of 5 and 10% MgO nanocomposite (MgO NC) and Low-density polyethylene (LDPE) films

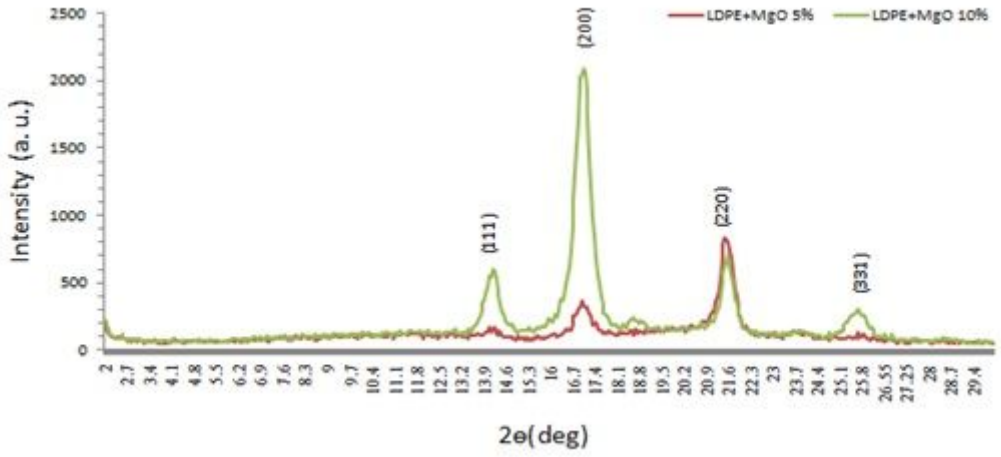

Figure 2

XRD pattern of 5 and 10% MgO nanocomposite (MgO NC) films

Supplementary Files

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