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REVIEW

Zein-Based Micro- and Nano-Particles for Drug and Nutrient Delivery: A Review

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ABSTRACT: Zein is the major storage protein from corn with strong hydrophobicity and unique solubility and has been considered as a versatile food biopolymer. Due to the special tertiary structures, zein can self-assemble to form micro- and nano-particles through liquid–liquid dispersion or solvent evaporation approaches. Zein-based delivery systems have been particularly investigated for hydrophobic drugs and nutrients. Recently, increasing attention has been drawn to fabricate zein-based advanced drug delivery systems for various applications. In this review, the molecular models of zein tertiary structure and possible mechanisms involved in zein self-assembly micro- and nano-particles are briefly introduced. Then, a state-of-the-art introduction and discussion are given in terms of preparation, characterization, and application of zein-based particles as delivery systems in the fields of food science, pharmaceutics, and biomedicine. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40696.

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INTRODUCTION

Zein is the major storage protein in corn or maize, accounting for 35–60% of total proteins present in corn, and it is exclusively found in endosperm.[1,2] Zein is considered as a prolamine due to its characteristic solubility. It is insoluble in water unless specifically defined conditions are applied, such as a certain concentration of alcohol, high concentrations of urea, extreme alkali condition (pH > 11), and/or anionic detergents.[3] This unique solubility behavior of zein is ascribed to its amino acid sequence, with more than 50% being nonpolar, including leucine, proline, alanine, phenylalanine, isoleucine, and valine. It is worth mentioning that zein has negative dietary nitrogen balance, being deficient in basic and acidic amino acids and particularly absence in tryptophane and lysine. The combination of imbalanced amino acid profile and water insolubility results in the poor nutritional quality of zein and becomes the major obstacle for its applications in food products for human consumption, but instead, it has been widely explored as a native biopolymer for novel applications in the fields of food science, pharmaceutics, and biomedicine.

Zein represents a mixture of many proteins or polypeptides, which are generally classified by their solubility, charges, and molecular weights. Three major fractions have been identified so far according to their molecular weight and solubility, i.e., α-, β-, γ-zein.[4,5] α-Zein is the major fraction which accounts for 75–85% of the whole zein in corn, whereas β- and γ-zein fractions only made up 10–15% and 5–10%, respectively, depending upon the genotype. The molecular weight of α-zein is identified within the range of 21–25 kDa plus a small subfraction of 10 kDa. α-Zein is soluble in 50–95% alcohol without any reducing agents or buffers, and this solubility is much better than the other two fractions, which can only be solubilized with the aid of reducing agents and/or buffers. Considering all the fractions present in zein, the maximum solubility for whole zein was achieved in 70% ethanol-aqueous solution, which was the solvent used for extraction of zein from dry-milled corn.[6] So far, α-zein is the main type of zein that is commercially available in the market.

The unique physicochemical properties of zein protein have brought a wide variety of applications in different industries, especially in the fields of food and biomedicine. Particularly, the development of delivery vehicles made from zein has begun receiving tremendous attention in recent years. The main interests of this newly discovered research trend of zein arise from the following aspects: (1) corn zein is a generally recognized as safe (GRAS) biomaterial that has promising characteristics, including biocompatibility, biodegradability, and low toxicity; (2) the hydrophobic property and unique solubility result in the easy fabrication of zein micro-/nano-particles which are the ideal vehicles for hydrophobic drugs and nutrients; (3) the relatively slower digestibility of zein than other proteins makes zein-based delivery systems more desirable for oral route; (4)

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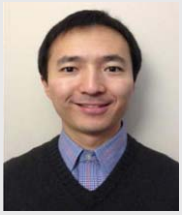

Qin Wang is an Assistant Professor of Food Nanotechnology at University of Maryland. She received her Ph.D. from Food Science and Human Nutrition at University of Illinois at Urbana-Champaign in 2004. The research in Dr. Wang's group focuses on design, characterization, and evaluation of natural polymers. Her research covers a broad scope of multidisciplinary areas including food science, materials science, nanotechnology, and biophysics.

the complex and/or conjugation formed between zein and other compounds are easy to fabricate, which convey advanced features to zein-based delivery systems.

MOLECULAR MODELS FOR ZEIN TERTIARY STRUCTURES

Based on the knowledge of amino acid sequences of α-zein and its largely distributed (50–60%) helical secondary structure, the nanostructure of zein molecules has been first proposed as a distorted cylinder model where 9–10 adjacent, topologically antiparallel, and homologous repeating helices are clustered.[7] Later, this model has been revised and further specified by Matsushima et al.,[8] based on small-angle X-ray scattering measurements. In this revised model, as shown in Figure 1, an elongated rectangular prism-like shape with three dimensions being 16, 4.6, and 1.2 nm, respectively, has been proposed.[9] In this model, the tandem helical units are linearly stacked glutamine-rich turns or loops and stabilized by intra-hydrogen bonds. Although the top and bottom surfaces containing glutamine-rich loops have a hydrophilic character, the other four surfaces are highly hydrophobic, and therefore, the two-dimensional aggregation may occur easily and extend infinitely.[8] This theory explains the reason that zein molecules exist as the aggregates instead of monomers even in 70% aqueous ethanol.

As an amphiphilic molecule, zein is able to self-assemble into a wide variety of mesostructures. In order to explore the practical applications of zein for constructing a delivery system, it is pivotal to understand detailed mechanisms of zein self-assembly, especially in the shape of sphere. Wang and Padua[10] recently investigated the possible mechanism at nanoscale for zein self-assembly from single molecules to nanospheres induced by ethanol evaporation during cast-drying process, using high-resolution transmission electron microscopy (TEM), fast Fourier transform image, as well as circular dichroism. The proposed mechanism with respective TEM images is illustrated in Figure 2. When the ethanol began to evaporate, the solvent hydrophilicity increased gradually, which induced conformational transition from α-helix to β-sheet. With the evaporation continued, the β-sheet would be packed in an anti-parallel way due to the hydrophobic interactions between two adjacent β-sheets, and then further form a long ribbon. Subsequently, the ribbon curled into a ring or toroid, followed by the closing of center hole. This four-step self-assembly process was evidenced by the TEM observed at different time point during ethanol evaporation. Wang and Padua[11] further established a model based on the hydrophobic and hydrophilic contribution to the interfacial free energy from zein and solvent. The model may be applied to predict solvent evaporation time and thus control over particle size of zein spheres. Therefore, understanding the self-assembly mechanism of zein spheres is crucial to develop micro- and nano-particles for encapsulation and delivery applications.

FABRICATION OF ZEIN PARTICLES AND ITS CHALLENGES

The strong hydrophobicity makes zein a unique natural biopolymer in solubility and therefore applications. Zein cannot be dissolved in water but is soluble in aqueous-alcohols, -glycols, and -acetone conditions. The manipulation of zein solubility via changing solvent conditions has resulted in various applications. The first report on producing zein particles by using its unique solubility can be traced back to 1991 in a U.S. Patent on preparation of hydrophobic protein microparticles,[12] and two other

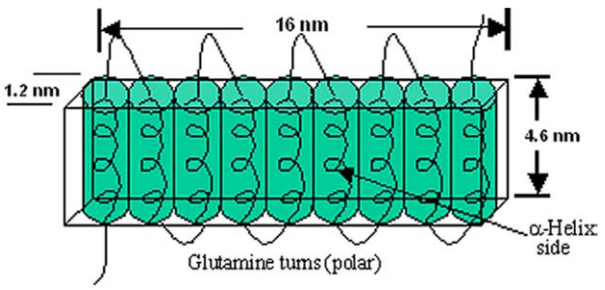

Figure 1. The tertiary structural model of zein protein (Reproduced from Ref. 9, with permission from Elsevier). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

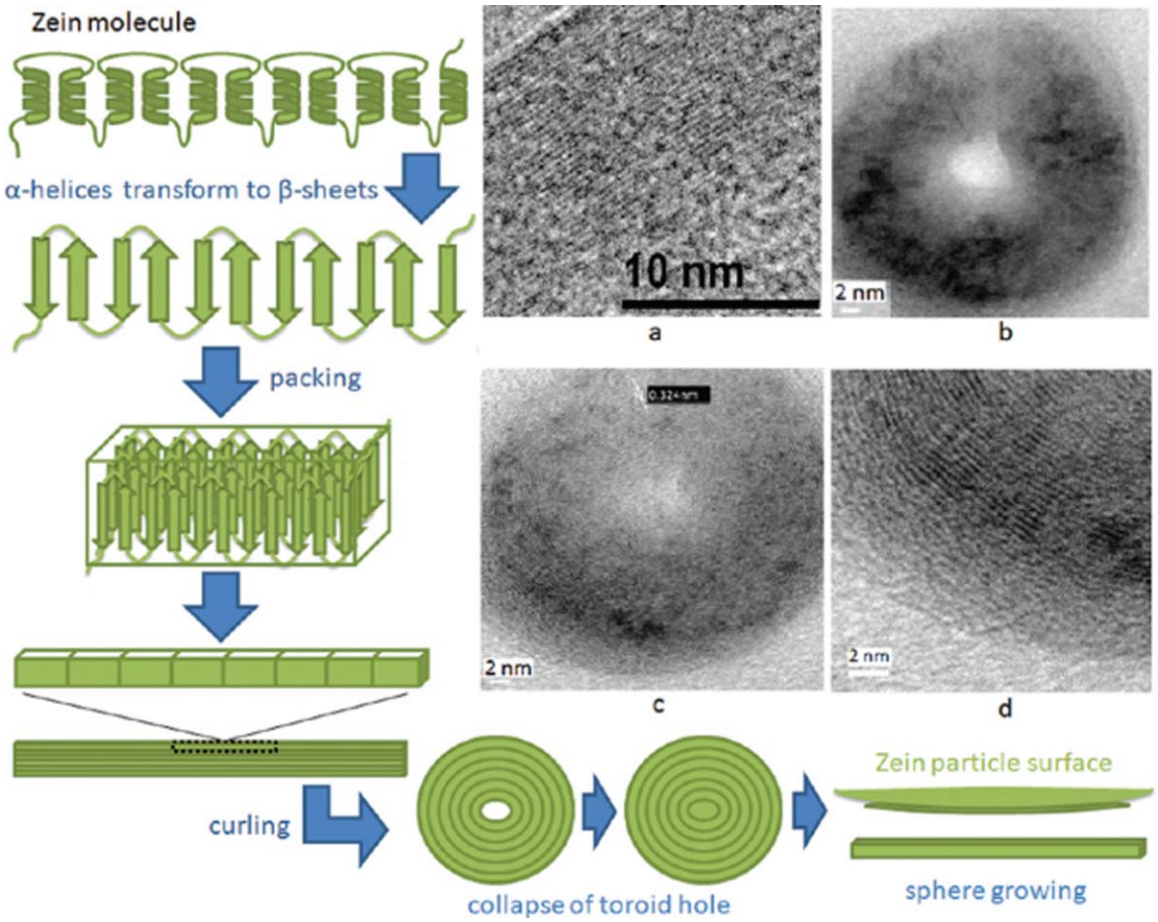

Figure 2. Possible mechanism for zein self-assembly from single molecules to nanospheres: (TEM image (A)) α-helices in the original zein solution transformed into *β*-sheet strands; (TEM image (B)) antiparallel *β*-sheets packed side by side forming a long ribbon, driven by hydrophobic interactions; the ribbon curled into a toroid or ring; (TEM image (C)) the center hole of the toroid was closed; and (TEM image (D)) disks enlarged by the addition of new *β*-sheet strands (Reproduced from Ref. 10, with permission from American Society of Chemistry). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

U.S. Patents issued in 1994 by the same inventor on the preparation of zein microparticles for coating applications in pharmaceutical industries.[13,14] Later on, one study was followed focusing on the preparation of zein microparticles as coating materials to develop tablets as solid dosage forms for drug delivery applications.[15] Although a few industrial patents have been published in 1990s, the systematic and detailed research studies investigating fabrication techniques on the formation of zein micro- and nano-particles have not been found until recent years.

Generally, the fabrication technique to prepare zein particles is considered as a liquid–liquid dispersion or antisolvent method. In this method, zein is first dissolved in aqueous-alcohol (55–90% v/v) solution, and then, the solution was poured or sheared into water to abruptly decrease the solvent concentration causing phase separation and formation of zein particles. The zein solution is transparent when the alcohol concentration is sufficiently high to completely dissolve zein; whereas it becomes opaque upon the change of solvent composition in which zein is no longer soluble and therefore phase separated. The major mechanisms involved are the solubility loss of zein molecules and simultaneous precipitation and solidification to particles upon decreasing of alcohol concentration. Solvent evaporation via cast-drying technique is another approach to fabricate films consisting of zein nanoparticles at basic pH conditions.[16] As shown in Figure 3, when zein was cast-dried at acidic condition it formed the films with holes, while if the pH raised to near neutral and basic conditions zein formed nanoparticles with uniform sizes ranging from 100 to 200 nm. This cast-drying process is also known as evaporation-induced self-assembly of zein particles, while the formation of nanoparticles may also depend on the surface where zein is cast-dried. This pH-dependent forming phenomenon was related to self-assembly behavior as affected by the rapid increase of environment polarity. During drying process, the gradual exposure of hydrophilic residues upon ethanol evaporation and the reinforced negative charges at basic conditions promoted formation of small particles. Another key element involved was the aluminum surface where zein solution was cast-dried. The high hydrophobicity of aluminum surface may also promote the exposure of zein hydrophilic residues to the air and hence facilitate the formation of zein nanoparticles during cast-drying

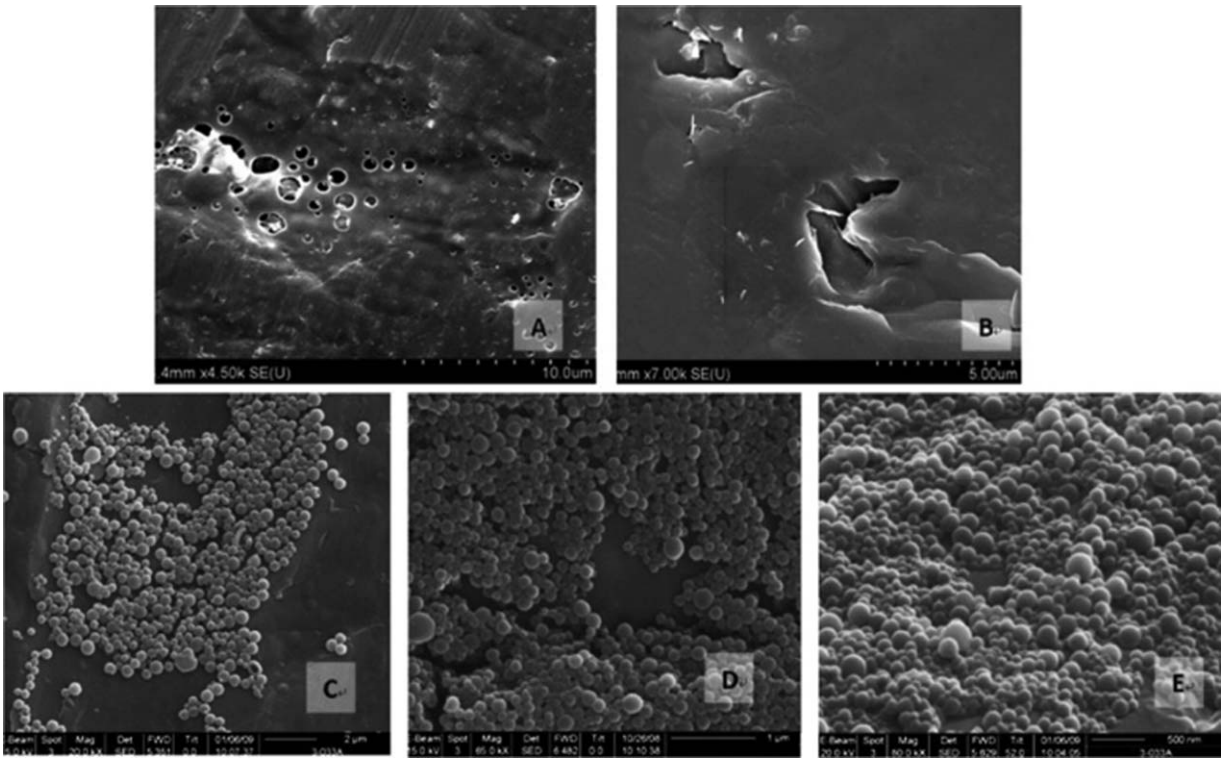

Figure 3. SEM images of pH-treated dried zein samples: (A) pH = 2.7; (B) pH = 3.3; (C) pH = 6.5; (D) pH = 10.5; and (E) pH = 12.5 (Reproduced from Ref. 16, with permission from Elsevier).

process. The morphology of evaporation-induced self-assembly of zein also depends on the zein concentration, if the pH of zein aqueous-alcohol solution is unadjusted. It was reported[17] that when zein concentration was around 2 mg/mL (in 70% ethanol), the self-assembly of zein molecules was triggered by ethanol evaporation (cast-drying), and formed free microspheres with diameter around 1 μm; whereas tightly packed microspheres were obtained at higher zein concentrations (10 mg/mL) with diameter around 3 μm. Further increasing zein concentration to 100 mg/mL the microspheres were fused into a film. Therefore, to precisely control the particle size of zein self-assembly, many factors such as pH, protein concentration, and ethanol content need to be taken into account, which will be discussed in detail in the following sections.

Typically, the particle size of zein nanoparticles ranges from 100 to 400 nm, depending on the fabrication parameters. For instance, increasing of shearing rate and alcohol concentration at pH conditions close to its isoelectric point (6.2–6.8) has been reported to produce zein nanoparticles with the smallest sizes.[18,19] The alcohols used to dissolve zein significantly influenced morphology and size of the particles. It has been reported that among different alcohols, ethanol was the best solvent to produce small particles with uniform sizes than isopropanol and methanol.[20] Recently, thermal treatment at 60°C was reported to affect the physicochemical of zein nanoparticles, by inducing aggregates of zein monomers to larger particles and exposure of hydrophobic domains to the surface of protein molecules, which may consequently provide new potentials for drug delivery applications.[21] To produce zein micro- and nanoparticles at industrial scale for encapsulation and delivery applications, several industrial scalable approaches have been recently reported, including supercritical anti-solvent,[22–24] flash nanoprecipitation,[25] and electrohydrodynamic atomization[26] technologies. However, these technologies have their own limitations and are to be further evaluated before industrialization. For instance, the supercritical antisolvent technique uses the supercritical CO_2 as an antisolvent to precipitate zein and solidify the particles; however, it is hard to produce zein particles at nanoscale with uniform sizes unless 100% methanol is used as the solvent and a strong flow rate of supercritical CO_2 is applied. This consequently increases the production cost and possible toxicity caused by the residue of organic solvent.[22] Flash nanoprecipitation is a relatively new technology to fabricate colloidal nanoparticles, employing the rapid mixing of a solvent with an antisolvent in a time less than the nucleation and growth time of the polymer.[27] This technique has been recently adopted to prepare zein nanoparticles, while the experimental setup was limited at laboratory scale using a lab-made confined impinging jet,[25] which can only process a small volume of zein at a time and its scale-up feasibility needs further investigation. For electrohydrodynamic atomization or electrospray, although it is a well-known technique to produce monodispersed spheres, it was recently reported that the morphology and dimension of zein particles were greatly affected by fabricating parameters.[26] For example, zein nanoparticles with compact and uniform structures were obtained only at the concentrations between 2.5% and 5%, whereas solutions at 10–15% yielded collapsed

and shrunken particles and transition of morphology from particles to fibers was observed if the concentration reached 20%. This phenomenon was due to the considerable changes of viscosity of zein solutions when the concentration was increased. If the viscosity is sufficiently high, the stable elongated jets were obtained and fibers were produced.

Although zein particulate delivery systems can be easily fabricated compared with other proteins, the redispersibility of zein particles after drying process, either freeze or spray drying, has become the major obstacle limiting its practical applications. Before drying, zein nanoparticles can be suspended well in aqueous phase with low alcohol concentrations (20–30%), but the dried powder of zein nanoparticles can no longer be redispersed in water due to the severe aggregation during drying process. Consequently, some surfactants are considered to enhance the redispersibility of zein nanoparticles, such as lecithin and pluronic F68.[19,28] However, those surfactants were not successful in producing small particles with narrow size distribution of zein particles. Recently, sodium caseinate has been explored as a stabilizer to form zein-caseinate complex nanoparticles that are freely redispersible in aqueous phase after freeze-drying. The isoelectric point (around pH 6.2) of zein protein was observed to shift to a lower value (pH 5.0) after the adsorption of caseinate on zein particles, thus preventing them from aggregation in the phosphate buffers with a wide range of ionic strength (15 mM−1.5M NaCl).[29] Sodium caseinate is an amphiphilic protein from milk,[30] and therefore, its hydrophobic portions can adsorb to the zein nanoparticles through hydrophobic interactions; meanwhile, the hydrophilic portions provide both steric and electrostatic repulsions to nanoparticles. Later on, another study explored the interactions between zein and caseinate under specialized conditions to further improve the dispersibility of zein nanoparticles after spray drying.[31] It was found that by heating zein and caseinate in 50% ethanol solution, κ-casein became a part of zein nanoparticles matrix and was not detached by increasing ionic strength during storage, resulting in the better dispersibility after spray drying. In this process, caseinate was dissociated in 50% ethanol upon heating and then disulfide bonds may be formed between κ-casein and zein protein molecules, making the complex nanoparticles more stable. In a recent study, we have discovered that the electrostatic deposition of a second layer of chitosan hydrochloride on the surface of zein-caseinate nanoparticles could further improve the dispersibility of zein nanoparticles and expand its encapsulation and delivery capacities.[32]

ZEIN PARTICLES FOR DELIVERY APPLICATIONS

Zein micro- and nano-particles have been studied as promising delivery systems specifically for hydrophobic drugs or nutrients. Generally, the hydrophobic bioactives are dissolved together with zein in aqueous ethanol binary solvent, and then mixed with an antisolvent, such as water, to co-precipitate bioactives with zein. During this process, bioactives would quickly migrate into zein particles due to strong hydrophobic interactions and hydrogen bonds. A variety of hydrophobic bioactives have been encapsulated into zein particles, including both drugs such as abamectin,[33] ciprofloxacin,[34] 5-fluorouracil,[35] and DNA,[36] and nutrients such as essential oils,[37,38] enzymes,[39,40] polyphenols.[41,42] The applications of zein particles are dependent upon the bioactives encapsulated, and thus, the following sections will discuss their applications as delivery systems for drugs and nutrients, separately.

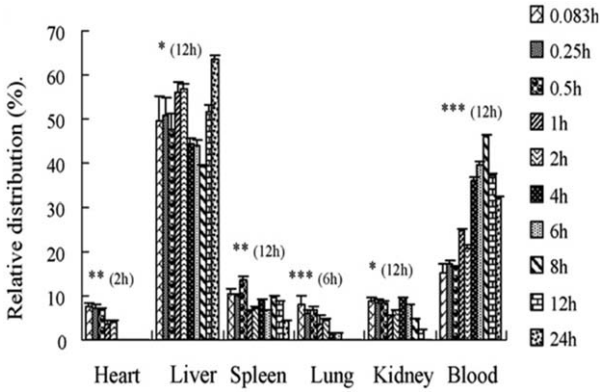

Figure 4. Relative fluorescence distribution in tissues and blood after intravenous administration of zein nanoparticles to mice (Reproduced from Ref. 35, with permission from Elsevier).

Zein Particles for Drug Delivery

For drug delivery applications, zein began to gain its attention since early 1990s, when a couple of U.S. Patents on zein microspheres preparation and applications were published by Mathiowitz and coworkers.[43–45] Then, a study on zein microspheres for oral drug delivery of ivermectin, an antiparasitic drug, was published in 2005.[46] Zein microspheres were prepared by phase separation technique and then further compressed into tablets. The diameter of zein microspheres was distributed between 0.3 and 1 μm, which was believed to provide optimal phagocytic uptake by macrophages and hence be delivered to targeted tissues. The high drug encapsulation efficiency and *in vitro* sustained release further implied potential of zein as oral drug delivery matrix. Zein microspheres similarly prepared were also applied to encapsulate heparin and cast-dried to form film by volatilization at 37°C, accelerating solvent evaporation.[47] Zein microspheres released heparin continuously and slowly in a controlled way for more than 20 days, while maintained good biocompatibility on the growth of human umbilical veins endothelial cells and excellent anticoagulant activity as determined with thrombin time assay. All of these features indicated that zein microspheres-based film may be a potential candidate for cardiovascular device to prevent acute or subacute thrombosis.

Zein nanoparticles encapsulating protein drugs have also been recently investigated to target activated macrophages.[40] Two antioxidant proteins (enzymes), catalase and superoxide dismutase, were encapsulated into zein nanoparticles. However, in this study, large amount of surfactants (sorbitol and glycerol) was used, in order to improve the miscibility of encapsulated proteins in aqueous alcohol solution as well as the stability and redispersibility of zein nanoparticles. Zein nanoparticles significantly preserved enzymatic activities of encapsulated enzymes from harsh conditions when tested in simulated gastrointestinal tract. The conjugation of folate onto therapeutic proteins in

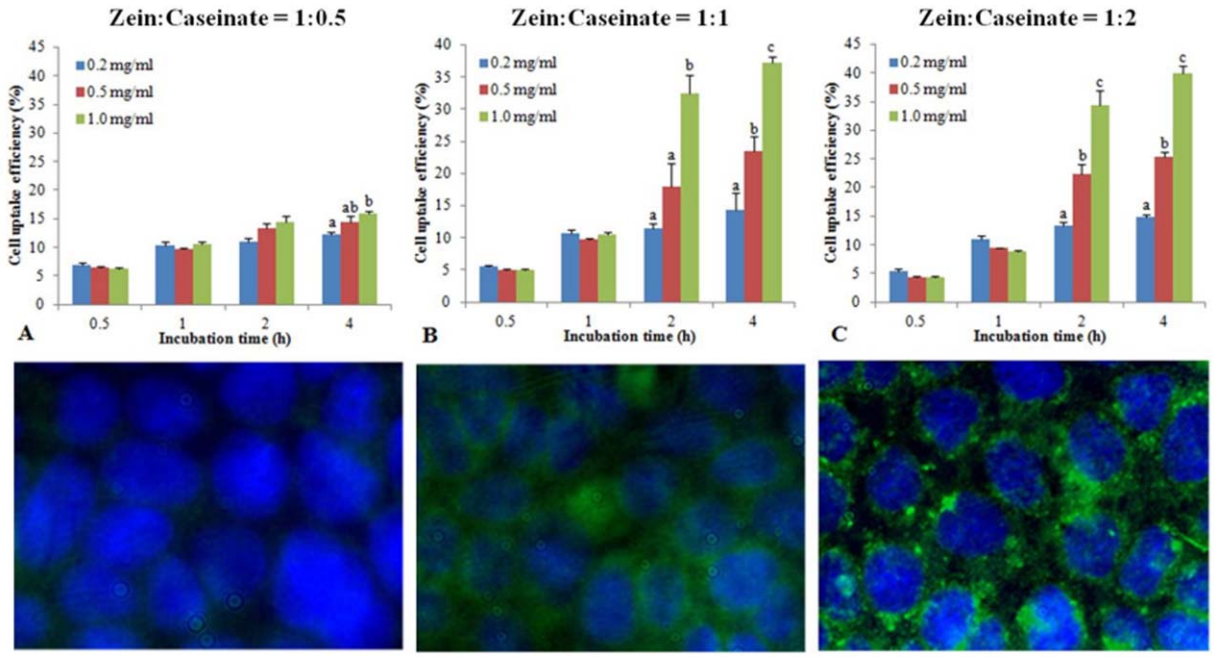

Figure 5. Quantitative measurement (upper panel) and qualitative visualization (lower panel) of cell uptake of coumarin-6 labeled zein-caseinate nanoparticles. In the lower panel, the blue color was the cell nuclei stained with DAPI, and green color was the intrinsic fluorescence from courmarin-6 (Reproduced from Ref. 50, with permission from American Society of Chemistry). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

zein nanoparticles improved targeting delivery to the activated macrophages and scavenge reactive oxygen species, holding promising potential to intervene the pathogenesis of rheumatoid arthritis. Besides the aforementioned *in vitro* evaluations, the *in vivo* delivery efficacy and biodistribution profiles of zein nanoparticles have also been recently addressed. Zein nanoparticles encapsulating 5-fluorouracil, a chemotherapeutic agent that has very low bioavailability even through injection, were prepared and labeled with rhodamine-B, and then its biodistribution was evaluated by intravenous injection through tail vein in mice model.[35] Zein nanoparticles distributed in following organs: heart, liver, spleen, lung, kidney, as well as blood, while the liver was the main targeted tissue with the majority of nanoparticles detected (Figure 4). Particularly, after 24-h administration, a significant amount of zein nanoparticles were still detected in the liver, while no fluorescent signals were found in other tissues. Interestingly, the study revealed that zein nanoparticles avoided rapid recognition by Kupffer cells and adequately remained in the bloodstream even after 24 h, which was probably ascribed to the small size (145 nm) of the nanoparticles composed of aggregated zein molecules. This finding showed the great potential of zein nanoparticles for liver targeting delivery of chemotherapeutic medicine.

In order to improve the encapsulating capability of zein nanoparticles for anti-cancer and anti-tumor drug delivery, Xu et al. developed for the first time the hollow zein nanoparticles.[48] Hollow zein nanoparticles were fabricated using phase separation technique with a two-step process. In the first step, zein dissolved in 70% aqueous ethanol was mixed with sodium carbonate crystals predispersed in 70% aqueous ethanol solution. Zein polymer would attach to sodium carbonate nanocrystals and prevent their agglomeration or growth. During the second step, the zein-sodium carbonate mixture was poured into a large amount of water to precipitate zein nanoparticles, while sodium carbonate core was dissolved out to the aqueous phase due to its high solubility in water, resulting in the formation of hollow zein nanoparticles. When metformin was tested as a model drug, the loading capacity of hollow zein nanoparticles reached 36.9%, being dramatically higher than that of solid zein nanoparticles that was typically less than 10%.[35,49] In comparison with solid nanoparticles, hollow zein nanoparticles had much smaller particle size (65 nm) with good biocompatibility as tested in 3T3 mouse fibroblast cells. The smaller particle size was attributed to the sodium carbonate nanocrystals in the solution, which acted as nanosized heterogeneous nucleation sites and thus prevented increase in diameter of zein nanoparticles. Besides encapsulating capability, cell uptake of zein nanoparticles is another key factor to consider when developing drug delivery systems. Recently, our lab fabricated zein nanoparticles stabilized by sodium caseinate and tested the cell uptake and transport using a Caco-2 cell model.[50] Sodium caseinate stabilized zein nanoparticles exhibited excellent redispersibility after freeze drying and maintained the particle size in cell culture medium and buffers at 37°C, thus enabling reliable cellular evaluation of zein nanoparticles. The cell uptake of coumarin-6 labeled zein nanoparticles were quantitatively measured by the fluorescent intensity and the results suggested that sodium caseinate significantly improved cell uptake of zein nanoparticles in a time- and concentration-dependent manner (Figure 5, upper panel). The cell uptake of zein nanoparticles was also

qualitatively visualized by fluorescent microscopy (Figure 5, lower panel), showing that the nanoparticles were found throughout the cytoplasm in Caco-2 cells. The epithelial transport through Caco-2 cell monolayer was also significantly improved by sodium caseinate. Our findings together with a previous study[41] showing that zein-caseinate nanoparticles exhibited strong mucoadhesive property by physical entanglement with mucin, suggested that zein-caseinate nanoparticles could be considered as a promising drug delivery system with ability to enhance the intestinal absorption of drugs.

Zein Particles for Nutrient Delivery

As a GRAS ingredient, zein particles find wide applications as delivery systems for nutrients, such as improving the bioavailability of food bioactives, preserving the stability of labile nutraceuticals, enhancing antimicrobial activity of essential oils, preserving the efficacy of food colorants, and so on. Similar for drugs, the nutrients investigated in literature are generally hydrophobic ones. The encapsulation of food ingredients in zein particles started from encapsulation of essential oils.[37,51] The sustained release (over 20 h) and high loading capacity (up to 25%) of fish oil in zein nanoparticles were achieved, and the encapsulated fish oil exhibited significantly better lipid oxidative stability during 4-week storage at room temperature. However, further increasing loading percentage of fish oil resulted in incomplete encapsulation in zein nanoparticles, attenuating the protection of fish oil against oxidation during storage. The other two essential oils, thymol and carvacrol, were also encapsulated into zein nanoparticles, and it was found that the encapsulation enhanced their dispersibility in aqueous phase while did not affect their antioxidant and antimicrobial activities.[52]

In recent years, the cytotoxicity and delivery efficacy of zein nanoparticles encapsulating food nutrients have also been evaluated, which may pave the path of developing zein nanoencapsulation based functional foods with enhanced health-promoting effects. Cranberry procyanidins, the oligomers and polymers of flavan-3-ols, are known to have potent antioxidant activity that may reduce the risk of chronic diseases. However, the low bioavailability and possible toxicity to multiple cell lines at high concentrations limit their food applications.[53] Zou et al. fabricated zein nanoparticles by phase separation to encapsulate cranberry procyanidins. They found that the encapsulated procyanidins exhibited a significant decrease of cytotoxicity compared with free procyanidins solution, by preventing the apoptosis of human promyelocytic leukemia HL-60 cells.[54] The alleviated cytotoxicity may be partially due to the sustained release of bioactives from zein nanoparticles. This finding may positively affect the health benefits of encapsulated procyanidins as a dietary intervention to prevent chronic diseases. Zein nanoparticles have been recently reported to encapsulate daidzin, an isoflavone glycoside.[42] In the study, the surfactant, tocopheryl polyethylene glycol succinate (TPGS), was applied to emulsify zein nanoparticles. The daidzin encapsulated in TPGS emulsified zein nanoparticles had a slower release profile in simulated gastrointestinal conditions and a significantly improved cell uptake by Caco-2 cells, compared with the one encapsulated in zein nanoparticles without TPGS. It was suggested that the hydrophobic end, α-tocopherol chain, of the TPGS was inserted in the zein nanoparticles, whereas the hydrophilic end, polyethylene glycol chain, protruded to cover the surface of nanoparticles. Thus, TPGS could decrease the surface hydrophobicity of zein nanoparticles and therefore improve the cellular uptake, which mechanism was also reported in our previous study.[50] The *in vivo* pharmacokinetic behavior of daidzin in TPGS emulsified zein nanoparticles were further evaluated in mice model. It was found that oral bioavailability of daidzin was significantly improved by more than twofold, compared with free daidzin solution. Therefore, zein nanoparticles are proved to be a promising system to deliver food ingredients and with improved oral bioavailability and reduced cytotoxicity.

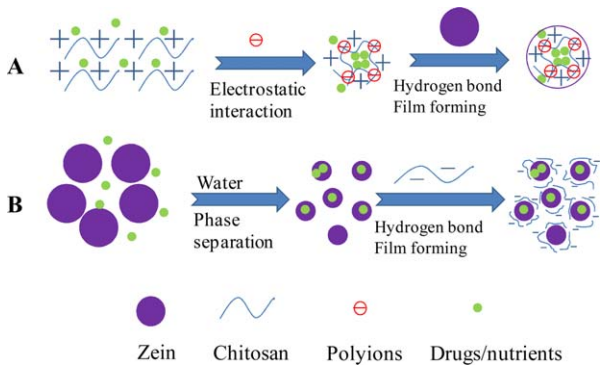

Figure 6. Two different designs for fabrication of zein/chitosan complex nanoparticles as delivery systems. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ZEIN-BASED POLYMERIC COMPLEX DELIVERY SYSTEMS AND THEIR APPLICATIONS

Delivery systems fabricated with polymer–polymer complex has received increasing attention in recent years.[55,56] The complexes-based drug delivery systems combine the advantages of multiple polymers, such as slower release, delayed digestion, and enhanced mucoadhesive property, etc. Different mechanisms and driving forces are involved in the formation of polymer–polymer complex delivery systems, including electrostatic interactions, hydrophobic interactions, hydrogen bonds, and van der Waals force. Being a weak polyelectrolyte, zein forms complexes with other biopolymers, either polysaccharides or proteins, by the driving force of hydrophobic interactions and hydrogen bonds.

Zein/Chitosan Complex Nanoparticles

Among zein-polymer complex delivery systems, zein/chitosan complex is the most investigated one. Zein/chitosan complex nanoparticles can be tailored in two ways for different applications, as shown in Figure 6. In the first design [Figure 6(A)], chitosan/tripolyphosphate nanoparticles are first fabricated through ionic gelation, and then zein (pre-dissolved in 70% ethanol aqueous solution) is added into nanoparticles dispersion with mild stirring. Subsequently, ethanol is removed under nitrogen stream or by rotary evaporation under reduced pressure. Because the acidic condition ($pH < 5.5$) of chitosan/TPP nanoparticles, zein will form films spontaneously upon removal of ethanol from the dispersion as aforementioned (Figure 3).

Zein/chitosan complex delivery system is therefore achieved in the design of chitosan nanoparticles as hydrophilic core and zein coating as hydrophobic shell. This specific design can be used for encapsulation and delivery of hydrophilic drugs or nutrients. Selenite, a hydrophilic trace element, has been encapsulated into such structured zein/chitosan complex delivery system.[57] When compared with chitosan nanoparticles without zein coating, the zein/chitosan complex nanoparticles provided significant improvement in functionalities, i.e., higher encapsulation efficiency and slower sustained release in gastrointestinal tract. The hydrophobic zein shell prevented dissolution of chitosan in the acidic condition of stomach and helped the complex maintain its structure, thus prolonged the release of hydrophilic selenite. The second design is aimed to encapsulate and deliver hydrophobic drugs and nutrients [Figure 6(B)]. In this design, zein nanoparticles along with hydrophobic bioactives are poured into chitosan solution to induce phase separation and form zein/chitosan complex nanoparticles. The encapsulation of fat-soluble vitamin, α-tocopherol, in this delivery system has been reported previously.[58] Although this design prevented enzymatic degradation of hydrophobic bioactives in gastric condition and achieved a slower release rate than pure zein nanoparticles, the zein/chitosan complex delivery systems had several limitations. For instance, the fast dissolution of chitosan in gastric condition resulted in only a limited improvement of release rate, unless high polymer concentrations were used. However, the microparticles were obtained at high concentrations of both zein and chitosan, which may lose some advantageous features of nanoscale structures.[59] Second, the water redispersibility of freeze dried powder of zein/chitosan complex nanoparticles was another obstacle for future applications. To address this issue, the water soluble chitosan derivative, carboxymethyl chitosan (CM-chitosan) is chosen as a chitosan alternative to form coatings on zein surface. CM-chitosan forms gel at acidic pH and acts as a better barrier and thus provides greater protection of zein protein against enzymatic degradation. Several hydrophobic nutrients have been tested in this zein/CM-chitosan complex delivery system, including vitamin D3[49] and indole compounds.[60] The strong negative surface charge of CM-chitosan favored the formation of zein nanoparticles with low energy input, by providing strong repulsive forces among nanoparticles and making them well separated.[49] Otherwise, high energy input is normally applied, such as high speed homogenization to obtain well-separated and mono-distributed zein nanoparticles. CM-chitosan coating also conferred thermal stability to zein nanoparticles that the complex nanoparticles were able to provide excellent protection of labile nutraceuticals against thermal degradation and oligomerization.[60] Recently, another positively charged chitosan derivative, glycol chitosan, has been investigated by our group to form complex with zein nanoparticles.[32] In this study, to improve the water redispersibility, zein nanoparticles were first stabilized by sodium caseinate and then coated by glycol chitosan. The glycol chitosan coating was reinforced by strong electrostatic interaction with sodium caseinate. The complex nanoparticles dramatically improved the encapsulation efficiency of thymol, an antimicrobial agent, and its antimicrobial efficacy was significantly enhanced. In summary, zein/chitosan complex nanoparticles demonstrate a great number of advantages compared to zein nanoparticles, securing a variety of novel applications for delivery of drugs and nutrients.

Besides particulate form, many researchers have been investigating the films/membranes and fibers of zein/chitosan complex. However, most of these studies focused on the physical structures and characterizations,[61,62] rather than their delivery properties. A few newly published studies demonstrated a potential trend that zein/chitosan complex films and fibers have a bright future as carriers for both nutrients and drugs. For instance, in a recent study, the electrospun zein/chitosan ultrafine (with diameter ranging from 300 to 500 nm) fibrous film exhibited good gastromucoadhesivity to porcine stomach mucosa due to the spider-web-like morphology.[63] A hydrophobic compound, α-tocopherol, was further tested in this delivery system fabricated with zein/chitosan electrospun fibers,[64] showing that the encapsulation of the hydrophobic nutrient further enhanced mucoadhesive property of the fibers due to the enhanced hydrophobic interactions with mucosa and a sustained release was achieved in simulated gastric fluid. These studies suggested that zein/chitosan fibrous film may be a potential carrier for delivery of nutrients to stomach mucosa and release them into the blood circulation.

Zein/Pectin Complex Microparticles

Zein/pectin complex has become another popular zein-based polymeric delivery system in recent years. Pectin is a ubiquitous and natural polysaccharide produced in cell walls of all higher plants. Pectin is well known for its unique digestibility that it remains intact in gastrointestinal tract but be degraded in colon due to pectinolytic enzymes and microflora, and therefore, it is extensively investigated for colonic targeting drug delivery.[65,66] Since the high solubility of pectin negatively affected its delivery efficiency because of rapid dissolution, the polymers with less water solubility could be beneficial to improve its drug delivery capability. Zein is one of the promising candidates to form complex with pectin for better drug delivery efficacy. Zein/pectin hydrogel beads/microspheres have been previously reported for such applications.[67,68] The beads were prepared by the coacervation of pectin with calcium together with zein in 75% ethanol aqueous solution. The fluorescence microscopic observation revealed that although zein was distributed throughout the matrix, it was mainly located in the periphery of the complex beads. This distribution resulted in such advantages that the outer layer of zein reduced the dissolution and restricted the swelling of pectin in simulated gastrointestinal fluid and the pectin network in turn shielded the bound zein from protease digestion. Therefore, zein/pectin complex hydrogel beads significantly improved encapsulation efficiency and provided sustained release in the colon, with minimal drug release in gastrointestinal tract. The drug release of piroxicam, a nonsteroidal anti-inflammatory drug, from zein/pectin has been evaluated via both *in vitro* simulated gastrointestinal tract[69] and *ex vivo* model using rat cecum.[70] Both evaluations suggested that increasing zein mass ratio in the complex apparently reduced the dissolution rate of the drug from the hydrogel microspheres and that the diffusion of drug from complex matrix played a dominant role as the release rate-limiting step. Recently, a new approach has been proposed to fabricate zein/pectin complex particles via electrostatic interaction.[71] In this approach, the pH

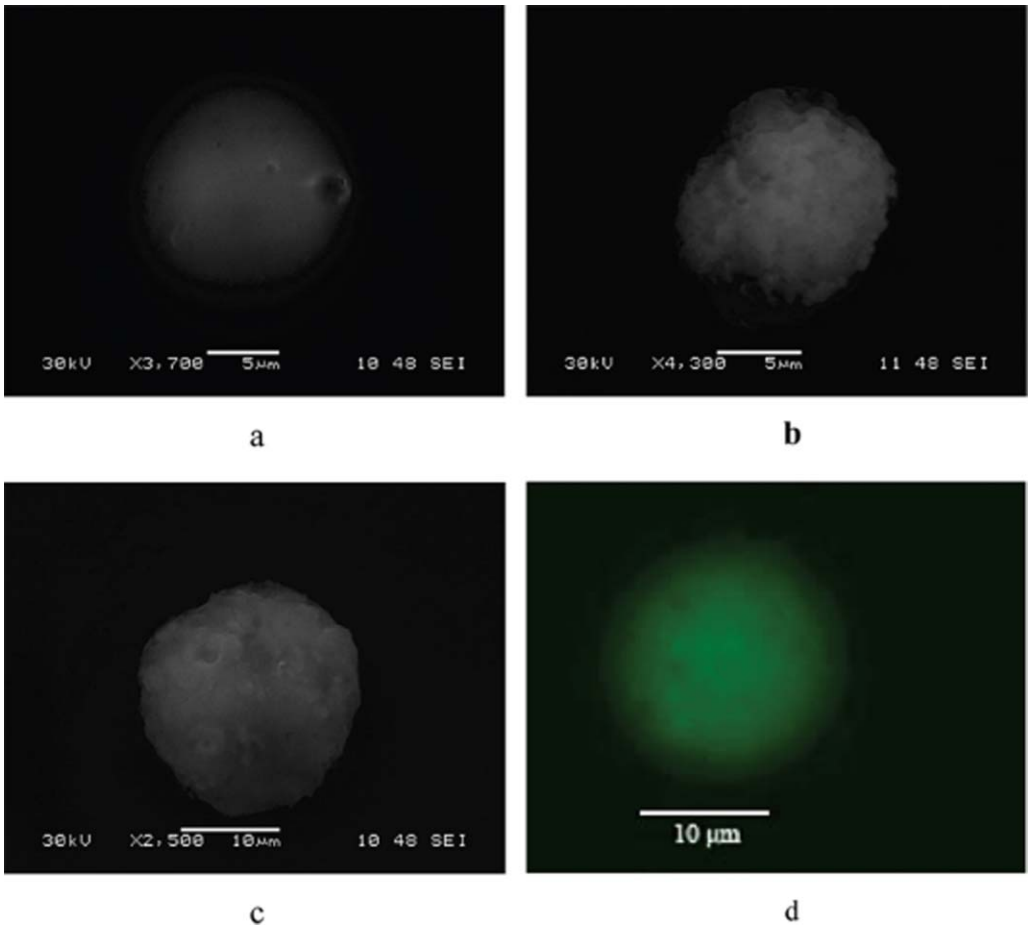

Figure 7. Photographs of (A) pure SPI microspheres, (B) pure zein microspheres, and (C) SPI/zein microspheres viewed by scanning electron microscopy and (d) SPI/zein microspheres labeled with phen green viewed by fluorescence optical microscope (Reproduced from Ref. 75, with permission from American Chemical Society). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of zein solution was adjusted to pH 4, which is below its isoelectric point (pH 6.2), to make zein carry positive charges. In this condition, the negatively charged pectin was then dropwise added to induce the electrostatic interaction between zein and pectin. It was found that zein concentration was the determining factor to induce the formation of zein/pectin complex particles, showing that higher zein concentration resulted in more complex particle formed. Although zein/pectin complex particles could be fabricated by this approach, the increase of pH to higher than 6.0 may cause the disintegration of the complex given that both zein and pectin carry negative charges. Thus, the pH dependence property may limit its applications in food or drug products.

Zein/Soy Protein Complex Microparticles

Soy protein (SPI) isolate, a water soluble plant protein, has been well documented for its capability to deliver nutraceuticals[72,73] and it is able to form complex structure with other biopolymers for fabrication of novel delivery systems.[74] A novel technique, cold gelation, has been reported to fabricate zein/SPI complex microparticles for delivery of hydrophilic nutraceuticals.[75] In this method, zein was dissolved at pH 11.0 in the absence of alcohol, and then mixed with pre-heated SPI and calcium carbonate. The mixture was then emulsified in soybean oil to form water-in-oil emulsion, followed by addition of acetic acid to lower the pH and induce the gelation of zein/SPI matrix. The microparticles prepared were spherical in shape with homogenous distribution of calcium-crosslinked SPI throughout the matrix (Figure 7). The pure SPI had a diameter of 15 μm with smooth surface, while the zein/SPI microparticles exhibited a larger dimension ranging from 20 to 25 μm with rough surface indicating the water-insoluble characteristic due to the presence of zein in the matrix. Interestingly, in this process without any alcohol involvement, phase separation did not occur between zein and SPI, which suggested excellent compatibility and miscibility. Because no organic solvent was involved, the zein/SPI microparticles were proposed as a delivery system of hydrophilic nutrients for food applications. The riboflavin, water soluble vitamin B2, was tested in this delivery system. When compared with pure SPI microparticles that showed first-order kinetic release of riboflavin in gastrointestinal conditions, zein/SPI microparticles demonstrated a zero-order kinetic release, resulting in the significant reduction (up to 30%) in the amount of accumulative release depending on the mass ratio of zein. Later on, the absorption rate and release performance of riboflavin in this delivery system were systematically evaluated with a dynamic artificial digestive system consisting of

four compartments in series simulating the stomach, the duodenum, the jejunum, and the ileum.[76] The study revealed that zein/SPI microparticles were surprisingly better than pure zein or SPI microparticles, in terms of slowing the release rate and increasing the absorption availability of riboflavin in jejunum, the main site of absorption. Zein/SPI complex microparticles encapsulating riboflavin were further tested in a food product, i.e., yogurt. Suspending microparticles in yogurt significantly delayed nutrient release, which would increase the likelihood of gastric-sensitive nutrients passing intact into the intestine for absorption. Thus, zein/SPI complex microparticles prepared by this cold gelation method exhibited outstanding features for delivery of hydrophilic nutrients or drugs with significantly improved bioavailability.

ZEIIN-BASED NANOCOMPOSITES FOR DRUG DELIVERY

Nanocomposites represent the assembly of organic–inorganic hybrid materials that are nanostructrued. The organic components refer to biopolymers, including proteins and polysaccharides. Generally, the techniques that are adopted to fabricate nanocomposites include both physical and chemical approaches. The physical approach refers to the incorporation and/or intercalation of inorganic nanoparticles into the biopolymeric matrix, whereas the chemical approach is achieved by conjugation of inorganic nanoparticles onto biopolymers chains. The drug delivery systems fabricated from nanocomposites are believed to be more effective in controlling the release of drugs from the matrix than polymers alone. Being a strong hydrophobic protein, zein with water-insolubility offers additional benefits than hydrophilic proteins in preventing the diffusion of drugs to aqueous phase. So far, several studies on zein-based nanocomposites drug delivery systems have been reported in recent years.

Physical Approach

Zein/alginate complex was recently prepared to form nanocomposites with layered double hydroxide (LDH) as an effective drug delivery system for oral administration of ibuprofen, a nonsteroidal anti-inflammatory drug.[77] In this study, the nanocomposites delivery system was processed as hydrogel beads, which were prepared by crosslinking zein/alginate complex, premixed with LDH and drug hybrid system, in a concentrated calcium solution. The presence of zein in hydrogel beads significantly reduced the dissolution (around 20%) of drug in stomach and impeded the swelling in intestine. Conversely, the beads without zein showed a significant dissolution of drug (more than 50%) in stomach and complete swelling in intestine due to replacement of calcium ions by phosphate ions at neutral pH.

Quantum dots (QDs) have also been recently studied to form nanocomposites with zein nanoparticles for bioimaging and drug delivery applications.[78] The strong hydrogen bond and hydrophobic interactions were the driving forces to incorporate QD (ZnS : Mn) into zein nanoparticles during the preparation of nanoparticles via phase separation technique. An anticancer drug, 5-fluorouracil, was encapsulated into zein-QD nanoparticles with particle size of 800 nm. Both drug-free zein and zein-QD exhibited excellent biocompatibility on two cancer cell lines tested (human breast cancer MCF-7 and mouse fibroblast L929) even at high concentrations up to 4 mg/mL, whereas the drug-encapsulated zein-QD possessed strong efficacy in killing cancer cells. The internalization of zein-QD nanoparticles were also visualized by fluorescent microscope.

Chemical Approach

Our group successfully fabricated zein-silver nanocomposites as a promising antimicrobial agent, by incorporating silver nanoparticles into zein matrix in 75% ethanol solution.[79] Zein was able to form composites with silver nanoparticles through silver-nitrogen (Ag-N) coordinate-covalent bond. When the composites were prepared at acidic pH (3.3), the electrostatic interaction between zein (positively charged) and silver nanoparticles (negatively charged) further promoted the formation of nitrogen-silver coordination bond and thus improved the stability of the as-prepared nanocomposites. When applied as dry coating, the zein-silver nanocomposites were found to greatly enhance the antimicrobial activity and significantly improved hemocompatibility, compared with the silver nanoparticles alone. However, the limitation still remains that when the nanocomposites were applied in solution the bactericidal efficacy was inferior to silver nanoparticles, possibly attributed to the low water solubility of zein.

To achieve the covalent conjugation of QD to zein protein, the QD is usually pre-treated with a hydrophilic coating (such as carboxylic acid), which can covalently interact with zein through the formation of amide bond. In a recent study, the hydrophilic QD was successfully conjugated onto zein molecules through amide bond in 80% ethanol solution.[80] The original size of QD was 4.6 nm, which was changed to 24 nm after conjugation, and QD/zein nanoparticles exhibited similar fluorescence emission behavior but lower intensity with QD, indicating that the successful conjugation was achieved. The cell uptake of QD and zein/QD nanoparticles varied significantly due to the size difference. The uptake of QD reached plateau within 1 h because the QD with less than 5 nm could penetrate into cells and reach saturation quickly, whereas zein/QD nanoparticles showed a continuous cell uptake in 24 h duration before reaching saturation. This gradual increase of uptake is of significance in reducing cytotoxicity for its biomedical applications, because the fast uptake of QD often causes cytotoxicity to the cells. The *in vivo* evaluation of transdermal delivery capacity of QD and zein/QD were also conducted in nude mouse skin. Interestingly, QD nanoparticles were able to penetrate through the stratum corneum barriers and localized within epidermal and dermal layers, whereas zein/QD nanoparticles were unable to penetrate the skin within 24 h, possibly ascribing to their larger particle size. The conjugation of QD to zein molecules dramatically altered its cell uptake behavior and skin penetration ability, which would significantly reduce its toxicity and therefore may provide solutions to the safety issue for QD applications such as in sunscreen products.

CONCLUSIONS

This review summarizes the technological strategies of fabrication techniques for zein-based micro- and nano-particles and their delivery applications, including delivery of drugs and

nutrients. The unique physicochemical characteristics and special nanostructures make zein molecules be intrinsically superior to many other natural and synthetic polymers. As the field of zein-based delivery systems is growing fast, the development of advanced zein-based micro- and nano-structured delivery systems will expand the possible applications. In the near future, the research trend on zein nanoparticles as drug delivery systems will be focused in three major directions. First, the fabrication of complex delivery systems composed of zein and other biopolymers will be one of the major directions that deserve more research. Those polymeric complex delivery systems can be tailored for diverse delivery applications, including colon targeting and transdermal delivery. Second, the development of zein-based nanocomposites with inorganic nanocrystal materials, such as QDs, will undoubtedly draw more attention. These nanocomposites-based delivery systems are beginning to demonstrate their superior capability to encapsulate and control release of drugs for a wide variety of applications. Third, the *in vivo* evaluations of zein-based delivery systems will be in great need for elaborating their biological fate after oral consumption and their efficacy and toxicity in physiological conditions. With more and more investigations, we envision that the area for zein-based delivery systems will continuously attract increasing interests and that the clinical applications and commercialization will be eventually realized in the near future.

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