Construction and functional assessment of zein thin film incorporating spindle-like ZnO crystals

Ying Cao, Ting-Ting Chen, Wei Wang, Meng Chen and Hua-Jie Wang*

1. Introduction

Food-borne diseases are becoming one of the serious problems faced by humans along with environmental contamination.1 Technological innovations in food safety related to consumer confidence and human health are becoming extremely urgent. Traditional synthetic plastics have been widely applied in food packaging in an accelerated mode. Although they are from low-cost raw materials and have good mechanical properties, poor biodegradability is one of their fatal flaws and it is resulting in a massive accumulation of plastic waste and subsequent serious environmental problems.2 Therefore, scientists are concentrating their efforts on finding new techniques and packaging materials.3

Biodegradable and natural polymers have recently aroused great attention for their relative abundance, film-forming ability or nutritional qualities.4,5 Especially, the appearance of slow-release technique drives the development of the native polymers-based active packaging, which is becoming a highlight in food industry.5–8 For example, Dalarbashi et al. fabricated a new active packaging film made from a soluble soybean polysaccharide incorporating ZnO nanoparticles, which showed good antibacterial, antifungal and yeasticidal activities.9

This study aimed to prepare an active packaging made of zein and ZnO crystals. Herein, biomimetic synthesis, characterizing with environmental-friend and facile conditions, has been used to fabricate spindle-like ZnO crystals. Zein, a predominant storage protein of corn, has exhibited great biodegradability and excellent biocompatibility, and can act as the structure-directing agent for nanocrystal synthesis and the formation of films.10–17 EDX mapping confirmed that ZnO crystals could homogeneously distribute in zein thin films. The zein thin film incorporating ZnO crystals could effectively inhibit the growth of Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus), and these antibacterial activities could maintain a long-term effect. All of these results support the conclusion that zein thin films incorporating ZnO crystals are suitable to act as a functional packaging materials.

2. Material and methods

2.1 Materials

Zein was purchased from Sigma (St. Louis, MO, USA). Zinc chloride (>99.999%, MW = 136.3 g mol⁻¹, AR) was purchased from Tianjin Chemical Reagent Factory (Tianjin, China). All other solvents and chemicals were analytical grade.

2.2 Biomimetic synthesis of spindle-like zein-conjugated ZnO crystals with hierarchical structure

Spindle-like zein-conjugated ZnO crystals with a hierarchical structure were prepared by a modified biomimetic method using zein as the structure-directing agent. Briefly, 20 mL of
2.6 Water vapour permeability (WVP)

The WVP of the zein thin film was determined according to the report of Abdollahi et al. Briefly, glass permeation cups (diameter = 30 mm, n = 3) containing distilled water were covered with the zein thin film and kept static at 20 °C and 1.5% relative humidity. The weight of the cup was recorded at 1 h intervals for 8 h. The WVP was calculated as follows:

\[
WVP = \frac{WVTR \times L}{\Delta P}
\]

where WVTR is the measured water vapour transmission rate (g m⁻² s⁻¹) through the film, \(L\) is the mean film thickness (m), and \(\Delta P\) is the partial water vapour pressure difference (Pa) across the two sides of the film.

2.7 Antibacterial activity

The antibacterial activities of the thin films were evaluated using the disc diffusion method and two microorganism strains of *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria) were chosen. Briefly, all microorganism pre-inoculum cultures were grown overnight at 37 °C in 20 mL of nutrient broth (made of 1 g L⁻¹ beef extract; 5 g L⁻¹ neutralized peptone; 2 g L⁻¹ yeast extract; 5 g L⁻¹ NaCl) and subjected to horizontal shaking at 100 rpm. The samples (diameter = 6 mm, \(n = 6\)) were placed onto the nutrient agar that was previously inoculated with different microorganisms. After incubation for 24 h, the presence of a zone of growth inhibition below and around the specimen was checked by visual inspection.

2.8 Statistics

All data represented as the mean ± SD and the number of the independent replicates is individually shown for each experiment. The analysis of data was carried out by one-way factorial analysis of variances (ANOVA) and multiple comparisons (Fisher’s method as post hoc test, \(p < 0.05\)).

3. Results and discussion

The specific requirements for environment protection and food safety drive the innovation of food packaging with an emphasis on the roles of biodegradability and antibacterial properties. This study focused on understanding the feasibility to construct a zein thin film incorporating ZnO crystals. We firstly developed a modified biomimetic synthesis route to fabricate zein conjugated ZnO crystals. This route had two advantages. First, the entire synthesis route could be accomplished under eco-friendly and gentle conditions, which will reduce the dubious factors from an organic system and investment cost from special equipment. Second, the participation of zein in spindle-like ZnO crystals will be helpful for their better distribution in a zein thin film. With our interest in active food packaging, we envisaged that a zein thin film incorporating ZnO crystals will generate a new class of active food packaging that could be biodegradable and antibacterial.

The XRD analysis clearly demonstrates the formation of ZnO crystals as shown in Fig. 1A. It can be seen that eight
characteristic peaks for ZnO appear at 2θ at 31.769°, 34.421°, 36.252°, 47.538°, 56.602°, 62.862°, 66.378°, 67.961° and 69.098°, corresponding to ZnO (100), (002), (101), (102), (110), (103), (200), (110) and (201) diffractions. These peaks are in good agreement with the reported data of the JCPDS card 36-1451. In this study, we supplemented 60% ethanol into the reactive system instead of high temperature to promote the transformation from Zn(OH)$_2$ to ZnO. Moreover, biomimetic synthesis allows us to control the morphologies and size of nanocomposites using native molecules as templates. Both SEM and TEM clearly confirm that zein acts as a good transport carrier in Ostwald ripening and induces the formation of a spindle-like structure that is comprised of nanoparticles (Fig. 1B-E).

Fig. 2 shows the FTIR spectrum of pure ZnO crystals, pure zein and zein conjugated ZnO crystals. It can be seen that the typical Zn-O stretching band in pure ZnO crystals appears between 430 cm$^{-1}$ and 520 cm$^{-1}$ (Fig. 2A). As for pure zein, the peaks at 3396 cm$^{-1}$, 2958 cm$^{-1}$, 1651 cm$^{-1}$ and 1539 cm$^{-1}$ are clearly separated and can be assigned to the stretching vibration of a hydroxyl group, amide A', and amide I and amide II, which are the typical protein absorption peaks (Fig. 2B). By comparing Fig. 2A and B, the FTIR spectrum of zein conjugated ZnO crystals supplies direct evidence of zein participating in the formation of spindle-like ZnO crystals (Fig. 2C).

According to TG/DTA analysis, we could easily quantify the zein content in ZnO crystals (Fig. 3A). The total weight loss of the ZnO crystals decreases to 44.42% from 250 °C to 650 °C according to the TG curve. In the DTA curve, there are three exothermic peaks, appearing at 365 °C, 485 °C and 531 °C,
activities are species-dependent.\textsuperscript{30,31} Moreover, these antibacterial activities are species-dependent.\textsuperscript{30,31} For example, Baek and An demonstrated that \textit{S. aureus} was more sensitive than \textit{E. coli} to ZnO nanoparticles.\textsuperscript{38} Sultana \textit{et al.} reported that ZnO nanoparticles exhibited stronger antibacterial activity against \textit{S. aureus} and \textit{Bacillus subtilis} compared to \textit{E. coli} and \textit{Pseudomonas aeruginosa}.\textsuperscript{34} In this study, we also tested the antibacterial activities of zein conjugated ZnO crystals against \textit{S. aureus} and \textit{E. coli} (Fig. 4). The results indicate that the ZnO crystals keep a positive correlation with ZnO concentrations. At the same time, \textit{S. aureus} shows higher sensitivity than \textit{E. coli} to ZnO crystals, with 1.14 $\mu$g mm$^{-2}$ and 1.89 $\mu$g mm$^{-3}$ of minimum inhibitory concentration, respectively.

Observations of the monodispersed and hierarchical structure and excellent antibacterial activity of zein conjugated ZnO crystals prompted us to focus on the fabrication of zein thin films incorporating ZnO crystals. The obtained thin films are yellow and transparent as shown in Fig. 5A. Moreover, we could easily control the thickness by adjusting the content of raw materials. Fig. 5B and C show the microstructure of the thin film observed by SEM and there are a few remaining air bubbles in the film. As one of the typical elements of a protein, N could be detected by EDX analysis (Fig. 5D). In addition, we also can detect Zn, which is from ZnO crystals. By the AAS test, the loading of ZnO in a thin film can get to 0.66 mg cm$^{-2}$. The homogeneity of the ZnO crystals in a thin film was directly observed by EDX mapping based on the distribution of Zn and N. As shown in Fig. 5E–G, zein has a homogeneous distribution according to N mapping, while the presence of only a slight aggregate of Zn signals suggests a homogeneous distribution of ZnO in a thin film.

Protein-based materials are known to undergo naturally-controlled degradation processes. Zein, the predominant storage protein of corn, has exhibited great biodegradability. In previous studies several groups including our group, have demonstrated that zein could be degraded via enzymolysis.\textsuperscript{14,15,32} In order to understand the biodegradability of a zein thin film, we further studied its degradation process \textit{in vitro}. A zein thin film ($d = 1.5$ cm) was immersed into 4 mL of an enzyme solution containing 12.5 U mL$^{-1}$ collagenase and incubated at 37 °C in an incubator under static conditions. As shown in Fig. 6, the degradation rate of the zein thin film increases gradually in 5 days and reaches 66.8%, suggesting its good biodegradability. In addition, we also tested the water vapour permeability of the zein thin film. The result shows that the WVP of the zein thin film incorporating ZnO crystals reaches 4.15 ± 0.12 (g ms$^{-1}$ Pa$^{-1}$) $10^{-11}$. The relatively lower permeability might result from the water-insoluble property of zein.

Active food packaging is currently a focus of attention that is linked to the development of the shelf-life and safety of packaged food.\textsuperscript{33,34} Herein, we investigated the antibacterial activities of a zein thin film incorporating ZnO crystals against various bacterial pathogens. As shown in Fig. 7, thin films can effectively inhibit the growth of \textit{S. aureus} and \textit{E. coli}. Furthermore, the inhibition zone size reached 25.39 ± 0.90 mm against
It can be seen that the thin films kept a high stability under different pH conditions ranging from 5.0 to 9.0 and the highest percentage of released Zn\(^{2+}\) from the thin films was only 17.4% in 4 weeks. It has been well documented that ZnO can release Zn\(^{2+}\) and the excess zinc can penetrate through the bio-membrane, affect the antioxidant defence system and further induce toxicity.\(^9,35,36\) Therefore, this result can explain why the thin film still has high antibacterial activities after 4 weeks of immersion. In summary, it will be very interesting to further study the application of a zein thin film incorporating ZnO crystals in food packaging due to its better film-forming effect and excellent antibacterial activities.

### 4. Conclusion

In summary, this study prepared a zein thin film incorporating ZnO crystals by the solution-casting method. Spindle-like zein conjugated ZnO crystals were synthesized via a green and gentle route. The participation of zein in zein conjugated ZnO crystals was key for the homogeneous distribution of ZnO crystals in the final zein thin film. At the same time, the synthesized zein thin film incorporating ZnO crystals showed high and long-term antibacterial activities against both Gram-negative and Gram-positive pathogens. Moreover, the bacteriostatic effect is due to the released zinc ion from the thin film. These data can serve as a guide for the application of a zein thin film incorporating ZnO crystals to food packaging material.

### Acknowledgements

This study was financially supported by the National Science Foundation of China (20971039 and 31000774), Program for Science & Technology Innovation Talents in Universities of Henan Province (HASTIT, 16HASTIT049), Innovation Scientists and Technicians Troop Construction Projects of Henan Province (C201500018), Henan Science and Technology Research Program (162102210257), and Zhengzhou Science and Technology Plan Project (20150484).

### Notes and references

1. N. B. Johnson, L. D. Hayes, K. Brown, E. C. Hoo and K. A. Ethier, *MMWR Surveillance Summaries: Morbidity and Mortality Weekly Report*, 2014, vol. 63, suppl. 4, p. 3.

2. H. Rostamzad, S. Y. Paighambari, B. Shabanpour, S. M. Ojagh and S. M. Mousavi, *Food Packaging and Shelf Life*, 2016, vol. 7, p. 1.

3. N. N. V. Long, C. Joly and P. Dantigny, *Int. J. Food Microbiol.*, 2016, 220, 73.

4. C. Pires, C. Ramos, G. Teixeira, I. Batista, R. Mendes, L. Nunes and A. Marques, *J. Food Eng.*, 2011, 105, 422.

5. P. Tongnuanchan, S. Benjakul, T. Prodpran and P. Songtipy, *Int. J. Biol. Macromol.*, 2011, 48, 758.

6. P. Appendini and J. H. Hotchkiss, *Innovative Food Sci. Emerging Technol.*, 2002, 3, 113.

7. L. Wang, F. Liu, Y. Jiang, Z. Chai, P. Li, Y. Cheng, H. Jing and X. Leng, *J. Agric. Food Chem.*, 2011, 59, 12411.
8 A. Llorens, E. Lloret, P. Picouet and A. Fernandez, *Int. J. Food Microbiol.*, 2012, **158**, 113.

9 D. Salarbashi, S. A. Mortazavi, M. S. Noghabi, B. S. F. Bazzaz, N. Sedaghat, M. Ramezani and I. Shahabi-Ghahfarrokhi, *Carbohydr. Polym.*, 2016, **140**, 220.

10 J. Dong, Q. S. Sun and J. Y. Wang, *Biomaterials*, 2004, **25**, 4691.

11 H. J. Wang, Z. X. Lin, X. M. Liu, S. Y. Sheng and J. Y. Wang, *J. Controlled Release*, 2005, **105**, 120.

12 H. J. Wang, S. J. Gong, Z. X. Lin, J. X. Fu, S. T. Xue, J. C. Huang and J. Y. Wang, *Biomaterials*, 2007, **28**, 3952.

13 Z. H. Qu, H. J. Wang, T. T. Tang, X. L. Zhang, J. Y. Wang and K. R. Dai, *Acta Biomater.*, 2008, **4**, 1360.

14 X. M. Liu, Q. S. Sun, H. J. Wang, L. Zhang and J. Y. Wang, *Biomaterials*, 2005, **26**, 109.

15 S. J. Gong, H. J. Wang, Q. S. Sun, S. T. Xue and J. Y. Wang, *Biomaterials*, 2006, **27**, 3793.

16 P. Argos, K. Pedersen, M. D. Marks and B. A. Larkin, *J. Biol. Chem.*, 1982, 257, 9984.

17 A. S. Tatham, J. M. Field, V. J. Morris, K. J. I’Anson, L. Cardle, M. J. Dufton and P. R. Shewry, *J. Biol. Chem.*, 1993, **268**, 26253.

18 M. Abdollahi, M. Rezaei and G. Farzi, *J. Food Eng.*, 2012, **111**, 343.

19 D. S. Cha and M. S. Chinnan, *Crit. Rev. Food Sci. Nutr.*, 2004, **44**(4), 223.

20 B. Liu and H. C. Zeng, *Langmuir*, 2004, **20**, 4196.

21 A. Vyalikh, P. Simon, E. Rosseeva, J. Buder, U. Scheler and R. Kniep, *Sci. Rep.*, 2015, **5**, 15797.

22 F. C. Meldrum, *Int. Mater. Rev.*, 2003, **48**(3), 187.

23 W. Z. Ostwald, *J. Phys. Chem.*, 1900, **34**, 495.

24 M. M. H. Farooqi and R. K. Srivastava, *J. Alloys Compd.*, 2017, **691**, 275.

25 R. Kripal, A. K. Gupta, R. K. Srivastava and S. K. Mishra, *Spectrochim. Acta, Part A*, 2011, **79**, 1605.

26 K. Raja, P. S. Ramesh and D. Geetha, *Spectrochim. Acta, Part A*, 2014, **131**, 183.

27 H. J. Wang, X. J. Yu, Y. Cao, B. Zhou and C. F. Wang, *J. Inorg. Biochem.*, 2012, **113**, 40.

28 Y. N. Chang, M. Zhang, L. Xia, J. Zhang and G. Xing, *Materials*, 2012, **5**(12), 2850.

29 L. C. Ann, S. Mahmud, S. K. M. Bakhori, A. Sirelkhatim, D. Mohamad, H. Hasan, A. Seeni and R. A. Rahman, *Ceram. Int.*, 2014, **40**(2), 2993.

30 Y. W. Baek and Y. J. An, *Sci. Total Environ.*, 2011, **409**(8), 1603.

31 S. Sultana, M. Z. Rafiuddin, M. Z. Khan and M. Shahadat, *J. Environ. Chem. Eng.*, 2015, **3**(2), 886.

32 H. J. Wang, J. C. Huang, L. Hou, T. Miyazawa and J. Y. Wang, *J. Mater. Sci.: Mater. Med.*, 2016, **27**, 92.

33 L. Gutierrez, A. Escudero, R. Battle and C. Nerin, *J. Agric. Food Chem.*, 2009, **57**, 8564.

34 L. Gutierrez, R. Battle, S. Andujar, C. Sanchez and C. Nerin, *Packag. Technol. Sci.*, 2011, **24**, 485.

35 U. I. Walther, S. C. Walther and O. Temruck, *Toxicol. In Vitro*, 2007, **21**, 380.

36 B. Wihelm, U. I. Walther and B. Fichtl, *Arch. Toxicol.*, 2001, **75**, 388.