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Review

Zein—A plant protein as a promising biopolymer for biomedical applications: A perspective

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Abstract: Recent technological advances in the fields of biomaterials and tissue engineering have spurred interest in biopolymers for various biomedical applications. The advantage of biopolymers is their favorable characteristics for these applications, among which proteins are of particular importance. Proteins are explored widely for 3D bioprinting and tissue engineering applications, wound healing, drug delivery systems, implants, etc., and the proteins mainly available include collagen, gelatin, albumin, zein, etc. Zein is a plant protein abundantly present in corn endosperm, and it is about 80% of total corn protein. It is a highly renewable source, and zein has been reported to be applicable in different industrial applications. Lately, it has gained attention in biomedical applications. This research interest in zein is on account of its biocompatibility, non-toxicity, and certain unique physico-chemical properties. Zein comes under the GRAS category and is considered safe for biomedical applications. The hydrophobic nature of this protein gives it an added advantage and has wider applications in drug delivery. This review focuses on details about zein protein, its properties, and potential applications in biomedical sectors.

Keywords: zein; drug delivery; nanoparticles; tissue engineering; wound healing; biopolymers

1. Introduction

The emergence of biopolymers has prompted a wave of exploration into the development of biomaterials possessing an array of characteristics that find value across a broad spectrum of biomedical applications. Notably, the biomaterials sector has witnessed significant advancement as these fabricated materials, with their diverse attributes, have contributed to progress in the medical and healthcare industries [1]. Biopolymers are employed in a wide range of applications, such as gels used to maintain moisture in eye drops or as laxatives for flexible scaffolds used for replacing different tissues, rigid materials that are required for support structures, and so on [2]. Furthermore, a variety of synthetic and natural polymers have been utilized in biomedical applications, such as surgical meshes, hard contact lenses, heart valves, enteric pharmaceutical coatings, drug encapsulation, wound dressings, and bone tissue regeneration, among others [3].

Protein-based polymers are being explored on a growing basis for use in biomedical applications owing to their superior processability, biocompatibility, and biodegradability [4]. Proteins are preferred over carbohydrates because they contain a variety of functional groups, such as carboxyl and amino groups, which can be exploited for labeling targeting molecules of interest or facilitate rapid and stable crosslinking during the development of three-dimensional scaffolds. Furthermore,

proteins possess an adaptable framework that allows them to easily and dynamically interact with lipids and carbohydrates, thanks to their amphipathic nature [5].

Zein is a biodegradable plant protein obtained from maize (*Zea mays*), an abundant and sustainable agricultural source [6]. Zein is a class of prolamin-rich alcohol-soluble protein that forms the major storage protein in the endosperm of corn, comprising almost 80% of the whole protein content of corn [7,8]. Considering that the corn market is massively widespread on a global scale, accounting for an annual production of over 1 billion tons, zein becomes readily available in the environment as organic waste [6]. Zein was previously thought of as more of a byproduct of the corn processing industries; general agreement suggested that zein was a low-value material with few practical technological applications. However, nowadays, there is a new perspective towards zein and zein-based materials, considering them as more valuable materials due to several recent technologies and developing processes allowing zein's applications in different sectors [7].

Zein is currently drawing research interest on account of its unique properties, such as biocompatibility, resistance to heat, water, abrasion, and humidity. It can be readily processed and can be applied to create films, sheets, microspheres, nanoparticles (NPs), and nanofibers, among others [4,8]. Zein has the potential to be used to generate biodegradable chewing gum, fibres, adhesives, coatings, ceramics, inks, cosmetics, and textiles. Zein finds applications in biomedical as well as pharmaceutical sectors. Zein-based biodegradable materials are most effectively employed in the food and pharmaceutical industries [7]. Moreover, zein was approved as a Generally Recognized as Safe (GRAS) excipient in 1985 by the United States Food and Drug Administration (USFDA, 21CFR184.1984) for application in the film coating of pharmaceuticals [6,8].

To improve the mechanical, physical, and biological properties of zein-based biomaterials, a number of chemical modifications, including plasticizing and crosslinking, as well as combinations with other materials, including biopolymers and inorganic materials, are being investigated. Inorganic fillers, like bioactive ceramics, glasses, and clays, are promising substances being combined with zein and zein-based biopolymers. Through this composite approach, zein can attain antibacterial, antioxidant, and anti-inflammatory functionalities [4]. Furthermore, zein protein can increase the possibility that the biomolecules will endure a longer shelf life. Zein is therefore regarded as a novel material that holds tremendous potential for advanced biomedical applications like tissue engineering, delivery systems, and wound healing. Numerous biomedical domains take advantage of zein, and research on its various characteristics is nowadays extensively prolific [8].

In this review, we attempt to highlight and describe general aspects of biomaterials and their contribution to biomedical research, focusing mainly on biopolymers, updating information concerning the source, structure, and general properties of zein, and potential applications of zein and zein-based materials in the fields of drug delivery, tissue engineering, and wound care.

2. Biomaterials and biomedical applications

A biomaterial is any material, synthetic or natural, that can be applied in medical

applications to carry out a bodily function or replace a tissue or body part. In 1987, the European Society for Biomaterials defined "biomaterial" as a non-biological material used in a medical device with the objective of interacting with biological systems [9]. Different biomaterials are designed based on their intended applications. It may be used as a potential carrier system for drugs or bioactive molecules, or it can act as a support matrix, providing a suitable microenvironment for cell growth and survival, etc. [10]. A biomaterial must be biocompatible, which means it should be friendly with the biological system without causing adverse effects on the cellular or system level [11].

2.1. Types of biomaterials and their biomedical applications

Broadly, biomaterials can be grouped into four major categories: i) metals; ii) ceramics; iii) composites; iv) and polymers [11].

2.1.1. Metals

Intrinsic properties of metals such as high mechanical strength, reliability, corrosion and wear resistance, toughness, elasticity, and thermal and electrical conductivity make them ideal for biomedical applications. The first successful implants were made of stainless steel and chromium alloys [12].

Pure titanium and titanium-aluminium-vanadium alloys (Ti-6al-4V) find application in dental implants, bolts and column slips, pacemaker housings, artificial heart valves, screws, and bone fixation staples as part of knee, hip, shoulder, elbow, and wrist joints. Chromium-cobalt alloys are also used in dental applications, artificial joints, and implants [13]. Gold is widely used in wiring for pacemakers, dental crowns, and other stomatological applications. Silver is used to treat burns, coat surgical instruments, in stethoscope diaphragms, in fibers used for wrapping wounds to prevent infections, etc. Platinum is incorporated as electrodes in pacemakers for stabilizing cardiac rhythm and in hearing aids. Metal alloys with memory capacity are used in orthopedic and orthodontic applications [9].

2.1.2. Ceramics

Ceramics exhibit properties such as resistance to wear, corrosion, and compression biocompatibility, low thermal and electrical conductivity, shinier surfaces, hardness, brittleness with minimum deformation, and greater density as compared to metals [9,10,14]. Ceramics are mainly used in the repair, regeneration, and augmentation of hard tissue, in particular in non-load-bearing applications or as metal implant coatings [11].

Alumina is employed in wear surfaces in joint prostheses such as the acetabulum and femoral heads in hip arthroplasty [9]. Polycrystalline tetragonal zirconia is used in prosthetic equipment, mainly in femoral heads [15]. Ceramic hydroxyapatite is ideal for application in dental implants, knee and hip replacement, polymeric prostheses, or metal coatings, as it releases calcium and phosphate ions into the body [9].

Carbon-derived materials such as carbon nanotubes, graphene, fullerene, quantum dots, non-crystalline diamond films, carbon nanofibers, etc. are promising materials for cancer diagnosis, bioimaging purposes, etc. [10]. Graphene and its associated materials are employed in biosensors, cancer therapy, imaging, tissue engineering applications, etc. [9].

2.1.3. Composites

A composite material is a heterogeneous material formed from the combination of two or more materials with different characteristics, with the aim of developing a novel material possessing properties superior to those of its individual materials [9]. For example, a combination of polymer and carbon fibre (like carbon-fiber-reinforced polyetheretherketone) is used in the development of screws for orthopedic purposes [10]. Ceramic microparticle polymer composites with polymers and bioactive glass as individual components have been applied in the orthopedic regeneration of tissue [16,17]. Hydroxyapatite/zirconia promotes the mechanical properties of HA, maintaining the properties of adhesion to bone tissue [18].

One of the recently emerged nanocomposite biomaterials is nanocomposite hydrogel. They can be developed as promising scaffolds due to their tailorable properties, and they closely mimic the ECM environment, thus providing a hydrated three-dimensional network. This network of nanocomposite hydrogels supports the transport of nutrients and promotes cell growth and maturation. The ability to selfheal in response to mechanical abrasion is one of the important features of nanocomposite hydrogels [19].

2.1.4. Polymers

Polymers, or polymeric biomaterials, are extensively used in biomedical uses such as cardiovascular, neural, and dermal tissues [10]. The biocompatibility of polymers depends on their chemical composition, molecular weight, solubility, geometry, water or liquid absorption, erosion or degradation processes, etc. [9]. Polymeric biomaterials can be degradable or non-degradable, synthetic or natural, or a combination of both [20].

Polymers can be classified into two categories: natural polymers and synthetic polymers. Natural polymers comprise proteins, polysaccharides, decellularized matrices of tissues, etc., and synthetic polymers include polyethylene, polyurethane, polyamide, polytetrafluoroethylene, polymethylmethacrylate, synthetic rubber, polystyrene, etc. An advantage of natural polymers over synthetic polymers is their non-toxic properties for our bodies [9,10].

Synthetic polymers such as PTFE have been employed in vascular graft applications as soft tissue fillers; PVC is used in tubes, blood bags, and catheters; silicon is used in ophthalmological aids; and encapsulation is formed in breast implants, Polymethyl methacrylate finds uses in orthopedic applications such as bone cement and ophthalmological applications such as contact lenses and intraocular lenses. Polyamides or nylon are used in suture lines, and a combination of nylon and polyurethane is used in balloons or catheters in angioplasty. Polyurethane is used as a coating in breast implants, aortic balloons, gastric balloons, male contraceptives, surgical gloves, etc. Ultra-high molecular weight polyethylene (UHMWPE) is used on sliding surfaces of artificial joints, surgical cables in bone fracture, high-strength orthopedic sutures for repair of soft tissues, catheters, stent-grafts, heart valves, and disc replacement for spinal issues [9].

3. Biopolymers as biomaterials

Biopolymers are a class of polymers that are produced by living organisms. They are also called polymeric biomolecules [21]. Natural polymers are non-toxic, biocompatible, biodegradable, and readily available from renewable sources such as plants, animals, microorganisms, etc. [9]. They possess properties such as low antigenicity, high bioactivity, providing support for cell growth and proliferation, appropriate mechanical strength, and processability for complicated shapes with sufficient porosity. Biopolymers can be classified based on different criteria. They can be classified based on the monomeric units present in them. These include polynucleotides (made up of nucleotide polymers like DNA or RNA), polypeptides (made up of short polymers of aminoacids), and polysaccharides (made up of different bonded monosaccharides). Biopolymers can be classified based on their origin or source of isolation. Polysaccharides of bacterial origin include xanthan, dextran, gellan, polygalactosamine, cellulose, etc. Fungal-based polysaccharides include pullulan, elsinan, yeast glucans, etc. Biopolymers such as starch, cellulose, agar, alginate, carrageenan, and pectin fall under plant or algal polysaccharides. Polysaccharides of animal origin are chitin and hyaluronic acid. Lignin, tannin, and humic acid are a category of polyphenols [21].

3.1. Polysaccharides

Biopolymers provide a plethora of applications in the biomedical sector. It includes drug delivery systems, wound dressings, tissue engineering, etc. [21]. Polysaccharide biopolymers that are employed in the biomedical sector include cellulose, chitosan, xylan, alginate, carrageenan, pectin, hyaluronic acid, etc. [9]. Cellulose is the most predominant polysaccharide found in nature. They are the main constituents of plants, and some bacteria, such as *Acetobacter xylinum*, can synthesize cellulose. Cellulose ether is used in solid tablets, enabling the swelling-driven release of the drug in the body [21].

Chitosan, a derivative of chitin, is present in the exoskeletons of crustaceans and insects [22]. Chitosan exhibits excellent biocompatibility, high bioactivity, biodegradability, selective permeability, polyelectrolyte action, antimicrobial and anti-inflammatory activity, gel and film formation, chelation, and adsorptive capacity, due to which it can be developed as a carrier for controlled release of the drug, promote osteogenesis, healing of lesions and ulcers, etc. [23]. A hydrogel sheet made up of powdered alginate, chitin, chitosan, and fucoidan is developed as a functional wound dressing to create a moist environment for rapid wound healing [24]. The chitosan/hydroxyapatite composite shows promise to mimic the organic portion as well as an inorganic component of bones. Chitosan gel is used in the prevention and treatment of dental caries [21].

Xylan, a hemicellulose, is a suitable biopolymer for developing drug delivery systems for the colon, as it gets degraded by colonic microflora enzymes. Pentosan polysulfate, a glucoxylan derivative anti-coagulant in gel form, can be applied in the treatment of infusion thrombophlebitis [25]. Alginate, a hydrocolloid polysaccharide extracted from sea weed, is used as a support matrix or delivery system for tissue repair and regeneration. Alginate is a US-FDA-approved polymer and finds application in

nutrition supplements. They are employed in making tooth impressions in dental clinics. Sodium and calcium-incorporated alginate-based wound dressings provide a moist environment at the wound bed, absorb the exudate, relieve wound pain, reduce the bio-burden around it, absorb proteinases, reduce odor, and help achieve hemostasis and wound closure. Wound dressings based on alginate formed as hydrogel, electrospun mats, or sponges have gel forming ability upon absorption of wound exudates and hemostatic ability. Moreover, alginate is used as a carrier to immobilize or encapsulate drugs [21].

Carrageenan is a polysaccharide present in seaweeds belonging to the class Rhodophyceae. It exhibits anticoagulant activity by inhibiting thrombin. They are also a potential substitute for gelatin in hard and soft gel capsules. Its addition to the glycerin-water mixture reduces the chalkiness of antacid gels. It is used in suppository or topical bases. It acts as a thickening agent and binder in dentifrices [21]. Pectin, is a heteropolysaccharide present in the primary cell wall of plants. Pectin in combination with Carbopol and chitosan, is employed in making mucoadhesive patches. It acts as a prophylactic substance against poisoning with cations, which are toxic. Pectin-based hydrogels have been used in tablet formulations as agents for binding and carrier material in colon-specific drug delivery systems. Hyaluronic acid is a biocompatible mucoadhesive polysaccharide with a negative charge. In the late 1950s, it was applied as a substitute for the vitreous humor of the eye during eye surgery [26]. Other applications include wound healing, epithelial regeneration, treatment of dry eye and Sjogren syndrome, osteoarthritic problems, preventing bacterial adhesion in dental implants, intraocular lenses, and catheters, etc. [27].

Gum Arabic is an edible gummy exudate from the stems and branches of the plant *Acacia Senegal*. Studies reported that gum Arabic could prevent the formation of dental plaque and improve tooth remineralization. These properties are due to the high content of Ca^{2+}, Mg^{2+}, and K^{+} salts [28]. Xanthan gum is a bacterial polysaccharide widely used in tooth pastes and cosmetics. The addition of xanthan gum to emulsions or suspensions of pharmaceutical formulations prevents the separation of insoluble components [29].

3.2. Proteins

Protein-based biopolymers applied in biomedical applications include collagen, gelatin, zein, etc. Collagen film/sheet/disc is used for the treatment of corneal tissue or liver cancer. A collagen-based soluble ophthalmic insert in the form of film or wafer was developed as a drug delivery system for the delivery of antibiotics such as gentamicin to treat corneal tissue infection. A collagen film matrix is employed as a gene delivery system for bone formation. Collagen-based wound dressings in the form of sponges, membrane sheets, and powder are used to manage severe burns. Nanoparticles based on collagen find use in sustained-release formulations for anti-microbial agents or steroids [30]. Gelatin, formed by partial hydrolysis of collagen, is used as a forming, emulsifying, and wetting agent in pharmaceutical applications due to its surface active properties [31]. Zein is another biopolymer abundantly present in nature. Its source is corn, and it comprises about 80% of total corn protein. Zein has been reported to be applicable in different biomedical applications such as drug

delivery, tissue engineering, wound healing materials, etc. This can be attributed to its unique properties such as hydrophobicity, biocompatibility, film formation properties, glossy, tough, and greaseproof nature, and water vapour permeability [7]. This review focuses on details about zein protein, its properties, and potential applications in the biomedical sector.

4. Zein

4.1. Zein—Source, structure and properties

Since the early twentieth century, zein has been investigated as a potential raw material for polymer applications. John Gorham first characterized zein from maize (*Zea mays*) in 1821 and named it 'zeine'. Gorham described zein as a soft, ductile, tenacious, and elastic protein resembling bees' wax. It resembled resin and possessed some of the qualities of wheat gluten. Ritthausen coined the term 'maize fibrin' to describe the same material. Chittenden and Osborne classified zein as a prolamine, as it is now [32]. Zein is abundant in maize and accounts for 44% to 79% of the endosperm content [33].

Unlike corn gluten, zein is not widely produced on a large scale, due to the high cost of the conventional extraction process [7]. The conventional process involves extraction in an aqueous alcohol solution and then clarification by centrifugation, followed by chilling to precipitate out zein. The composition, with respect to the relative proportion of different fractions of zein and the fine structure of zein, is affected by raw material, solvent type, method of extraction and purification, concentration, temperature of alkali or other reducing agent used, drying method, etc. [34–36].

Zein is commercially available in two forms: yellow zein and white zein [37]. Yellow zein has a purity range of 88%–90%. It carries a high concentration of xanthophyll with lutein, zeaxanthin, and β-cryptoxanthin [38]. The presence of xanthophyll bound to zein has a significant impact on the solubility of zein and is responsible for the large particle size distribution and low drug encapsulation in the zein matrix during nanoparticle formation. On the other hand, white zein has a purity of more than 96%. It is obtained from the decolourization of yellow zein and has negligible xanthophyll content [39].

Zein is found in whole maize as a heterogeneous mixture of aggregates linked by disulfides, with an average molecular weight of 44 kDa. Zein truly represents a combination of distinct peptides that have differing molecular sizes, charges, and solubility. Zein is classified into four types based on its molecular weight and solubility: α-zein, β-zein, γ-zein, and δ-zein. With a molecular weight of 19–22 kDa and soluble in 70%–95% aqueous ethanol solution, α-zein accounts for 35% of total zein content as well as 80% of prolamine content. β-zein is made up of α-zein linked by disulphide bonds, so its molecular weight is higher. In reducing SDS PAGE studies, β-zein displayed 3 distinct bands of 14 kDa, 22 kDa, and 24 kDa. It is soluble in 60% ethanol and insoluble in 95% ethanol [40,41]. β-zein has a high content of methionine. δ zein is the minor fraction with 10 kDa. The solubility of β-zein and δ-zein is comparable with that of α-zein. The γ-zein consists of two parts: 27 kDa and 18 kDa [37]. More than 50% of zeins' amino acid residues are hydrophobic—20% leucine,

9%–10% proline, and 10%–14% alanine. It also possesses 21%–26% of glutamine, a hydrophilic amino acid [7,40,41].

Various models to interpret the tertiary structure of α-zein have been proposed, including the cylinder model, ribbon-like model, hairpin model, super helical structural model, etc. [42]. Argos et al. [43] proposed the molecular structure of zein to be a helical wheel conformation, in which the protein is believed to possess nine homologous consecutive units stacked in an anti-parallel manner, stabilized by hydrogen bonds, and exhibit asymmetric geometry. According to the circular dichroism and optical rotatory dispersion measurements, the helical content of zein ranges between 50%–60% in 80% ethanol [43–45]. The α and β zeins are present almost in the same proportion. Zein takes on a globular structure in non-aqueous solutions with conformational properties comparable to those of other globular proteins like insulin and ribonuclease [45]. The primary structure of each zein molecule consists of 20 amino acid units that are repeated nine times. Physical characteristic analysis (hydration potential, polarity, turn, and helix formation tendency) reveals that the amino acid sequence, including the repeat fragments, is made up of α-helixes with turn areas on both sides [42].

The helix comprises several polar amino acids as well as numerous hydrophobic amino acid residues, whereas the turn regions are rich in glutamine. The van der Waals force and intra- and intermolecular hydrogen bonds tend to stabilize this structure. The glutamine-rich turns are present between the helix and the cylindrical cap, assisting the intermolecular stacking into a plane by the interaction between side chains [43]. On the contrary, Matsushima et al. [44] modified the model proposed by Argos et al. on the basis of small angle X-ray scattering studies and proposed that reduced α-zein exists as asymmetric particles of length 13 nm with an elongated prism-like molecular structure with an axial ratio of 6:1 [44].

This ribbon-like model of zein defines it to be 9–10 topologically antiparallel homologous helices, clustered and arranged adjacently to form a slender rectangular prism-like structure possessing an edge length of 13 nm, 3 nm, and 1.2 nm and an axial ratio of 6:1. The side chain residues in the helices accumulate into the hydrophobic exterior as a result of intermolecular hydrophobic aggregation. Thus forms the zein structure of considerable stability with both hydrophilic and hydrophobic sides. The two-dimensional ribbon-like model of zein is extensively used to demonstrate various orientations of zein molecules on hydrophilic and hydrophobic surfaces and their self-assembly from single molecules to nanoparticles [42,44].

Furthermore, the hairpin model of zein is primarily based on nuclear magnetism resonance (NMR), small angle X-ray diffraction (SAXS), and Fourier transform infrared spectroscopy (FTIR). According to this model, zein is made up of a succession of helix configurations. Unlike the previous two models, these helix structures are connected and stretched in the shape of rings, sheets, or loops, allowing zein to fold or expand in diverse ways in different solvent systems. At the same time, it is assumed to be related to zein's fibrillation ability [46]. Regardless of disparities in the molecular models of zein, it is widely assumed that zein exhibits a slender confirmation formed by a large number of alpha-helical structures [42].

The characterization of α-zein by FTIR is well-studied. The amide A band between 2800 cm^{-1} and 3500 cm^{-1} corresponds to N-H stretch and O-H stretch in

amino acid residues. The carbonyl, C=O stretch of amide groups appears at 1650 cm^{-1} and is referred to as amide I. The band at 1540 cm^{-1} is named amide II and corresponds to the angular deformation vibration of the N-H bond, whereas the band at 1230 cm^{-1} defines the axial deformation vibrations of the C-N bond [47,48].

Different factors affect the 3-dimensional structure of zein, particularly the solvent type. The somewhat larger sizes of α-zein in acetic acid, 70%, and 80% ethanol aqueous solutions imply that the α-zein structure is locally unfolded in these solutions when compared to the folded structure model. The circular dichroism data reveal that the secondary structure of α-zein does not change much in acetic acid or in 70% and 80% ethanol aqueous solutions, which might possibly be due to local unfolding occurring largely in the coil area. The size of α-zein in acetic acid is bigger than in ethanol aqueous solutions, showing that the degree of α-zein expansion and swelling is greater in the presence of acid. The conformation and dissolution behavior of alpha-zein vary with the nature of the solvent. This may be attributed to three main causes. The dissolution behavior of α-zein is closely related to the solvent polarity, hydrophilicity/hydrophobicity of the protein surface, its protonation, etc. [49].

Zein behaves as an amphiphilic protein but also displays a hydrophobic nature [40]. Therefore, zein is soluble in ethanol, acetone, or acetylacetone and insoluble in water. This characteristic property is due to the presence of more hydrophobic non-polar amino acids than polar hydrophilic amino acids. The existence of numerous uncharged amino acid residues also reinforces this property. Studies showed that zein is approximately 50 times more hydrophobic than albumin and fibrinogen [40]. Although zein is insoluble in water, it becomes soluble in the presence of alcohol, a high concentration of urea, reducing agents, anionic surfactants, or a pH above 11 [37]. The presence of the following functional groups determines zein's solubility and chemical reactivity: amines, amides, hydroxyls, carboxylates, and phenols. Because of the presence of such various types of groups, zein can be physically and chemically transformed to enhance its functional qualities. The addition/grafting of groups with adequate hydrophilicity may be applied to control zeins' hydrophilicity/hydrophobicity. Chemicals such as formaldehyde, glutaraldehyde, citric acid, and others may trigger cross-linking between zein molecules [32].

Despite zein's poor water solubility, low nutritional value, and unequal amino acid composition, its unique solubility allows it to self-assemble into micro- or nanoparticles. In this context, zein serves as an excellent carrier for hydrophobic bioactive molecules. Moreover, it is less digestible than other proteins, which makes it suitable as an oral delivery carrier [50]. As the isoelectric point of zein is 6.2, colloidal systems prepared at neutral pH or in PBS pH 7.4 tend to aggregate and display a large size distribution. This can be overcome by the addition of a moderate concentration of stabilizers or surfactants such as casein, lecithin, tween 20, and polyvinylpyrrolidone, thus altering the surface charge of the preparation medium and facilitating the formation of more stable zein micro- or nanoparticle dispersion [37,51].

Zein has grease-proof properties and low water vapor permeability. Zein is a completely amorphous polymer with plasticizing viscoelasticity and a glass transition temperature (Tg) of about 165 °C, although its Tg decreases significantly with increasing degree of plasticization. Zein exhibits a thermal degradation temperature of

320 °C, a degree of polymerization of 210–245, and a partial specific volume of 0.771 [48].

Zein finds application in various industries. Zein films appear tough with glossy surfaces, and so they find application in the food and pharmaceutical industries. Zein is modified for the delivery of drugs and vaccines owing to its mucoadhesiveness and ability to withstand gastric conditions. The brick-like structure of the protein offers adequate space for drug entrapment [37]. The gel-forming capacity of zein, with or without other polymers, has been exploited for the delivery of active biomolecules. In recent years, zein has attracted the attention of the pharmaceutical sector due to its important qualities, such as its natural renewable origin, biodegradability, non-toxicity, and biocompatibility [33]. **Figure 1** depicts the biomedical applications of zein.

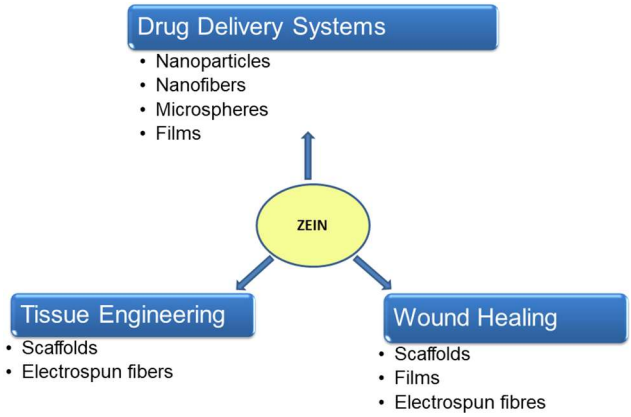

Figure 1. Schematic diagram of different biomedical applications of zein.

4.2. Zein-based drug delivery systems

Drugs are compounds used to prevent, control, and treat diseases. Drug delivery systems (DDS) have several advantages over conventional drug delivery methods and have now reached the third generation of DDS. With DDS, therapeutic efficacy is increased significantly, and there are advances in developing potential drugs with the help of bioinformatics. Even with the use of technological advances, there are still limitations in terms of solubility, stability, toxicity, and bioavailability. Scientists have been motivated by this situation to come up with technologies for enhancing therapeutic efficiency by developing novel carriers that release the molecule at a regulated pace while posing minimal toxicity. Among the widely explored new carriers, the demand for zein formulations to convey bioactive ingredients has continued to grow. Zein-based formulations were developed based on the many features of this natural substance for use in medication and biological delivery systems. Its resistance to heat, moisture, abrasion, and humidity increases the probability that the formulated product will have a longer shelf life. Moreover, zein consists of a large number of non-polar amino acids with aggregation formation ability, which in turn helps in the entrapment of drugs. These physical properties of zein allow for a wide range of usage in different formulations, including films, fibers, gels, controlled drug delivery systems such as tablets, and micro- or nano-particulate systems.

4.2.1. Zein nanoparticles

Zein is one of the most intensively investigated plant proteins for drug delivery applications due to its amphiphilic nature, bio-adhesive properties, and tendency to self-assemble into nanoparticles of sufficient stability. Nanoparticles are used to encapsulate, protect, and deliver therapeutic agents. For effective delivery of active ingredients, nanoparticles should withstand environmental stresses and effectively release the active material at the desired site [52]. Protein-based nanoparticles offer advantages over other materials in terms of biodegradability, safety status, and commercial scale-up [37].

Zein nanoparticles have been found to be effective in cancer therapy. In general, nanoparticles exhibit the ability of size dependent passive targeting through leaky tumor capillary fenestrations into the tumor vasculature through enhanced permeability and retention effects. As a result, tumour cells carry elevated concentrations of loaded cytotoxic agents, thereby facilitating the rapid and effective death of cancer cells. In a study, Lai et al. encapsulated 5-fluorouracil, an anticancer drug, into zein nanoparticles via the phase separation method for liver targeting and reported more than 31% targeting efficiency. The relative uptake of the loaded nanoparticle in the liver was reported to be 2.79 times greater than that of the 5-fluorouracil drug solution when administered through the intravascular route [53].

Resveratrol is a drug that displays poor solubility and bioavailability but has the potential to treat cancer. It is naturally present in grapes, berries, and peanuts, but after consumption, it is quickly metabolized into glucuronides and sulfates and excreted. Moreover, resveratrol can be degraded by heat, light, and enzyme exposure. To circumvent this problem, resveratrol can be preserved by nanoencapsulation, which will improve the drug's oral administration. In this regard, Huang and colleagues developed zein-pectin core/shell nanoparticles loaded with resveratrol by combining antisolvent precipitation and electrostatic deposition. It was found that the antioxidant activity of resveratrol was greatly improved when it was encapsulated into biopolymer-based nanoparticles. With the help of a pectin-based shell, the zein can act as a core and avoid aggregation caused by pH, high salt concentrations, and high temperatures [54].

A novel drug delivery system where the drug, honokiol, was encapsulated in a zein/hyaluronic complex was developed by Zhang et al. [55]. The complex exhibited anti-carcinogenic activity against breast carcinoma and anti-inflammatory, antioxidant, and neuroprotective properties. Honokiol targets the CD44 receptors that are overexpressed in cancer cells. Hyaluronic acid-zein-honokiol complex reduces tumor growth by 77.3% when compared with free honokiol with only 25.8% of tumor inhibition.

Nanoparticles have been shown to improve the oral bioavailability of compounds, either by protecting them from degradation by the gastric environment, improving the solubility of less water soluble drugs, or improving drug permeability through the intestinal lumen. Zein nanoparticles can also be utilized for enhancing the oral bioavailability of therapeutic molecules [56]. Zou et al. [56] generated zein nanoparticles and emulsified these particles with a surface active agent, D-α-tocopheryl polyethylene glycol succinate (TGPS), to form TGPS emulsified zein

nanoparticles and employed them for oral administration of daidzin, which is an isoflavone 7-glycoside with poor bioavailability in mice. It was inferred from the results that zein nanoparticles enhanced the bioavailability (2.64 times higher), and the cellular uptake and drug release were elevated in the Caco-2 cell line. It was reported that the enhanced absorption of the drug was due to P-glycoprotein inhibition by TGPS and transcytosis of particles.

According to Lee et al. [57], zein nanoparticles synthesized by the phase separation method have the ability to protect therapeutic proteins, antioxidant enzymes such as catalase, and SOD from digestion by pepsin and acidic conditions in the stomach. Zein NPs were loaded with SOD and catalase to protect them from gastrointestinal degradation following oral administration and also to target them to activated macrophages through folate receptors. The particle size of this system was 255.21 nm, with a loading efficiency of 30%–50%. The encapsulation of these enzymes into zein increased the proteins' therapeutic function from 5%–10% with free catalase and SOD to 40%–60%. The complex is believed to protect the loaded enzymes from pepsin as well as the acidic conditions of the stomach.

Moreover, the antioxidant compound, procyanidin, extracted from cranberries, was successfully loaded into zein nanoparticles by employing the liquid-liquid dispersion method. The procyanidin-zein nanoparticle showed an average size of 392 nm to 447 nm, and cell culture studies reported the cytotoxicity of procyanidins in pro-myelocytic leukemia L-60 cells in comparison with plain procyanidin solution [58]. Similarly, using the liquid-liquid dispersion method, essential oils such as thymol and carvacrol, were encapsulated in the zein nanoparticles. The morphology, structure, antioxidant capacity, and antibacterial activity of the nanoparticles with respect to different pH conditions were studied. Samples under acidic conditions tended to form films after lyophilization, whereas samples under neutral and basic conditions formed nanoparticles. Ferric ion spectrophotometric assay and DPPH analysis were used to evaluate the antioxidant qualities. Depending on the formulation, DPPH was lowered by 24.8%–66.8%, and samples quenched more than 65% of the hydroxyl free radicals. Furthermore, it was reported that *Escherichia coli* reductions of 0.8–1.8 log CFU/mL were achieved in the presence of essential oil-encapsulated nanoparticles [59].

Drugs can be encapsulated in zein nanoparticles. A zein-based hollow nanoparticle with sodium carbonate as a template has been employed in the delivery of the anti-diabetic drugs, metformin. A phase separation technique was used to fabricate these hollow zein nanoparticles. The development of sacrificial cores from sodium carbonate (2.0–9.1 wt%) and the precipitation of zein onto cores were the two steps involved in the preparation of hollow zein nanoparticles. The dissolved zein polymers were found to be attached to sodium carbonate nanocrystals, thereby preventing their agglomeration. The mixture was added to 200–500 g of distilled water in various proportions to precipitate sodium carbonate-cored zein nanoparticles. The sodium carbonate cores exhibited high water solubility, causing them to disintegrate quickly when the zein polymers on the surface precipitated, creating hollow zein nanoparticles [60].

In the studies conducted by Podaralle and Perumal, zein nanoparticles were fabricated using a pH-controlled nano-precipitation method. The model hydrophobic drug, 6,7-dihydroxy-coumarin (DHC) was encapsulated. It was observed that DHC,

in spite of being a hydrophobic drug, exhibited 78% encapsulation efficiency with white zein [61]. Luo et al. developed zein nanoparticles coated with carboxy methyl chitosan (CMC) and further encapsulated with vitamin D3. On comparing CMCs-coated zein nanoparticles with plain zein nanoparticles, the former displayed an encapsulation efficiency of 87.9% and sustained release in PBS medium [62]. Retinol, one of the hydrophobic vitamins, was effectively encapsulated into zein chitosan nanoparticles. Before chitosan coating, the retinol-loaded zein nanoparticles had a particle size of about 6 nm; however, after coating, the particle size increased to about 400 nm. As the concentration of zein increased, the encapsulation efficiencies marginally increased from 66.2% to 72.8%. These findings indicate that processing parameters such as the molecular weight of chitosan and a ratio of weight of zein/chitosan may have a significant impact on the characteristics of zein-chitosan nanoparticles [63]. **Table 1** summarizes some of the zein nanoparticle-based drug delivery systems.

Table 1. Zein nanoparticle-based drug delivery systems.

Carrier system	Approach/Method	Drug/bioactive compound	Biomedical applications	Reference
Zein	Phase separation	5-Fluorouracil (5-FU)	Liver targeting	[53]
Zein/Pectin	Anti-solvent precipitation and electrostatic deposition method	Resveratrol	Oral delivery system, Improved bioavailability	[54]
Zein/Hyaluronic acid	Anti-solvent precipitation and electrostatic deposition method	Honokiol	Delivery system for metastatic breast cancer therapy	[55]
Zein/D-α-tocopheryl polyethylene glycol succinate (D-α-TGPS)	Antisolvent precipitation method	Daidzin	Improving oral bioavailability of isoflavone glycosides	[56]
Zein	Phase separation method	Catalase, superoxide dismutase (SOD)	Reactive oxygen species (ROS) scavenging, targeting activated macrophages	[57]
Zein	Liquid-liquid dispersion method	Cranberry Procyanidins	Leukemia treatment	[58]
Zein	Liquid-liquid dispersion method	Thymol, carvacrol	Antioxidant, anti-microbial activity	[59]
Zein	Phase separation method with nucleation	Metformin	High payload, intracellular delivery	[60]
Zein	pH-controlled nanoprecipitation	6,7-Dihydroxy coumarin	Optimization of formulation factors	[61]
Zein/Carboxymethylcellulose (CMC)	Phase separation method	Cholecalciferol (Vitamin D3)	Delivery system for hydrophobic nutrients/drugs	[62]
Zein/Chitosan	Anti-solvent precipitation and solvent evaporation	Retinol	Increasing nanoparticle stability	[63]
Zein/Sodium caseinate	Modified anti-solvent approach	Thymol	Anti-microbial, antioxidant activity	[64]
Zein	Solution-enhanced dispersion by supercritical fluids	Lutein	Controlled drug release	[65]
Zein	Anti-solvent precipitation method	Hyperoside	Oral delivery system	[66]

4.2.2. Zein based nanofibers

Nanofibers find diversified uses in the biomedical sector. The natural origin polymeric nanofibers incorporated with different therapeutics, growth factors,

vitamins, etc. have been investigated for various delivery applications through various administration routes such as oral, pulmonary, transdermal, and ocular routes [67]. Zein fibres can be fabricated using two different techniques. Melt-spinning is applied for the preparation of conventional fibres whereas electrospinning is used for nanofibers. The nanofibrous structure of electro-spun nanofibres, mimics the ECM environment, offers a large surface area for drug loading and cell attachment, and hence is more advantageous in biomedical applications than conventional fibers [68]. Moreover, electrospinning is the most widely used technique for the fabrication of nanofibers from natural as well as synthetic sources for controlled delivery of drugs, gene delivery, tissue engineering, etc. [69]. However, poor morphological stability and low mechanical strength of zein in wet conditions are limitations of native zein fibers [70].

Many attempts are being made by scientists to amplify its mechanical strength by making use of other polymers with zein or chemical crosslinking agents, which include formaldehyde or glutaraldehyde. The latter is toxic for human use and, hence, not preferable. Citric acid was used as a crosslinker for zein electrospun fibers. It was observed that the crosslinked electrospun fibers were able to maintain their ultrafine fibrous structure in phosphate-buffered saline at 37 ℃ up to 15 days [70]. In another similar study, citric acid cross-linked electrospun zein scaffolds exhibited better biocompatibility, attachment, migration, and proliferation of fibroblast cells than uncross-linked electrospun zein fibers, cross-linked zein films, and electrospun polylactide fibers [71]. Similarly, zein/citric acid crosslinked fibers catalyzed by sodium hydroxide exhibited a drug-loading efficiency of 58% along with a prolonged release of drug in artificial gastric juice. The mechanical properties, such as dry and wet tensile strength, increased by 183% and 448%, respectively [70]. Gallic acid, a food-grade antioxidant, when electrospun into zein produced ultra-fine fibers of diameters of 327 to 387 nm and an in vitro DPPH assay concluded that loaded gallic acid could preserve its phenolic character and retain its antioxidant activity after electrospinning [72].

Nanofibers based on zein, poly (ethylene oxide), and chitosan (zein/PEO/CS fibres) were synthesised by Wongsasulak et al. and exhibited good mucoadhesion features in the gastrointestinal tract. Wetting and swelling of the fiber's polymeric molecular chains caused a molecular interaction between the fiber and mucin molecules, (a glycoprotein found in epithelial tissues) due to the gastro-mucoadhesion [73]. Similarly, Wang and Chen formulated hordein-/zein-based nanofibers. These fibers with 30% zein content demonstrated a well-interconnected network, sufficient tensile strength, and stability in water. The release profile experiments revealed that the three-dimensional porous structure of the fibers may possibly serve as carriers for the regulated release of bioactive substances such as riboflavin in phosphate-buffered saline. Interestingly, the fibers were found to be stable in simulated gastric fluid as they were found to be resistant to pepsin, but underwent digestion in simulated intestinal fluid (where they are normally absorbed), thereby progressively releasing the incorporated compound [74].

Zein/Eudragit S 100 was electrospun to generate composite nanofibers that were utilised for carrying pantoprazole and aceclofenac simultaneously. Aceclofenac was readily loaded into zein, while Eudragit S 100 was intended to deliver pantoprazole,

an acid labile proton pump inhibitor that inhibits gastric acid secretion. The medication was then loaded into nanofibers after the development of nanocarriers using a single-nozzle electrospinning technique. Both in vitro and in vivo studies revealed sustained drug release for up to 8 h; hence, it was concluded that NSAIDs with controlled release triggered less toxicity in the GI tract. The electrospun composite zein/eudragit nanofibers developed by Karthikeyan et al. [75] aimed to simultaneously deliver two distinct drugs that would mitigate or lessen the side effects of NSAIDs. In this study, a single nozzle electrospinning process was applied to generate zein/Eudragit S 100 nanofibers loaded with aceclofenac and pantoprazole. Pantoprazole is an acid-labile drug, and hence, to prevent its degradation in an acidic environment, Eudragit S 100 was used as a carrier for its delivery via the oral route. The effectiveness of the developed fibers to sustain the release of both drugs for up to eight hours was demonstrated by in vitro release studies. It was observed that the co-administration of pantoprazole and aceclofenac lessened the NSAID-induced gastrointestinal toxicity, as further evidenced by in vivo animal studies. Zein nanofiber-based drug delivery systems are listed in **Table 2**.

Table 2. Zein nanofibers as drug carriers for drug delivery systems.

Carrier system	Fiber type	Drug/bioactive compound	Biomedical applications	Reference
Zein	pure	Citric acid	Improving cytocompatibility	[70]
Zein	pure	Citric acid	Sustained drug release, improved mechanical properties	[71]
Zein	pure	Gallic acid	Antioxidant activity	[72]
Zein/Poly (ethylene oxide) (PEO)/Chitosan	Hybrid	-	Gastro mucoadhesive delivery vehicle improves bioavailability	[73]
Zein/Hordein	Hybrid	Riboflavin	Intestine-targeted drug delivery systems, delivery of bioactive compounds for wound healing	[74]
Zein/Eudragit S100	Hybrid	Pantoprazole, Aceclofenac	Oral drug delivery, sustained release of non-steroidal anti-inflammatory drugs (NSAIDs)	[75]
Zein	Core sheath	Ferulic acid	Sustained drug release	[76]
Zein	Pure	Curcumin	Sustained drug release	[77]
Zein/Polycaprolactone	Hybrid	Tetracycline	Sustained antibiotic release	[78]
Zein/Polyurethane/Cellulose Acetate	Hybrid	Streptomycin sulphate	Antimicrobial activity	[79]
Zein/Collagen	Hybrid	Berberine	Antibacterial activity, sustained drug release	[80]

4.2.3. Zein based microspheres

Protein-based microspheres and microparticles are used in mucoadhesive vaccine delivery, depot preparations, and controlled release in the GI tract [37,81–83]. Zein microspheres were developed by Mehta et al. in which various antitubercular drugs such as rifampcin, isoniazid, and pyrazinamide were loaded using a solvent evaporation technique. Among these drugs, rifampicin and isoniazide exhibited higher and lower encapsulation, respectively, than others [84]. Similarly, α-tocopherol was encapsulated in zein/chitosan to form a complex possessing a size of 200–800 nm and a zeta potential of 22.8 to 40.9 mV. It was reported that electrostatic interactions and hydrogen bonding aided the complex formation. This complex provided better

protection to this antioxidant from adverse conditions in the GI tract as compared with plain zein nanoparticles, thereby revealing their potential efficacy in delivering hydrophobic drugs [85].

Researchers developed a novel delivery system consisting of chitosan/zein nano-in-microparticles loaded with DNA. The chitosan/DNA particles represented the core, which was encapsulated in zein microspheres. The study revealed the protective nature of zein microspheres towards the encapsulated DNA in a harsh GI environment [86]. Zein-based microspheres were conjugated with the chemoimmunotherapy drug polysaccharide-Kureha used for cancer treatment [87]. In a similar study, antitumor drugs such as mitomycin C, daunomycin hydrochloride, and peplomycin sulfate were successfully conjugated with zein microspheres using a DMSO-water solvent system [88]. Zein-based microspheres for controlled delivery of antibiotics such as ciprofloxacin have also been reported [89]. In a study conducted by Liu et al. [90], the phase separation method was used to generate a novel microsphere drug delivery system for ivermectin, an antiparasite drug, by making use of zein. Release studies of this model drug from zein microspheres as well as from tableted microspheres were carried out in vitro. The outcomes demonstrated that the tableted and zein microspheres are appropriate for use as a sustained-release form of ivermectin. As the diameters of microspheres are suitable for macrophage phagocytosis, it could be helpful in drug targeting systems.

In the same study, the release kinetics of tableted microspheres were explored [90]. Pepsin enzyme degraded tableted microspheres exhibited zero order kinetics for drug release. Hence, the formulation can be applied to orally administer drugs to maintain a constant concentration of drug in plasma in vivo. Furthermore, they could be developed as scaffolds consisting of microspheres that can steadily release bioactive compounds that could promote cell differentiation in tissue engineering applications. Muthuselvi and Dhathathreyan [91] formulated simple coacervates of zein to encapsulate a cardiotonic glycoside, gitoxin. Zein microspheres and gitoxin-loaded zein microspheres were prepared using different solvents such as methanol, ethanol, and isopropyl alcohol. In-vitro release studies of gitoxin from zein microspheres in different solvent systems were conducted, and results revealed zein microspheres developed in ethanol were best suited as the sustained release form of gitoxin. **Table 3** summarizes some of the zein microsphere-based drug delivery systems.

Table 3. Zein microsphere-based drug delivery system.

Carrier system	Approach/method	Drug/bioactive compound	Biomedical applications	Reference
Zein	Emulsification and solvent evaporation method	Antituberculosis drugs – Rifampicin, Isoniazid, pyrazinamide	Tuberculosis chemotherapy	[84]
Zein/Chitosan	Anti-solvent precipitation and solvent evaporation	α-tocopherol	Hydrophobic drug delivery to the GI tract	[85]
Zein/Chitosan	Water in oil emulsion	DNA	Oral gene delivery	[86]
Zein	High dilution process	Polysaccharide-K	Cancer immunotherapy	[87]

Table 3. (*Continued*).

Carrier system	Approach/method	Drug/bioactive compound	Biomedical applications	Reference
Zein	Emulsification	Antitumour drugs –Mitomycin C, Daunomycin, Peplomycin sulphate	Cancer immunotherapy	[88]
Zein	Phase separation method	Ivermectin	Drug targeting system	[90]
Zein	Coacervation	Gitoxin	Sustained drug release	[91]
Zein	Coacervation method	Ovalbumin	Vaccine delivery, to improve adjuvanticity and immunogenicity	[92]
Zein	Emulsification and solvent evaporation method	Aceclofenac sodium	Sustained delivery systems for NSAIDs	[93]

4.2.4. Zein based films

Zein can be developed into films either by interaction with other materials or by a chemical or heat-based crosslinking procedure. This property is attributed to the presence of numerous amino acid side chains. Zein films are tough, glossy, hydrophobic, biodegradable, resistant to microbial attack, and safe for internal human use [7,37,94,95]. Wang et al. [96] reported zein films to be biocompatible with human umblical vein endothelial cells (HUVECs). Zein microspheres as well as heparin-loaded zein microspheres suspended in a 40% ethanol solution were prepared and subsequently volatilized at 37 ℃ to form zein microsphere films. It was observed that zein films and heparin-loaded zein microsphere films were able to suppress platelet adhesion. Moreover, heparin-loaded zein film exhibited better anti-coagulation properties. It was reported in a study that ciprofloxacin-loaded zein films could possibly prevent bacterial infection on implanted devices [89]. Singh et al. [97] prepared zein films loaded with salicylic acid and acetyl salicylic acid, with or without glycerol. The resultant films containing glycerol, salicylic acid, and acetylsalicylic acid showed a decrease in tensile strength. Studies reported that oleic acid plasticized zein films withstand gastric fluids and are soluble in intestinal fluids [98].

Zein films were investigated for their ability to enhance the resistance of cells to flow-shear-stress after the implantation of vascular implants. The films were made of three types of zein, collagen, and poly-L-lactic acid. The flow shear stress resistance of NIH3T3 and EA.hy926 cell lines on films was studied. It was observed that cell retention of EA.hy926 on zein film with a rough surface was better than that of films with a flat surface. The cell retention of NIH3T3 on a rough surface was better under flow-shear stress for 6 hours. This study concluded that zein films with more roughness could possibly enhance the cell's flow shear stress resistance and may be applied to vascular implant coatings [99]. Another study has reported that zein-based films act as a good matrix for the sustained release of drugs such as nisin, iodine, thymol, catechin, lysozyme, and gallate esters [100]. A novel technique was developed by Gao et al. for the preparation of films from zein thermo-modified starch by using dry heating of starch or di-starch phosphate with zein. It was observed that the dry heating reduced the pasting properties of zein and distarch phosphate. These zein/starch films exhibited good compatibility, a higher water contact angle, and tensile strength, implying that dry heating is a possible method to improve the properties of starch films [101].

4.3. Zein in tissue engineering

The extracellular matrix (ECM), as is well known, is made up of a variety of biomolecules, notably proteins, and polysaccharides such as hyaluronic acid, chondroitin sulphate, heparin, heparin sulphate, etc. [102], and proteoglycans, among others, which aid cell attachments as well as proliferation, differentiation, and migration [33]. Tissue engineering typically involves the culturing of living human cells, usually in polymeric (ceramic) scaffold materials, ex vivo, and eventually permitting them to develop into a three-dimensional tissue. With the use of tissue engineering, it is possible to regenerate tissues that have been damaged by disease or trauma, and in some situations, to develop new tissues or substitute failing or malfunctioning internal organs. Degradable biomaterials generally serve this purpose by either promoting the ingrowth of surrounding tissue and cells or acting as temporary scaffolds on which transplanted cells can adhere, proliferate, and retain their differentiated functions. Hence, biomaterials play a transient but vital role in the success of tissue engineering [103]. Therefore, in the tissue engineering process, cells are exposed to a fabricated scaffold that mimics the ECM, and under this new and artificial environment, the cells are expected to carry out the same functions and regenerate or heal the damaged tissue [33,102].

It is feasible to elaborate the extracellular matrix by different methods to produce fibers, both from natural and synthetic sources. Fibers from natural sources are biocompatible but show low mechanical firmness. Although the synthetic fibers lack cell binding and acid production ability, they show sufficient mechanical stability. The nanofibers from natural and synthetic sources can be combined to fabricate an ECM matrix by different methods, thus providing a synergetic effect. Zein has been shown to be an exceptional biopolymer in tissue engineering on account of its flexibility, resistance to microorganisms, compatibility with the human body, and biodegradability [33].

The successful implementation of tissue engineering depends on the choice of scaffold, which has a sponge-like structure with high porosity and optimum thickness. Three-dimensional porous zein fibers have also been manufactured to replicate the structure of human tissue as scaffolds for tissue engineering [37]. Zein scaffolds were developed in combination with other fibers such as cellulose acetate, polyurethane, and gum Arabic [33]. Rad and colleagues designed and characterized an electrospun nanocomposite scaffold for skin tissue engineering using polycaprolactone/zein/gum Arabic. Zein and gum Arabic provide protein and polysaccharide content, respectively, for the regeneration of skin, while PCL provides mechanical stability. The PCL/zein/gaum Arabic scaffold exhibited high hydrophilicity, a tensile strength of 1.36–3 MPa, and an elongation of 19.13%–44.06%, thus making it ideal for skin tissue engineering applications. In addition, the porosity of the scaffold was greater than 77%, which was appropriate and suggested for cell infiltration. Furthermore, due to the presence of cyanogenic glycosides in gum Arabic, the composite scaffolds showcased antimicrobial characteristics as well as improved hydrophilicity, which in turn enhanced cell viability and proliferation [104].

Dong et al. prepared zein films using solutions of varying concentrations, and by SEM analysis, it was found that these films were formed of zein particles with varying

sizes ranging from 100–500 and 500–2500 nm in diameter. Human liver cells (HL-7702) and mouse fibroblast cells (NIH3T3) were cultured in these films, and the ability of cells to adhere, grow, and proliferate on zein films served as a measure of their biocompatibility. The results showed the attachment of more than 60% of cells to zein films at 3 h after seeding [105]. Zein exhibited excellent biocompatibility with platelets and human umbilical vein endothelial cells. This property was explored by Wang et al. [96] to develop zein film as a coating material. It was observed that platelet adhesion was suppressed by both zein film and heparin-loaded microsphere film, while the latter showed anticoagulation properties. In another study conducted by Dhandayuthapani et al., a novel fluorescent nanocomposite nanofiber made up of cadmium sulphate and zein was developed through an electrospinning technique. These composites tend to support the mesenchymal stem cells and fibroblasts for their attachment and proliferation. It was further noted that these cells hold normal cell shapes and merge well with surrounding fibers, emphasizing the importance of quantum dot-encapsulated fluorescent zein nanofibers as scaffolds for tissue engineering [106].

Studies have reported that three-dimensional fiber scaffolds developed from a homogeneous blend of gelatin/zein (1:4) via the force-spinning technique exhibited improved tensile strength and good hydrophobic properties, with a water contact angle of 115° and low cytotoxicity in human fibroblasts, and facilitated sustained release of the drug berberine for over 15 days [107]. The gelatin ought to be responsible for the scaffold's improved elastic modulus, hydrophilicity, and cytocompatibility [108]. In a study conducted by Saowakon et al. [109], ultrafine zein-based electrospun composite fibers with mucoadhesive properties were developed. The fibers were composed of zein, polyethylene oxide, and chitosan in the ratios 87.5, 10, and 2.5, respectively. A hydrophobic antioxidant compound, –α tocopherol was added 20 wt% into the fiber matrix. This fiber displayed adequate gastro-mucoadhesive and release characteristics, which makes it capable of delivering hydrophobic compounds to the GI tract.

As is well known, the crosslinking method and the addition of plasticizers can strengthen the mechanical properties of membranes. For example, hexamethylene diisocyanate is often used as a crosslinker agent for zein-based nanofibers. Besides, zein nanofibers in combination with palmitic acid/zein (1:2) and stearic acid/zein (1:4) displayed better mechanical stability, as palmitic acid contributes to its plasticization property. Likewise, the addition of mica, kaolinite, montmorillonite, and zeolite to zein fibers enhanced their mechanical properties [4].

Zein is an excellent choice for fabricating biomaterial scaffolds for bone regeneration due to its biocompatibility, biodegradability, remarkable mechanical properties (including strength, flexibility, and compressibility), antioxidant capacity, and microbial resistance. It has been established that scaffolds made of zein-based composites enhance osteogenesis differentiation, which renders them ideal for bone tissue regeneration [4]. Because of probable interactions between cells and the protein layer, zein-based biomaterials are favourable for cell adhesion [110].

Periodontitis is a prevalent dental disease in which electrospun NFs could provide effective treatment and aid in the recovery of the paradentium. Yang et al. [108] employed the co-electrospinning method to develop zein/gelatin nanofibres using 1,1,1,3,3,3-hexafluoro-2-propanol as the solvent. The fibers were characterized, and

human periodontal ligament stem cells were used to evaluate the cell affinity towards zein/ gelatin nanofibers. On comparison with the native zein scaffold, the zein/gelatin scaffold demonstrated good cytocompatibility, and the addition of gelatin promoted the development of periodontal ligament stem cells. Furthermore, by altering the gelatin-to-zein ratio, surface shape, and fibre diameter, the mechanical performance and cell adhesive capacity in vitro may be easily adjusted. In addition, the constructed scaffold promoted cell adhesion and expedited periodontal ligament stem cell development.

Shrestha et al. [111] succeeded in the development of a novel bone regenerating scaffold using a zein/chitosan/polyurethane composite membrane incorporated with functionalized multiwalled carbon nanotubes using the electrospinning method. The obtained scaffold exhibited good antibacterial efficacy against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Staphylococcus epidermis*. According to in vitro investigations, it was observed that the scaffold significantly enhanced the regenerative effect of pre-osteoblast (MC3T3-E1) by facilitating rapid cell-to-cell communication through a bio-interface and promoting cell growth, proliferation, and differentiation. The excellent osteoinductive properties of the scaffold were indicated by the nucleation of hydroxyapatite nanocrystals and the expression of osteogenic markers, as confirmed by Alizarin red staining analysis, alkaline phosphatase activity, and western blotting. These findings suggested that the designed PU/Zein/CS-carbon nanotube fibrous scaffold has the biological properties necessary to function as an artificial bone extracellular matrix, ensuring bone cell regeneration.

The solvent casting particulate leaching method was applied to fabricate a zein/poly(caprolactone) biocomposite scaffold with sodium chloride particles as porogen. This scaffold enhanced hydrophilicity, and faster in vitro degradation as compared to the PCL scaffold. In addition, the in-vitro degradation behaviour of scaffolds in PBS was studied for 28 days and reported a slight weight loss of the zein/PCL scaffold with increasing the zein content in the composite. These studies concluded that zein/PCL biocomposite could be a promising material for bone tissue engineering [112]. The zein-based scaffold generated by Yan-Zhi et al. [113] exhibited better porosity, an open pore wall structure, and excellent biocompatibility with periodontal ligament cells, and hence was applied for periodontal tissue healing.

Moreover, zein blended with poly (glycerol sebacate) finds application in soft tissue engineering [114]. The incorporation of hydroxyapatite (HA) improves tissue regeneration as well as minimizes scaffold degradation rate. In this regard, zein with hydroxyapatite has proven to improve osteoblasts [115]. Yao et al. [116] and Zhang et al. [117] developed nanofibers of zein/hydroxyapatite with a reasonable tensile strength and subsequently utilized these materials as scaffolds for tissue reconstruction in separate investigations. The mineralized zein nanofibrous membranes had an excellent impact on osteoblast growth and did not generate cytotoxicity, as demonstrated by osteoblast adhesion tests and in vitro cytotoxicity experiments. The zein/HA membrane had distinct nanofibrous structural properties, and a coating of HA nanocrystallites can be used in bone repair and regeneration, indicating that the electrospun zein/HA fibrous membranes show potential for bone tissue engineering applications.

Poly (3-hydroxubutyrate-co-4hydroxubutyrate) (PHB), a bacterial thermoplastic, when incorporated with zein in appropriate concentrations (20%–80%) and subsequently electrospun, formed ultrathin fibres (60–650 nm in diameter) with a tensile strength of 4.8–7.1 MPa and elongation at break between 15%–69%. The in-vitro experiments demonstrated that the blended scaffolds are non-cytotoxic for NIH3T3 fibroblast cells and MG-63 osteoblast cells and offer support for cell adhesion, distribution, and proliferation [118].

A three-dimensional zein porous scaffold with and without rabbit mesenchymal stem cells was assessed for its osteogenic activity by Tu et al. [119]. Earlier studies from this group proved that zein scaffolds have good mechanical properties suitable for bone-tissue engineering applications, and their porous nature encouraged cell migration and ingrowth [120]. In this study, mesenchymal stem cells were seeded onto zein scaffolds, and the cell-seeded scaffold was evaluated for the formation of ectopic bone in the thigh muscle pouches' model of nude mice. Further to this, the MSC-seeded zein scaffolds were implanted into a segmental bone defect of 1.5 cm length created in the middle of the radial shafts. These studies revealed that MSC-seeded zein scaffolds could successfully induce ectopic bone formation in the thigh muscle pouches of nude mice, and they also demonstrated a good ability to repair rabbits' critical-sized radial bone defects, which were accompanied by blood vessel formation. These findings suggest that stem cell-seeded zein scaffolds could be a promising strategy for treating bone abnormalities [119].

A three-dimensionally stable zein scaffold with an interconnective and open pore structure was produced using the salt leaching process. The particle size and porosity of the scaffold were 300 mm and 75.3%–79.0%, respectively. The mechanical properties, such as Young's modulus and compressive strength, of the scaffold were comparable to those of the cancellous bone, implying its possible application in non-load-bearing regions. Additionally, in-vitro cell culture studies showed that rat mesenchymal stem cell adhesion, growth, and proliferation could be successfully achieved on zein porous scaffolds and that these scaffolds possessed demonstrable osteoconductive qualities in the presence of dexamethasone, further indicating zein as a promising material for bone tissue engineering [121].

Hadavi et al. synthesized a zein nanoparticle-based delivery system incorporated with bone morphogenic protein-6 (BMP6), which is a peptide growth factor in bone formation, using a liquid-liquid phase separation method. This was investigated for its in vitro osteoinduction property in mouse myoblast C2C12 cells. The study reported 72% encapsulation and a prolonged release pattern of the loaded peptide, as well as high biocompatibility of the BMP-6 loaded zein, with 90% cell viability after 48 and 96 h incubation. Furthermore, on day 14 after incubation, the level of enzyme alkaline phosphatase, an osteocyte marker, in C2C12 cells treated with BMP-6-loaded nanoparticles and those treated with blank nanoparticles was compared, and an elevated level of ALP was observed in the former. The BMP6 peptide-loaded zein nanoparticles substantially improved Runx2, a transcription factor for osteoblast differentiation gene, expression compared to blank nanoparticles, implying that the BMP pathway was active. The study suggested that BMP6 peptide-loaded zein nanoparticles may be a good candidate for bone regeneration [122].

In a study conducted by Turner et al. [123], to improve hydrolytic stability, zein was cross-linked with trimethylolpropane triglycidyl ether to form a fibrous scaffold using the electrospinning method. The scaffolds were characterized and assessed for their ability to support the osteogenic differentiation of MC3T3-E1 cells. It was observed that even in the absence of induction factors, MC3T3-E1 cells were able to grow and differentiate on the zein scaffolds, as evidenced by the early upregulation of Runx2 gene expression and increased alkaline phosphatase activity. These studies show that zein fibrous scaffolds promote cell growth and differentiation without the aid of growth factors, and these cross-linked scaffolds are stable and are proposed to be useful in bone repair applications.

According to recent research, the bioactive glass-based scaffold covered with zein excels the bare scaffold in bone regeneration. Arango-Ospina et al. [124] created a multifunctional bone regeneration scaffold using a foam-replica approach by coating bioactive glass with zein and Manuka honey. The study aimed at combining the biodegradability and biocompatibility of zein with the bioactive properties of bioactive glass and the antimicrobial effect of manuka honey. This scaffold outperformed uncoated bioactive glass scaffolds in terms of mechanical properties; the compressive strength of the uncoated scaffold was as low as 0.04 ± 0.01 MPa, whereas it increased to 0.14 ± 0.05 MPa for the coated one. The increased compressive strength of the coated scaffolds might be linked with the reinforcement effect of the infiltration of the polymer into the cracks and open hollow struts of bioactive scaffolds. The uncoated scaffolds had a porosity of $95.6\% \pm 0.3\%$, while the scaffolds coated with zein and 20 wt.% Manuka honey had a porosity of $77.0\% \pm 3.0\%$. The formation of a hydroxycarbonate-growing apatite layer on the surface of scaffolds was detected using an SEM and FTIR analysis as a part of bioactivity studies in simulated bodily fluid. The coated scaffolds generated an apatite-like layer on their surfaces after 1 week of incubation, but the untreated scaffolds developed the apatite layer after only 1 day. The release studies showed a fast release of honey from the scaffold within 1 hour.

It is worth mentioning the studies carried out by Eldeeb et al. [125], in which the dual-drug-loaded implant was developed using a zein matrix to deliver pitavastatin calcium, an osteogenic drug, and tedizolid, an antibiotic for bone regeneration. In order to improve the bone proliferative effect of the formulated implants, a titanium-doped bioactive glass was used, as well as sodium hyaluronate, which served as a porogenic agent to produce the porosity essential for cellular infiltration and proliferation. The fabricated implant demonstrated persistent release of both drugs for 28 days. Besides, in vivo studies in Sprague Dawley rats showed a strong bone regenerating effect.

A study conducted by Mariotti et al. [126] applied electrospinning to generate fiber mats of zein mixed with non-doped or copper-doped bioactive glass. A 14-day degradation study in DMEM media revealed a slow development of halite crystals with a gradual loss of fibrous structure of the scaffolds; nonetheless, some fibers contained bioactive glass at the end of the study, indicating that the scaffolds were sufficiently strong. In vitro cell viability investigations, using zein fiber mats loaded with copper-doped bioactive glass indicated enhanced cell proliferation of up to 61% on the human osteosarcoma cell line MG-63 and 59% on the mouse muscle cell line C2C12 after 7 days.

Another study was conducted by Ranjbar et al. [127], in which a permeable scaffold was constructed from 58S bioactive glass with zein to strengthen its mechanical properties and serve as a medium for the controlled release of the drug kaempferol. The scaffolds coated with a 7% w/v zein solution possessed superior mechanical strength and an extremely porous structure. Furthermore, the data suggested that the generated scaffolds could promote optimal kaempferol release and a favorable environment for cell adhesion. In summary, zein-based composite scaffolds can be utilized as promising and nontoxic alternatives in bone tissue regeneration. The hybrid zein-based fibrous scaffold has the potential to serve as a future biomaterial for the repair of a wide range of tissue defects.

4.4. Zein in wound dressings

Over the past few years, scientists have tried to make advanced wound dressings from zein-based biomaterials due to their specific properties, including biodegradability, biocompatibility, film formation, and antioxidant capacity [128]. Zein can be fabricated as films or bandages that bind to the wound, form a protective barrier, and expedite wound healing. This can help save a moist wound environment and promote a natural healing process.

An ideal wound dressing for commercial use ought to promote the natural healing process of the wound while also protecting it from external conditions. The wound dressing should maintain an appropriate moist environment that prevents the wound from drying out, and it must not create excessive moisture that can lead to bacterial growth. Another property of wound dressing is its ability to absorb excess exudate and prevent leakage to the surrounding healthy skin. The commercial wound dressing should also be easy to apply, remove, and dispose of, possessing antimicrobial properties to manage infection in the wound area. Moreover, the dressing material should be biocompatible, non-toxic, non-allergenic, and cost-effective. The combination of these properties can help promote wound healing, reduce pain, and thereby improve the quality of patients' lives [129]. These dressings can be customized for different types of wounds, including acute and chronic wounds [130].

Proteins in the form of film or nanofibrous scaffolds have been explored for wound healing applications [125,126]. Recently, the function of nanofiber membranes designed through an electrospinning process in accelerating wound healing has attracted wide attention [132]. A blend combination of zein and poly(ethylene oxide) (PEO) fabricated by coaxial electrospinning, in which zein occupied the core and PEO in the shell, also exhibited wound healing properties. Interestingly, it was found that the electrospun formation of zein improved with the addition of PEO and increased the elasticity of these microfiber mats [133].

Miyoshi et al. reported [134] that when zein was hydrolyzed with thermolysin, angiotensin inhibitory peptides, mainly tripeptides, were produced, and further oral administration of these hydrolysates of α-zein to hypertensive rats brought about a reduction in their blood pressure. Further studies from the same group reported that this inhibitor, when administered topically and orally, hastens wound closure and prevents the formation of scar tissue by inhibiting angiotensin receptors or by converting angiotensin I to angiotensin II [130]. Interestingly, the wound site showed

more structurally organized collagen fibers that resembled normal skin texture. This might be due to inhibition of expression of TNF-α, fibroblast proliferation, and collagen production after ACEI production.

Antimicrobial wound dressing using electrospun zein, along with synthetic polymers like polycaprolactone (PCL), poly(ethylene oxide) (PEO), polyurethane (PU), and polylactic acid (PLA), and natural polymeric substances like gum arabic, hyaluronic acid, cinnamaldehyde, gum tragacanth, etc., were explored for their wound care properties. A wide range of antibiotics, antibacterial nanoparticles, and antimicrobial plant extracts could be incorporated into zein-based fibers. The role of zein-based electrospun nanofibers in combination with antimicrobial agents in enhancing wound healing is widely studied [131]. In a study conducted by Dashdorj et al. [135], silver nanoparticles loaded with electrospun zein fibers exhibited higher cytocompatibility and showed antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*.

Ghalei and collaborators developed a bioactive nanofiber wound dressing matrix from poly (vinyl alcohol) PVA/zein nanoparticles loaded with diclofenac (DLF) by the single jet electrospinning method. The drug loading efficiency was reported to be 47.80%. According to release profile studies, incorporation of zein nanoparticles (NP) into PVA nanofibers ensured a slower, sustained, and controlled release of the drug with prolonged release time instead of burst release of the drug. This makes PVA/zein NPs/DLF nanofibers a promising drug delivery system. Moreover, the cytotoxicity tests of PVA, PVA/zein NPs, and PVA/zein NPs/DLF nanocomposite dressings in L929 cell lines showed no signs of toxicity, and the addition of NPs to PVA fibers led to better cellular proliferation [136].

In a study conducted by Kimna et al. [130], gentamicin-loaded zein fibers were fabricated by electrospinning on the surface of the membrane. These fibers, which possessed a film thickness ranging from 311 to 361 μm and a fiber diameter between 350 and 425 nm, displayed structural features comparable to the layers of skin tissue. Moreover, the mechanical characteristics of the fiber were compatible with the skin tissue. Furthermore, membranes exhibited antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Gentamicin displayed a sustained cumulative release of 94%. According to the in-vitro cell culture results, membranes were found to be non-toxic, and proliferation of NIH/3T3 and HS2 cell lines was observed in each layer of the fiber, mimicking the multilayer skin tissue. These studies shed light on the possibility of developing a zein-based bilayer as a potential antimicrobial wound dressing for skin tissue regeneration.

Zein, in combination with other polysaccharides, also displayed wound-healing properties. Nanofibrous membranes of zein/collagen exhibited better surface wettability, mechanical and in vitro degradable properties, and cell adhesion ability [137]. In a study conducted by Unnithan et al. [138], the electrospinning method was adopted for the fabrication of a streptomycin sulfate-loaded composite nanofiber blend composed of polyurethane (PU), cellulose acetate (CA), and zein. Here, PU was used as the core polymeric component, while CA and zein were intended to improve hydrophilicity, permeability to air and moisture, cell attachment, proliferation, and blood thickening ability. The in-vitro antimicrobial activity of the nanofiber membranes was evaluated for use in wound dressings. There was a favorable

interaction of cells with the PU-CA-zein-drug composite scaffold. These scaffolds showed enhanced blood clotting and platelet activation. To add to above-mentioned properties, CA and zein also increased liquid uptake and created a moist environment for the wound, which is necessary to hasten wound recovery.

Application of natural medicinal extracts such as essential oils can enhance the antibacterial properties of zein-based wound dressings significantly. Thyme oil incorporated zein wound dressing was prepared by the in-situ electrospinning method. These fibres exhibited superhydrophilicity with a 0° contact angle and a gas permeability of 154 ± 20.9 m^2, as well as antibacterial activities. The in-vivo experiment data revealed that the fibres hastened wound recovery in mice within 11 days [139]. In a study carried out by Qin et al. [140], an antibacterial wound dressing was fabricated by incorporating clove essential oil (CEO) into the fibrous membranes of zein and deposited directly on the wound area of experimental mice via the in situ electrospinning method. The wound healing process was observed to be promoted as the zein/CEO fibrous membrane exhibited sufficient porosity, good gas permeability (168.2 ± 43.3 mm s^{-1}), biocompatibility, and an antibacterial effect. Moreover, higher hydrophilicity to absorb wound exudate was observed. Zinc oxide nanoparticles and aloe vera incorporated in zein/PCL/collagen electrospun nanofibers were studied by Ghorbani et al. [141]. The developed mats had strong mechanical properties, conductivity, antibacterial qualities, and a unique surface for cell adhesion and growth. They also exhibited outstanding biocompatibility.

An interesting study was conducted by Gunes et al. [142], in which a novel hybrid bilayer wound dressing was designed from zein. The upper layer, composed of zein film with montmorillonite (MMT) nanocomposite incorporated with *Hypericum perforatum* oil, and three-dimensional electrospun zein/MMT nanofibers represented the bottom layer. This layer induced wound healing with the controlled release of *H. perforatum* oil. The bilayer composites proved to have appropriate gas barrier properties, surface wettability, and antimicrobial efficacy against *Esherichia coli, Staphylococcus aureus*, and *Candida albicans.* The cell culture studies indicated no signs of toxicity on NIH3T3 mouse fibroblast and HS2 keratinocyte cell lines. The scratch wound assay indicated a wound-healing effect by enhancing fibroblast attachment, proliferation, and collagen secretion.

One of the drawbacks of electrospun zein matrices is their poor mechanical stability. This limitation can be overcome by mixing zein with other compatible polymers or cross-linking. Surendranath et al. [143] have reported post-electrospinning UV-mediated crosslinking for the zein/PEO blend wound dressing matrix to achieve mechanical stability and water resistance. Furthermore, the fabricated membrane elevates collagen production and aids in wound healing in human dermal fibroblast cells with no in-vitro cytotoxicity.

Zein has been explored for its efficacy in treating diabetic wounds. Liu and team developed a cellulose acetate-zein composite nanofiber incorporated with 5% sesamol for the treatment of diabetic wounds. This membrane turned down the expression of inflammatory cytokines IL-10 and sesamol, up-regulated IL-6 expression, and stimulated TGF-α signaling pathway transduction, and together enhancing the growth and proliferation of keratinocytes in diabetic mice [132]. A study conducted by Gough

et al. revealed air-jet spun zein nanofibers to be an effective carrier of sodium citrate that is applied topically to treat diabetic ulcers [144].

Attempts have been made by Wang et al., to develop composite films of zein and hydrogel poly (acrylic acid) to heal skin burns. These composite films showed better elasticity, better adhesiveness to human finger skin, and stretching properties under strenuous joint exercises. The burn wound in vivo data indicated that film improved wound healing properties and suggested that it could be a promising wound dressing material [145].

5. Conclusion

Biomedical applications based on protein-derived materials are of great interest now. Proteins, particularly those derived from plants, have tunable properties, safety, and biocompatibility characteristics that render them suitable for biomedical purposes. The efficiency of zein in being shaped and chemically handled provides an incredible tool for the biomedical world. The physical and chemical characteristics and unique structure (at molecular, nano, and micro levels) indicate that zein is inherently superior to many other natural as well as synthetic polymers. The chemical modification of zein is a critical aspect that could be improved in every scope, which is crucial to work on the zein-based biomaterial for enhancing the mechanical properties of biomaterials. Thanks to its remarkable physicochemical properties, zein protein has a glowing future in the intracellular delivery of therapeutic peptides and genetic materials, making it a viable substitute for the currently employed synthetic polymers. Moreover, the attempt to create zein-based 3D structures also sheds light on zein as a very promising material in regenerative medicine and tissue engineering. New research focusing on the chemical modification of zein, biodegradability, and cytotoxicity of zein-based materials is still required. The fact is that the biodegradability and biocompatibility of zein and other inherent characteristics associated with zein's chemistry allow various applications of zein and zein-based biomaterials with great potential in the near future.

Conflict of interest: The authors declare no conflict of interest.

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