Pertanika J. Sci. & Technol. 30 (4): 2805 - 2829 (2022)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Review Article

A Review on the Effect of Zein in Scaffold for Bone Tissue Engineering

Istikamah Subuki[1,2]*, Khairun Nor Ashikin Nasir[1] and Nur Azrini Ramlee[1,2]

[1]School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia
[2]Circular Industrial Research Laboratory, School of Chemical Engineering, College of Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor Darul Ehsan, Malaysia

ABSTRACT

Natural pharmaceutical ingredients have been widely used in recent decades due to their safety and biocompatibility. Zein, a plant-derived natural protein, has several advantages over other synthetic polymers in bone tissue engineering (BTE). This study of zein protein focuses more on its application in BTE as potential biopolymer material used in scaffold development. The use of zein in BTE has shown its benefits in the production of scaffolds. Therefore, attention has been given to studies of the effect of zein usage in bone scaffold development, as it offers a great ability based on its porosity, mechanical strength, in vitro degradation study, cell proliferation, and osteogenic differentiation, which is important for healing bone tissue damage. Therefore, this review aims to critically analyze the current research on the method of scaffold fabrication and the effect of zein usage in scaffolds for BTE. In addition, the common methods used in creating the scaffold are addressed.

Keywords: Bone tissue engineering, protein, polymer, scaffold, zein

ARTICLE INFO

Article history:
Received: 22 November 2021
Accepted: 14 February 2022
Published: 23 September 2022

DOI: https://doi.org/10.47836/pjst.30.4.28

E-mail addresses:
istikamah@uitm.edu.my (Istikamah Subuki)
khairunashikin7@gmail.com (Khairun Nor Ashikin Nasir)
azrini@uitm.edu.my (Nur Azrini Ramlee)
*Corresponding author

INTRODUCTION

Zein consists of 45–50% of the protein in maize and is the primary storage protein of maize. It was first identified in 1897 due to its solubility in aqueous alcohol solutions (Shukla & Cheryan, 2001). Corn, often known as maize, is the only cereal crop native to the Americas and one of the essential food and industrial crops in the United States (Shukla & Cheryan, 2001).

ISSN: 0128-7680
e-ISSN: 2231-8526

Because of its negative nutrient supply and low water solubility, zein isolate is not instantly consumed by humans (Labib, 2018). However, commercial value has been placed on the ability of zein and its resins to make strong, glossy, hydrophobic grease-proof coatings resistant to microbes. Zein offers promising applications in fiber, adhesives, coatings, ceramics, paint, cosmetics, textiles, chewing gums, and biodegradable plastics (Shukla & Cheryan, 2001; Subuki et al., 2018). Table 1 shows the usage of zein in the earliest years of its discovery. Zein can be categorized into four main groups based on its solubility and sequence homology which is α-zein, accounting for 70–85% of the total zein mass; β-zein, and γ-zein, the second most common component; and δ-zein (Esen, 1987). Several hydrophobic and neutral amino acids, such as leucine, proline, and alanine, are present in all zein classes, as well as some polar amino acid traces such as glutamine. Zein differs from other proteins as it contains a very small amount of lysine and tryptophan, including a few arginine and histidine residues. In addition, it has unique solubility characteristics due to its amino acid composition, which is limited to acetone, acetic acid, aqueous alcohols, and aqueous alkaline solutions (Lawton, 2002). The features of zein-based formulations have shown remarkable properties of this natural material, including excellent heat, water, abrasion, and humidity resistance. In addition, zein protein from maize can increase the potential of biomolecules having a longer shelf-life (Tortorella et al., 2021).

In this manuscript, the effect of zein in scaffolding for bone tissue engineering (BTE) is assessed in view of the characteristics and advantages of zein as the material used, scaffolding fabrication, and current research on the zein addition effect in BTE.

Table 1

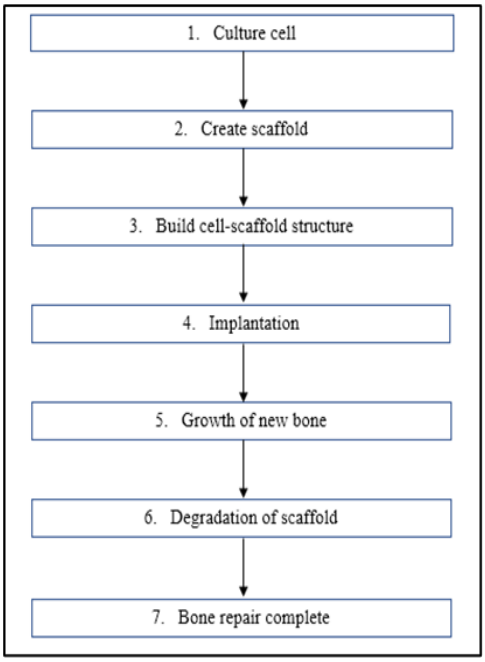
Zein usage in the industry (Shukla & Cheryan, 2001)

Applications	Year
Adhesive, binders	1944
Fibers	1950
Printing inks	1951
Medical tablets coating	1970
Drug controlled release application	1984
Paper surfaces, glossy magazine covers	1994
Biodegradable plastics, films	1997
Chewing gum	1999
Coating	1999

Current Research on Zein Protein in Bone Tissue Engineering (BTE)

The present study of zein protein focuses more on its application in bone tissue engineering (BTE) as a potential biopolymer material used in scaffold development. The use of Zein

in BTE has shown benefits in the production of scaffolds, where it is required in BTE application to build adequate scaffolding materials that can help heal tissue damage by rejuvenating new tissues (Bharadwaz & Jayasuriya, 2020; Gong et al., 2006). Biomaterials, cells, and signaling factors are the combination for BTE to heal or restore bone defects through scaffolding. Figure 1 shows the steps involved in the repair or regeneration process: (1) Culture cells and creates a scaffold; (2) Introduce the cells into the scaffold, creating a "cell-scaffold" structure; (3) Implantation; (4) Formation of new bone and degradation of the scaffold; and (5) Bone repair is achieved (Tong et al., 2017).



.*Figure 1.* Steps involved in the repair or regeneration process (Tong et al., 2017)

Biodegradable polymers based on proteins are widely used in biomedical applications because they show excellent biocompatibility, particularly those similar to the components of the extracellular matrix (ECM) (Demir et al., 2017). Therefore, zein protein has been widely studied and used as a scaffold material; outcomes of the research have shown acceptable mechanical properties and degradation portrayed by the zein-based scaffold (Hum & Boccaccini, 2018; Jing et al., 2018; Qu et al., 2008). Most scaffolds are analyzed on their biocompatibility, biodegradability, mechanical properties, and degradation rate. In the design of bone scaffolds, the following considerations should be taken:

Biocompatibility

In BTE application, biocompatibility is one of the key characteristics of a scaffold, as the scaffold can allow the cells to bind, normal function, proliferate, differentiate, and create new cell structures (Ghassemi et al., 2018). Scaffold, transplant, template, or artificial ECM are all terms for support. Biocompatibility is essential in a scaffold (Qu et al., 2019). Biocompatibility of a scaffold is also related to the condition of the pores where the required pore size is sufficient to allow for cell migration and is adequate for the cell binding of the scaffold (Chocholata et al., 2019). A highly porous microstructure with interconnected pores and a wide surface area is widely recognized to be favorable for tissue ingrowth in the scaffold. Pore diameters between 100 and 350 μm and porosities greater than 90% are preferable for bone regeneration (Yoshimoto et al., 2003).

Biodegradability

The scaffold must degrade in a timely manner after implantation to facilitate adequate tissue regeneration (Bitar & Zakhem, 2014). In addition, biodegradable scaffolds have gained much attention because of their irremovable features after the first implantation surgery, as they may be absorbed naturally by the body (Patel & Fisher, 2008). In summary, materials from scaffold degradation must also be non-toxic, so they will not cause any harm to the human body during the degradation process (Qu et al., 2019; Tariverdian et al., 2019).

Mechanical Strength

Next, in the creation of a scaffold for BTE application, mechanical strength is an important characteristic that is mostly regulated by the volume or size of the pore (Bose et al., 2013; Roseti et al., 2017). Therefore, the scaffold must provide sufficient mechanical strength to enable new tissue replacement (Prasadh & Wong, 2018). Cortical bone has a strength of 100 to 230 MPa on average. Scaffold biomaterials should fall within this range. However, the scaffold is supported by plates, screws, wires, or pins to avoid failure (Prasadh & Wong, 2018). Table 2 shows some characteristics of the human bone.

Table 2

Mechanical properties of human bone tissue (Shamaz & Halima, 2015)

Property	Cortical bone	Cancellous bone
Compressive strength (MPa)	100–230	2–12
Tensile strength (MPa)	50–150	10–20
Strain to failure (%)	1–3	5–7
Young's modulus (GPa)	7–30	0.5–0.05

Advantages of Zein Proteins for Bone Tissue Engineering Compared to Other Proteins.

Many commonly used proteins act as polymers in BTE application, including collagen, silk, zein, soy protein, and wheat gluten. Zein is categorized as one of the plant-based protein biopolymers, which makes it preferable to other proteins. In comparison to animal proteins and synthetic polymers, plant proteins have various advantages, including relatively inexpensive, safe, biocompatibility, and the ability to be manufactured from renewable sources (Berardi et al., 2018; Chen et al., 2019; Reddy & Yang, 2013). Moreover, zein can be processed using a variety of solvents compared to other plant-based proteins, which makes them easy to handle (Anderson & Lamsa, 2011; Reddy & Yang, 2011). In addition, in comparison to wet mechanical properties, zein protein shows a better performance than other proteins, as it is an important property because the scaffold is accessible to the physiological fluid inside the body (Maji & Dasgupta, 2017).

FABRICATION OF SCAFFOLD

The use of zein in BTE has shown to be advantageous in the production of scaffolds. Zein is classified as a natural polymer that can be used as one of the biomaterials for scaffold fabrication. Bio-functional elements in the polymers ensure bioactivity, a biomimetic surface, and natural remodeling. However, immunogenicity, microbial contamination (i.e., endotoxin), limited tunability, unstable degradation rate, and low mechanical strength are among their key drawbacks, which limit its application in bone tissue engineering (Francesca et al., 2020). Therefore, researchers often combine two or more materials with diverse qualities to enhance the properties of materials. Examples of these combinations are copolymers, polymer-polymer blends, and polymer–ceramic.

Bone scaffolds can be created in a few ways and categorized into two types which are conventional and additive manufacturing (AM). Conventional techniques have limitations in building structures with complex porosity (Bajaj et al., 2014). AM techniques in producing three-dimensional scaffolds have progressed to the point that they can now construct complicated scaffolds. Furthermore, because harmful solvents are not employed in making three-dimensional (3D) scaffolds, biocompatibility issues observed in some conventionally manufactured scaffolds are no longer an issue (Torabi et al., 2015). The three-dimensional scaffold design is sent to the machine using computer-aided design (CAD) and computer-aided manufacturing (CAM) software in AM technology used to create the scaffold (Sah & Pramanik, 2011).

Salt Leaching

The salt leaching method entails mixing the polymer with a water-soluble porogen, such as sodium chloride or sodium citrate, after dissolving it in an organic solvent and then transferring the mixture into a mold (Chocholata et al., 2019; Mikos & Temenoff, 2000; Rezwan et al., 2006).

In 2006, a study conducted by Gong et al. (2006) used the salt leaching method using several different measurements of the porogen's mass fraction and particle size. First, the mixture was shaped into three-dimensional scaffolds, with different particle sizes ranging from 38.5 to 220 mm and mass fraction ratio of 1:1.4 and 1:2.5 w/w (zein:NaCl). Then, the samples were leached using a water bath and lyophilized. Scaffolds were 10 mm diameter cylindrical rods with 3–25 mm height. Additionally, this method has been employed by Wang et al. (2008) to demonstrate the application of zein in bone tissue engineering, where porous zein scaffolds were produced using the salt leaching technique based on its adhesive characteristics and solubility, which differ significantly from other native protein biomaterials and demonstrate remarkable compressive performance.

Meanwhile, another study on using PCL and zein in the fabrication of scaffolds for BTE application was conducted by Wu et al. (2012). The sample was made by employing

a solvent casting–particulate leaching process with sodium chloride particles to combine poly(ε-caprolactone) (PCL) with zein. The method used is quite similar to work done by Gong et al. (2006). In addition, Hum (2016) utilized the leaching approach to create porous zein scaffolds, with salt as the porogen. As a result, zein and sodium chloride particles were homogeneously mixed. Subsequently, the mixture was compressed into cylindrical specimens. Finally, the samples were leached in a water bath, rinsed in ultrapure water, then lyophilized for later usage to obtain a porous structure.

Gas Foaming

Gas foaming is a way to avoid utilizing harmful organic solvents. Gas-foaming agents, such as carbon dioxide and nitrogen, are employed in this approach, known as non-flammable, low-toxic gas (Ji et al., 2011; Mikos & Temenoff, 2000). This method allows high-pressure water to enter biodegradable polymers, resulting in a porous structure with an average pore size of 30–700 μm and a porosity of up to 85% (Thavornyutikarn et al., 2014b). In addition, it is an easy and cost-effective scaffolding method (Nam et al., 2000).This method was used by Salerno et al. (2010) to prepare the multi-phase PCL/TZ and PCL/TZ– hydroxyapatite particles (HA) scaffold. Firstly, in a batch foaming device, chosen protein or plasticizer systems were foamed with CO_2 and N_2 mixtures as blowing agents (Salerno et al., 2006). Salerno et al. (2010b) studied using the CO_2 foaming method. The scaffold samples were created using PCL, thermoplastic zein (TZ), and HA. Another study by Salerno et al. (2011) used a combination of gas foaming–leaching methods to create porous multi-phase PCL–TZ and PCL–TZ–HA composite scaffolds. PCL, TZ, and HA were first mixed in an internal mixer. The most recent study on the usage of zein in BTE applications using the supercritical CO_2 foaming method was conducted by Subuki et al. (2020).

3D Printing

Several commonly used approaches for creating these 3D scaffolds use a conventional method. However, they all have the same drawbacks, including poor scaffold design, pore network and size, and unsatisfactory 3D scaffolds (An et al., 2015). In addition, the processes of these methods are not adaptable enough (Gungor-Ozkerim et al., 2018). On the other hand, rapid prototyping, solid free-form fabrication, biofabrication, bioprinting, and additive manufacturing are all 3D printing processes that could tackle all the issues regarding the usage of conventional methods (Hospodiuk et al., 2016).

Three-dimensional (3D) printing is additive manufacturing (AM) method, and fast prototyping is a method of layer-by-layer combining of materials to create products from 3D model data, as opposed to subtractive manufacturing methods (Wang et al., 2020). Ru et al. (2018) study create ternary scaffolds using 3D printing. The scaffolds were created using three components: zein (ZN), PCL, and nano magnesium silicate (n-MS). The ingredient was chosen due to their advantages given to the BTE application.

Freeze Drying

Freeze drying, also known as emulsification, is a promising scaffold preparation procedure. Freeze drying works on sublimation, in which frozen water in polymer nanocomposites is immediately transformed from solid to a gas state without liquefaction (Wahid et al., 2018).

The resulting pores using this method are constantly produced, but their porosity is greater than 90%, and their size ranges from 20 to 200 μm (Xiaohao & Peter, 2004). The emulsion freeze-drying process can be paired with particle leaching, sucrose, or sodium chloride, which can be added to the emulsion to create porosity. Particles can be cleaned after freeze drying (Alizadeh et al., 2013). This method was used by Shahbazarab et al. (2018) to produce the ZN/chitosan (CS)/nHAp biocomposite scaffold. The CS solution was mixed with the ZN solution and swirled. The nHAp was introduced to the CS-ZN solution and mixed for 24 hours. The sample was then subjected to 24-well culture plates and pre-frozen; it was freeze-dried at -80°C for 48 hours before being stored.

Polymer Coating

Polymer coatings are regularly used for integrating drug delivery transport and bone tissue engineering scaffolds (Philippart et al., 2015; Li et al., 2014; Yao et al., 2013). Polymer coatings are thin polymer layers applied on flat or uneven surfaces (Francis & Roberts, 2016). For example, in BTE, scaffolds are usually coated with polymer to enhance scaffold performance.

A study by Fereshteh et al. (2015) has produced highly porous bioactive glass (BG)-based scaffolds with a porosity of 90%, where it applied a dual PCL/zein coating on the scaffold surface to improve the mechanical properties of the scaffold. The scaffolds were made via the foam replication technique (Chen et al., 2006). In addition, the potential of zein as a covering material for such scaffolds was studied by Hum (2016). Dip coating was used to make zein-coated BG scaffolds, which were then characterized in microstructure, bioactivity, and mechanical strength. Another study conducted by Arango-ospina et al. (2021) used zein and manuka honey (MH) coating to BG scaffold. Table 3 shows the method used by researchers in the production of the scaffold.

Table 3

Summary of the current progress of zein (ZN) usage in BTE applications

Method	Material	Reference
Salt leaching	ZN	Gong et al. (2006)
Salt leaching	ZN, plasticizer	Wang et al. (2008)
Salt leaching	ZN, PCL	Wu et al. (2012)

Table 3 *(Continue)*

Method	Material	Reference
Salt leaching	ZN	Hum (2016)
Gas foaming	PCL, TZ, HA	Salerno et al. (2010a)
Gas foaming	PCL, TZ, HA	Salerno et al. (2010b)
Gas foaming-leaching	PCL, TZ, HA	Salerno et al. (2011)
Gas foaming	PCL, TZ, HA	Subuki et al. (2020)
3D printing	ZN, PCL, nMS	Ru et al. (2018)
Freeze drying	ZN, CS, nHAp	Shahbazarab et al. (2018)
Polymer coating	PCL, ZN	Fereshteh et al. (2015)
Polymer coating	ZN	Hum (2016)
Polymer coating	ZN, MH	Arango-ospina et al. (2021)

EFFECT OF ZEIN IN SCAFFOLD

Most scaffolds are analyzed on their biocompatibility, biodegradability, mechanical properties, and degradation rate. In addition, considerations such as scaffold porosity, mechanical properties, in vitro degradation study, cell proliferation, and osteogenic differentiation should be taken when designing a bone scaffold. This section reviews the effect of zein usage in bone scaffolds based on those considerations mentioned.

Scaffold Porosity

Macroporosity (pore size more than 100 μm) is frequently necessary for enhancing osteogenesis and angiogenesis (Murphy et al., 2010). In addition, interconnected macropores are required to increase the body's fluid circulation and the cell growth to the core of the implant (Reinwald et al., 2014). More notably, researchers discovered that microporosity (pore size less than 10 μm) plays a vital role in scaffold osteoinduction (Bohner et al., 2017; Polak et al., 2011).

Figure 2 depicts the scaffold's shape and microstructure, as Gong et al. (2006) reported. The pores on the surface of scaffolds produced were bigger and more uniform when manufactured with large porogen particle sizes. The porosities of zein scaffolds with various sodium chloride mass fractions and particle sizes range from 75.3 to 79.0%. Despite the pores' interior morphology and structure, all produced scaffolds had high porosity above 75%, which was deemed favorable to cell growth and survival (Thavornyutikarn et al., 2014a).

Wu et al. (2012) discovered that the incorporation of zein in PCL scaffold could produce macropores larger than 300 μm, with many micropores ranging from 5 to 20 μm. Moreover, Figure 3A shows that the 3D model displays a highly porous structure and pore linkages. Meanwhile, the micro-CT scan in Figure 3B reveals a well-connected pore structure. For

cell migration and proliferation, consistent and suitable size of well-connected pores is required for 3D porous scaffolds (Chocholata et al., 2019; Murphy & O'Brien, 2010).

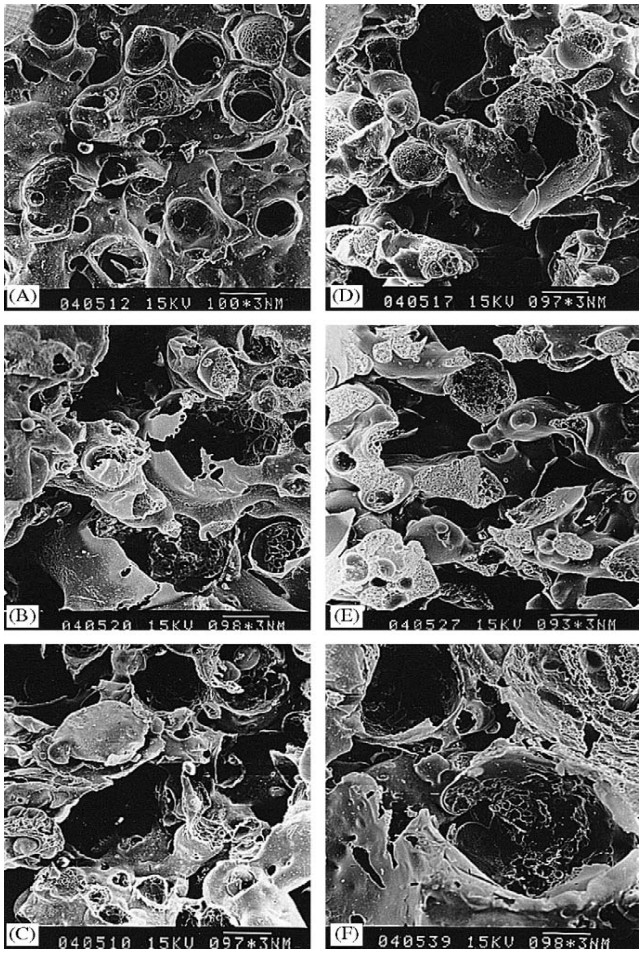
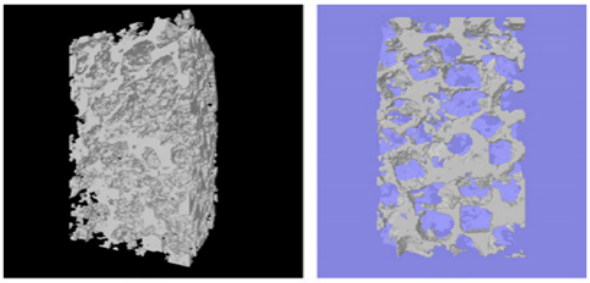


Figure 2. SEM illustrates the outer and inner morphology of scaffolds constructed with a porogen mass fraction of 1.4 and different particle sizes (Gong et al., 2006)



(A) (B)

Figure 3. Micro-CT images of the zein/PCL-40 scaffold: (A) 3D model; and (B) semi-transparent overlay of a cross-section of the 3D model (Wu et al., 2012)

Besides that, Salerno et al. (2010) compared the structures of the PCL/TZ–HA with the PCL/TZ. As a result, the PCL/TZ–HA has smaller pores and a narrower pore size distribution, as demonstrated in Figure 4. Moreover, Salerno et al. (2010b) observed an increase in pore size as the foaming temperature (T_f) went from 80 to 100°C.

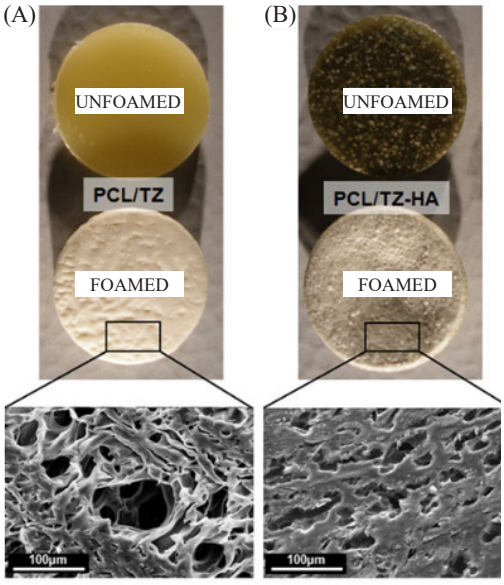
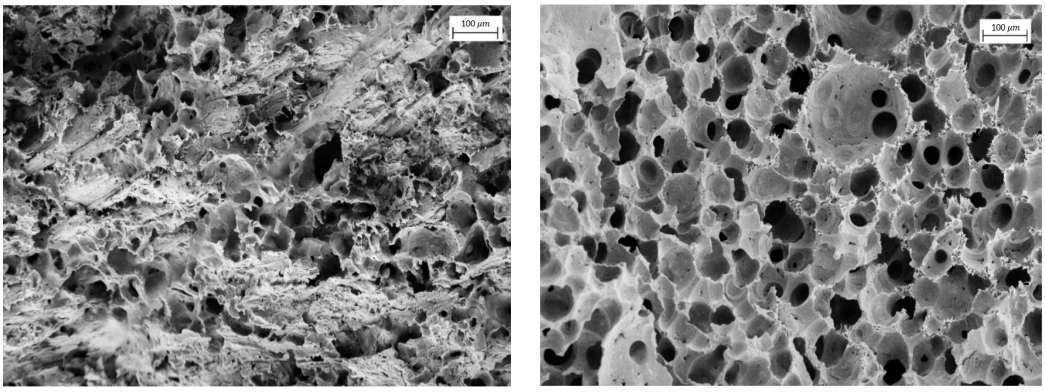


Figure 4. Optical and SEM microscope images of (a) PCL/TZ; and (b) PCL/TZ–HA before and after undergoing the gas foaming step (Di Maio et al., 2010)

In addition, Subuki et al. (2020) discovered that biocomposite at 20 wt% incorporations with TZ exhibits strong visible interconnection of the porous structure, as seen in Figure 5(a) when contrasted to Figure 5(b), in which the effect of the foaming process at 10% displays low interconnection.

Furthermore, Shahbazarab et al. (2018) performed SEM analysis, revealing that the ZN comprises particles with a diameter of 100–500 nm and a high amount of interconnected pores in pure ZN. Besides that, according to SEM images, the pore size of the PCL/zein coating experiment conducted by Fereshteh et al. (2015) was in the range of 200–450 nm, which is ideal for use in bone tissue engineering.



(a) (b)

Figure 5. SEM Micrograph Images of the Cross-Section of: (a) 20 wt% TZ; and (b) 10 wt% TZ (Subuki et al., 2020)

Mechanical Properties

The scaffold must provide sufficient mechanical strength to enable new tissue replacement (Prasadh & Wong, 2018). The biostability of the scaffold is improved by the material interface's strength, flexibility, and absorption. The mechanical properties of the scaffold should be closely related to the mechanical properties of the surrounding bone. Wang et al. (2008) reported that Young's modulus and strength increased with decreasing mass percentage of the porogen. As a result, the compressive modulus and compressive strength of the porous zein scaffold were in the trabecular bone range (compressive modulus: 10–2000 MPa; compressive strength: 2–180 MPa).

On the other hand, with increasing zein content, the compressive stress value of the composite scaffolds decreased, as Wu et al. (2012) and Shahbazarab et al. (2018) reported in Figures 6 and 7, respectively. In addition, the tensile properties analysis conducted by Salerno et al. (2010) shows that the yielding stress, the stress at break, and the elongation at the break of the scaffold decrease with the incorporation of ZN. It was observed that the compressive strength of the tested scaffold decreased with increasing wt% of ZN in the scaffold (Ru et al., 2018), which could be due to the presence of zein that caused a reduced degree of crystallinity.

Although the creation of porous scaffolds made of natural materials for bone regeneration has yielded promising results, they all have one weakness: lack of mechanical strength due to their porosity. It means that they are insufficiently strong to meet the demands of load-bearing applications (Wang et al., 2008). Furthermore, larger porosity was associated with decreased Young's modulus and compressive strength, consistent with previous research by Zhao et al. (2018) and Sabudin et al. (2019) that revealed an inverse relationship between porosity and mechanical characteristics.

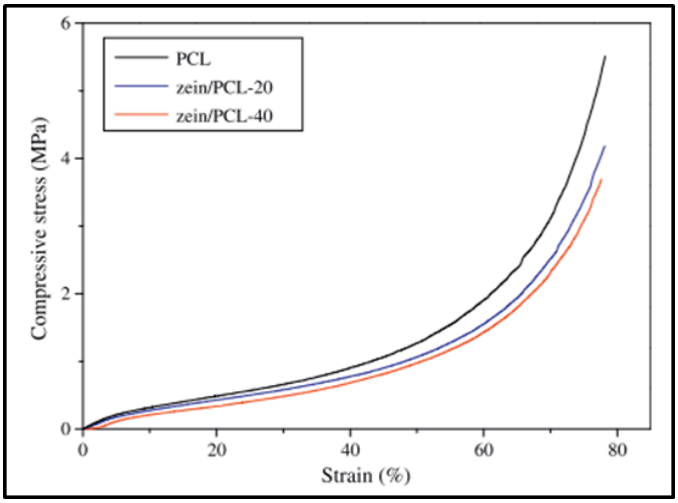


Figure 6. Compressive stress-strain curves of the different scaffolds (Wu et al., 2012)

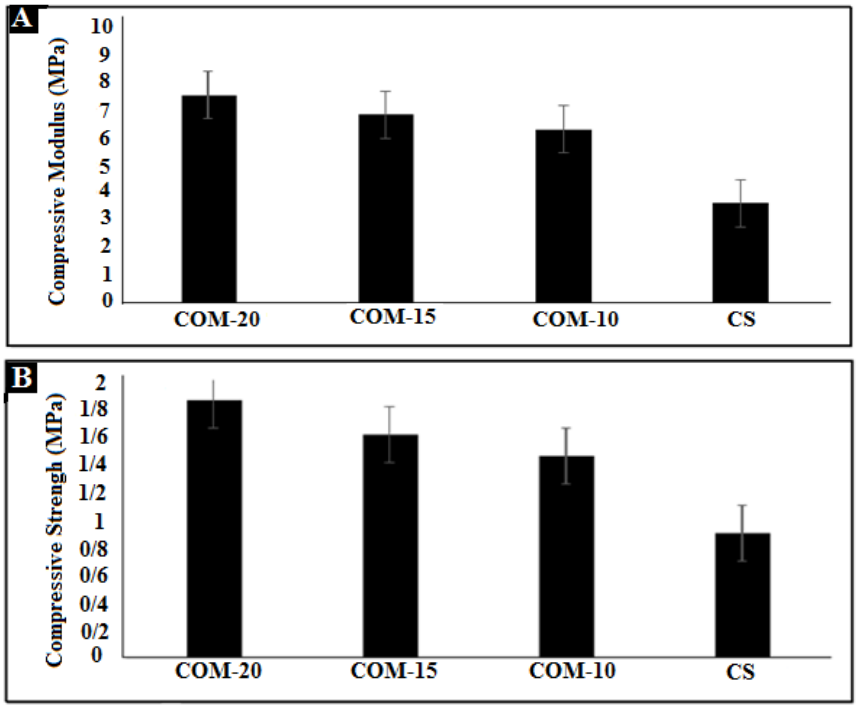


Figure 7. Mechanical properties of tested composite scaffolds (Shahbazarab et al., 2018)

In Vitro Degradation Study

The scaffold must degrade in a timely manner after implantation to facilitate adequate tissue regeneration (Bitar & Zakhem, 2014). Gong et al. (2006) reported that during the 14-day in the vitro incubation period, up to 36% and 89% rates of degradation were recorded when incubated with collagenase and pepsin, respectively. This result indicated that a greater porogen mass proportion results in increased porosity, leading to a faster scaffold degradation rate. Wu et al. (2012) found that the weight loss of the zein/PCL composite scaffolds was significant, and it increased as the amount of zein in the composite increased, as shown in Figure 8. According to Salerno et al. (2010), during the rate of degradation analysis, PCL/TZ and PCL/TZ–HA showed considerable variations, as shown in Figure 9. A study by Fereshteh et al. (2015) also shows that during the 28-day immersion in PBS, the PCL/zein coating degraded noticeably. The quicker degradation rates found for the PCL/TZ and PCL/TZ–HA may be due to the capacity of TZ to swell and degrade in water.

Moreover, Ru et al. (2018) and Shahbazarab et al. (2018) reported that scaffolds with higher wt% of ZN lose significantly more weight than other scaffolds. The analysis of the degradation rate obtained shows that the scaffold degradation rate increases with the increasing mass fraction of zein. Furthermore, in water-based systems, zein-coated scaffolds could exhibit a high level of stability. The steady degradation posed by zein is advantageous to the goal of tissue engineering strategy for natural tissue replacement of

the temporary matrix (Gomes et al., 2008). From the analysis obtained, it can be said that the rate of degradation of composite scaffolds can be controlled to match the rate of tissue regeneration by manipulating the amount of zein in the composite.

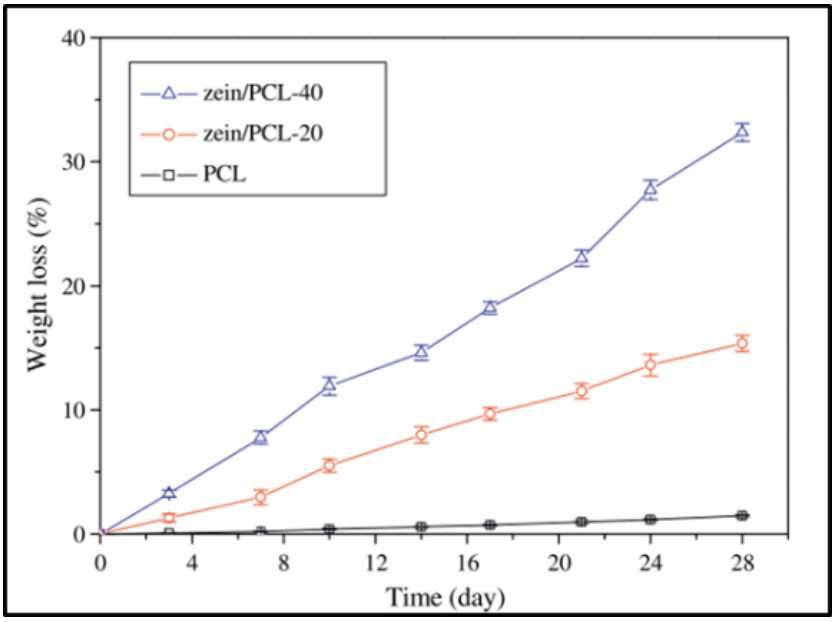


Figure 8. Weight loss of scaffold analysis graph (Wu et al., 2012)

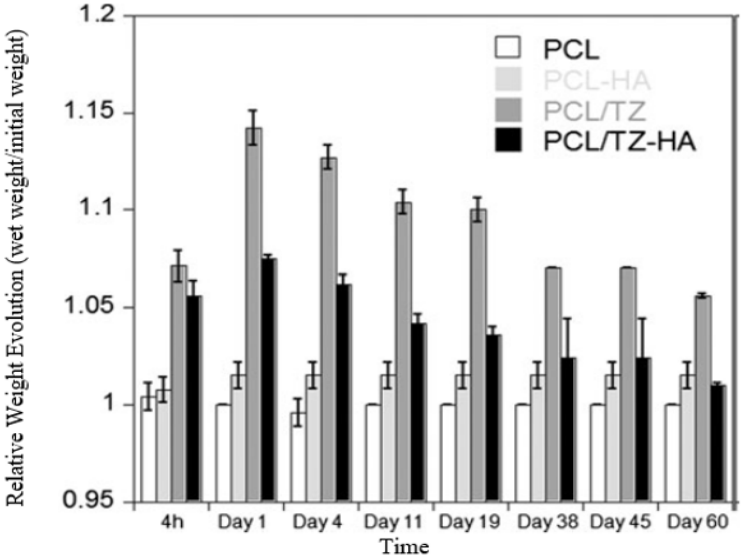


Figure 9. Effect of biomaterial composition on relative weight loss during degradation analysis (Salerno et al., 2010)

Cell Proliferation and Osteogenic Differentiation

For materials used in tissue engineering, good biocompatibility is required in addition to adequate mechanical capabilities, outstanding pore characteristics, and degradation behavior. Gong et al. (2006) show that the dexamethasone group had much more activity than the control group, showing that MSCs successfully differentiated themselves towards osteoblasts and proliferated on the porous zein scaffolds. The findings of SEM and CLSM investigations in Figure 10 verified the ability of multi-phase scaffolds to allow for both cell types' attachment and colonization, as Salerno et al. (2010b). The cells adhered to the pores of the scaffolds and colonized them, expanding and forming bridges between opposite pore walls, as seen in Figure 10a. Meanwhile, the CLSM results shown in Figure 10b demonstrate that the hMSCs were stable on Day 1 after seeding, adhering to and populating the scaffold surface, and following the topology of the pore walls.

According to Ru et al. (2018), the OD values (cell proliferation) for 20 wt% and 10 wt% of ZN incorporated in scaffolds were higher than no ZN at all, indicating that ZN scaffolds increased cell proliferation. The number of newly generated bone tissues (NBs) in the tested scaffolds grew. Furthermore, as the ZN content in the scaffolds grew, the amount of NBs development in the scaffolds increased, as seen in Figure 11 according to Ru et al. (2018). Shahbazarab et al. (2018) also showed that cells were adhered and spread out through SEM images of cells cultured on scaffolds. It was also discovered that adding CS and ZN to porous scaffolds improved MG-63 cell adhesion, growth, and proliferation. Therefore, it is deemed that the topography of the scaffold surface was influenced by ZN, which could have had a significant impact on cell adhesion, proliferation, and osteogenic differentiation. One possible reason for this impact is the higher hydrophilicity and degradation rate of TZ, which may increase serum protein absorption and HA particle exposure, ultimately increasing rMSC osteogenic development. All these findings show that ZN had improved osteogenic properties. Table 4 shows the summary of the outcome of the findings from the previous paper.

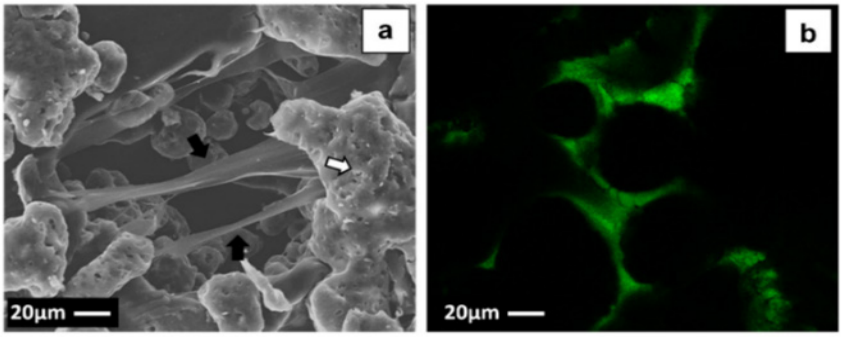


Figure 10. a) SEM image of MG63 seeding; b) CLMS image of hMSCs seeding Salerno et al. (2010b)

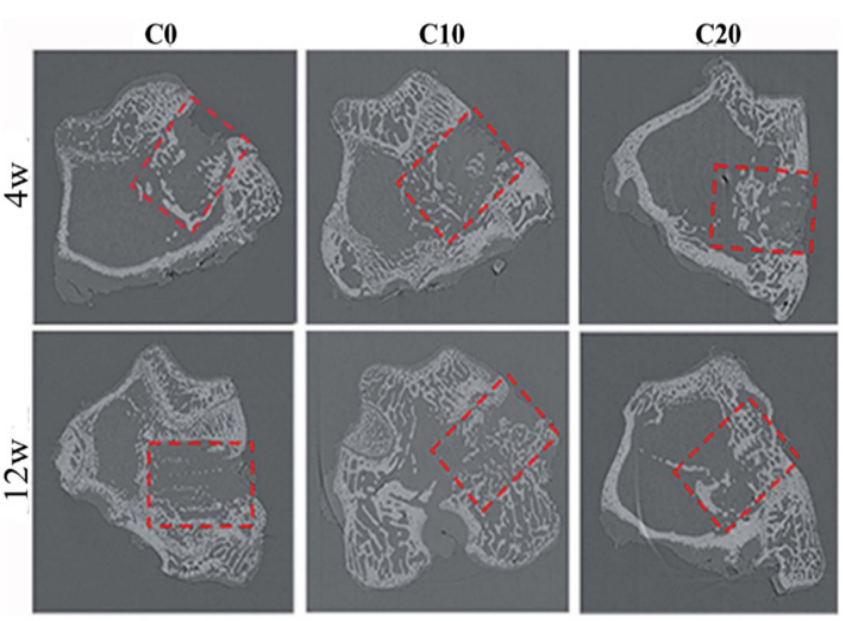


Figure 11. The 2D graphics of bone-scaffolds from micro-CT after tested scaffolds implanted to femoral defects of rabbits at 4 and 12 weeks (Ru et al., 2018)

Table 4

Summary of findings outcome from the previous paper on the usage of ZN and various materials used to combine with ZN, along with technique employed and its advantages/disadvantages

Method	Material	Advantages	Disadvantages	Reference
Salt leaching	ZN	Reliable scaffolds with an interconnective, open pore structure and pore size up to 300 mm were produced using the salt leaching technique. Mesenchymal stem cells (MSCs) can successfully adhere, develop, and proliferate on the zein scaffold.	The mechanical properties of the scaffold decrease with the increased mass fraction of zein.	Gong et al. (2006)

Table 4 *(Continue)*

Method	Material	Advantages	Disadvantages	Reference
Salt leaching	ZN, plasticizer	Plasticizer concentrations of 20% for (oleic acid) OA and 15% for stearic acid (SA) were sufficient to improve the flexibility and tensile properties of porous zein scaffolds.	Even when the mechanical properties of the OA plasticized zein scaffold meet the requirements for a hard tissue engineering replacement, it is not strong enough.	Wang et al. (2008)
Salt leaching	ZN, HA	The pore interconnectivity of the HA-coated scaffolds was comparable to that of the zein scaffolds. The HA-coated zein scaffolds exhibited the typical structure of HA crystals, indicating the influence of immersion and apatite production. The compressive modulus of HA-coated zein scaffolds was acceptable for bone tissue engineering. hBMSC development was better on both the HA-coated and uncoated zein scaffolds.	N/A	Qu et al. (2008)

Table 4 *(Continue)*

Method	Material	Advantages	Disadvantages	Reference
Salt leaching	ZN, PCL	The incorporation of zein showed a high hydrophilic nature that could help cell adhesion and proliferation. The scaffold's degradation rate was found to be higher in the zein/PCL scaffold.	Increasing zein content will cause the scaffold to have low mechanical strength.	Wu et al. (2012)
Salt leaching	ZN	The compressive strength of pure zein is found to be within the ranges required by natural cancellous bone.	Because of its insolubility, pure zein did not lose weight after 14 days in PBS, implying that it has high stability in water-based solutions. There is no hydroxyapatite on the surface of zein; hence no bioactive behavior can be discovered.	Hum (2016)
Gas foaming	PCL, TZ, HA	The increased relaxation temperature of the PCL/TZ and PCL/TZ–HA, which is close to human body temperature, may allow a cyclic mechanical solicitation to be dissipated. TZ improved the wettability of PCL. The topography of the scaffold surface was also changed by TZ and HA, which could have had a significant impact on cell adhesion, proliferation, and osteogenic differentiation.	N/A	Salerno et al. (2010)

Table 4 *(Continue)*

Method	Material	Advantages	Disadvantages	Reference
Gas foaming	PCL, TZ, HA	Satisfactory correlation with those found in the literature on the capability of PCL and TZ to help adhesion and proliferation of the cell. HA content increases, and the mechanical properties of the scaffold also increase. PCL-TZ has the fastest degradation rate. Scaffold managed, allowing the cell for adhesion and colonization in the tested culture cell. The findings are consistent with previous research that has combined natural polymers such as chitosan and starch with PCL to create scaffolds with improved hydrophilicity and a faster breakdown rate.	N/A	Rosa et al. (2005); She et al. (2007); Salerno et al. (2010b)
Gas foaming-leaching	PCL, TZ, HA	The use of PCL and TZ together allowed for precise control of scaffold degradation.	N/A	Salerno et al. (2011)
Gas foaming	PCL, TZ, HA	At temperatures up to 250°C, all the composite samples showed a stable thermal property. The composite that contains 20 wt% of TZ exhibited better morphology of pore structure in the sample composite than other compositions of TZ.	N/A	Subuki et al. (2020)

Table 4 *(Continue)*

Method	Material	Advantages	Disadvantages	Reference
3D printing	ZN, PCL, nMS	The combination of those advantages of both biodegradable polymers and bioactive materials would result in enhanced bio-performance of the composite scaffolds. The apatite-mineralization capability of the scaffolds was enhanced with an increase in ZN content. The rate of degradability of the scaffolds has been improved with the addition of ZN. Tested cell culture responses, such as replication and division to the scaffolds, have been promoted with increasing ZN.	N/A	Ru et al. (2018)
Freeze drying	ZN, CS, nHAp	Including CS and ZN (with a reduction in the amount of nHAp) improved the adhesion, growth, and proliferation of MG-63 cells on porous scaffolds.	N/A	Shahbazarab et al. (2018)

CONCLUSION

With excellent flexibility and compressibility, zein can create strong, glossy, hydrophobic, greaseproof coatings prone to microbial harm. Due to its advantages in the specialty food, pharmaceutical, and biodegradable plastic industries, zein has been of interest for commercialization. As a result, zein protein is widely used in many sectors due to its usefulness as an industrial and specialty polymer. In addition, the current research on zein has been mostly found in BTE applications, as zein displays great ability for use in the scaffold.

From the current research on the zein addition effect in BTE, it can be concluded that zein shows suitable interconnectivity as a material for scaffolding, which aids in achieving optimum vascular ingrowth. The scaffold degradation rate also increases with the increasing mass fraction of zein. In addition, the tested cell culture can successfully adhere, develop, and proliferate on the zein scaffold. Furthermore, the incorporation of zein shows high hydrophilic nature that could assist in the adhesion and proliferation of the cell. This research also demonstrates that the 3D printing method is preferred for scaffold fabrication, as it could overcome all issues regarding the use of the conventional method. Moreover, scaffold produced by the 3D printing method shows better performance for bone tissue engineering applications.

However, this research shows that the increased zein content will cause the scaffold to have low mechanical strength. Therefore, zein has to be combined with other components such as PCL, HA, and n-MS to enhance the mechanical strength of the composite scaffolds.

ACKNOWLEDGMENT

The authors would like to thank for the financial support by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2019/TK02/UITM/02/19), Universiti Teknologi MARA (UiTM), Malaysia.

REFERENCES

Alizadeh, M., Abbasi, F., Khoshfetrat, A. B., & Ghaleh, H. (2013). Microstructure and characteristic properties of gelatin/chitosan scaffold prepared by a combined freeze-drying/leaching method. *Materials Science & Engineering: C*, *33*(7), 3958-3967. https://doi.org/10.1016/j.msec.2013.05.039

An, J., Teoh, J. E. M., Suntornnond, R., & Chua, C. K. (2015). Design and 3D printing of scaffolds and tissues. *Engineering*, *1*(2), 261-268. https://doi.org/10.15302/J-ENG-2015061

Anderson, T. J., & Lamsa, B. P. (2011). Zein extraction from corn, corn products, and coproducts and modifications for various applications: A review. *Cereal Chemistry*, *88*(2), 159-173. https://doi.org/10.1094/CCHEM-06-10-0091

Arango-ospina, M., Lasch, K., Weidinger, J., & Boccaccini, A. R. (2021). Manuka honey and zein coatings impart bioactive glass bone tissue scaffolds antibacterial properties and superior mechanical properties. *Front Mater, 7*, Article 610889. https://doi.org/10.3389/fmats.2020.610889

Bajaj, P., Schweller, R. M., Khademhosseini, A., West, J. L., & Bashir, R. (2014). 3D biofabrication strategies for tissue engineering and regenerative medicine. *Annual Review of Biomedical Engineering, 16*, 247-276. https://doi.org/10.1146/annurev-bioeng-071813-105155

Berardi, A., Bisharat, L., AlKhatib, H. S., & Cespi, M. (2018). Zein as a pharmaceutical excipient in oral solid dosage forms: State of the art and future perspectives. *AAPS PharmSciTech*, *19*, 2009-2022. https://doi.org/10.1208/s12249-018-1035-y

Bharadwaz, A., & Jayasuriya, A. C. (2020). Recent trends in the application of widely used natural and synthetic polymer nanocomposites in bone tissue regeneration. *Materials Science and Engineering: C*, *110,* Article 110698. https://doi.org/10.1016/j.msec.2020.110698

Bitar, K. N., & Zakhem, E. (2014). Design strategies of biodegradable scaffolds for tissue regeneration. *Biomedical Engineering and Computational Biology*, *6*, 13-20. https://doi.org/ 10.4137/BECB.S10961

Bohner, M., Baroud, G., Bernstein, A., Do, N., Galea, L., Hesse, B., Heuberger, R., Meille, S., Michel, P., von Rechenberg, B., Sague, J., & Seeherman, H. (2017). Characterization and distribution of mechanically competent mineralized tissue in micropores of b -tricalcium phosphate bone substitutes. *Materials Today, 20*(3), 106-115. https://doi.org/10.1016/j.mattod.2017.02.002

Bose, S., Vahabzadeh, S., & Bandyopadhyay, A. (2013). Bone tissue engineering using 3D printing. *Materials Today*, *16*(12), 496-504. https://doi.org/10.1016/j.mattod.2013.11.017

Chen, H., Wang, J., Cheng, Y., Wang, C., Liu, H., Bian, H., Pan, Y., Sun, J., & Han, W. (2019). Application of protein-based films and coatings for food packaging: A review. *Polymers*, *11*(12), Article 2039. https://doi.org/10.3390/polym11122039

Chen, Q. Z., Thompson, I. D., & Boccaccini, A. R. (2006). 45S5 bioglasss-derived glass-ceramic scaffolds for bone tissue engineering. *Biomaterials, 27*(11), 2414-2425. https://doi.org/10.1016/j.biomaterials.2005.11.025

Chocholata, P., Kulda, V., & Babuska, V. (2019). Fabrication of scaffolds for bone-tissue regeneration. *Materials*, *12*(4), Article 568. https://doi.org/10.3390/ma12040568

Demir, M., Ramos-Rivera, L., Silva, R., Nazhat, S. N., & Boccaccini, A. R. (2017). Zein-based composites in biomedical applications. *Journal of Biomedical Materials Research Part A, 105*(6), 1656-1665 https://doi.org/10.1002/jbm.a.36040

Esen, A. (1987). A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*Zea mays* L.). *Journal of Cereal Science*, *5*(2), 117-128. https://doi.org/10.1016/S0733-5210(87)80015-2

Fereshteh, Z., Nooeaid, P., Fathi, M., Bagri, A., & Boccaccini, A. R. (2015). The effect of coating type on mechanical properties and controlled drug release of PCL/zein coated 45S5 bioactive glass scaffolds for bone tissue engineering. *Materials Science and Engineering: C*, *54*, 50-60. https://doi.org/10.1016/j.msec.2015.05.011

Francesca, D., Emanuela, J., Monica, S., & Manuela, T. R. (2020). Natural and synthetic polymers. In S. Chandra & Y. Ohama (Eds.), *Polymers in concrete*, (pp. 5-25). CRC Press. https://doi.org/10.1201/9781003068211-2

Francis, L. F., & Roberts, C. C. (2016). Dispersion and solution processes. In *Material Processing* (pp. 415-512). Academia Press. https://doi.org/10.1016/B978-0-12-385132-1.00006-9

Ghassemi, T., Shahroodi, A., Ebrahimzadeh, M. H., Mousavian, A., Movaffagh, J., & Moradi, A. (2018). Current concepts in scaffolding for bone tissue engineering. *Archives of Bone and Joint Surgery*, *6*(2), 90-99. https://doi.org/10.22038/abjs.2018.26340.1713

Gomes, M. E., Azevedo, H. S., Moreira, A. R., & Ell, V. (2008). Starch-poly (lactic acid) fibre-mesh scaffolds for bone tissue engineering applications: Structure, mechanical properties and degradation behaviour. *Journal of Tissue Engineering and Regenerative Medicine, 2*(5), 243-252.

Gong, S., Wang, H., Sun, Q., Xue, S. T., & Wang, J. Y. (2006). Mechanical properties and *in vitro* biocompatibility of porous zein scaffolds. *Biomaterials*, *27*(20), 3793-3799. https://doi.org/10.1016/j.biomaterials.2006.02.019

Gungor-Ozkerim, P. S., Inci, I., Zhang, Y. S., Khademhosseini, A., & Dokmeci, M. R. (2018). Bioinks for 3D bioprinting: An overview. *Biomaterials Science, 6*(5), 915-946. https://doi.org/10.1039/c7bm00765e

Hospodiuk, M., Dey, M., Sosnoski, D., & Ibrahim, T. (2016). The bioink: A comprehensive review on bioprintable materials. *Biotechnology Advances, 35*(2), 217-239. https://doi.org/10.1016/j.biotechadv.2016.12.006

Hum, J., Naseri, S., & Boccaccini, A. R. (2018). Bioactive glass combined with zein as composite material for the application in bone tissue engineering. *Biomedical Glasses*, *4*(1), 72-81. https://doi.org/10.1515/bglass-2018-0007

Hum, J. K., (2016). *Bioactive glass combined with natural derived proteins as composite materials for the application in bone tissue engineering* (Doctoral dissertation). University of Erlangen-Nuremberg, Germany. https://d-nb.info/1103801953/34

Ji, C., Annabi, N., Khademhosseini, A., & Dehghani, F. (2011). Fabrication of porous chitosan scaffolds for soft tissue engineering using dense gas CO_2. *Acta Biomaterialia*, *7*(4), 1653-1664. https://doi.org/10.1016/j.actbio.2010.11.043

Jing, L., Wang, X., Liu, H., Lu, Y., Bian, J., Sun, J., & Huang, D. (2018). Zein increases the cytoaffinity and biodegradability of scaffolds 3D-printed with zein and poly(ϵ-caprolactone) composite ink. *ACS Applied Materials and Interfaces*, *10*(22), 18551-18559. https://doi.org/10.1021/acsami.8b04344

Labib, G. (2018). Overview on zein protein: A promising pharmaceutical excipient in drug delivery systems and tissue engineering. *Expert Opinion on Drug Delivery*, *15*(1), 65-75. https://doi.org/10.1080/17425247.2017.1349752

Lawton, J. W. (2002). Zein: A history of processing and use. *Cereal Chemistry*, *79*(1), 1-18. https://doi.org/10.1094/CCHEM.2002.79.1.1

Li, W., Nooeaid, P., Roether, J. A., Schubert, D. W., & Boccaccini, A. R. (2014). Preparation and characterization of vancomycin releasing PHBV coated 45S5 Bioglass® -based glass-ceramic scaffolds for bone tissue engineering. *Journal of the European Ceramic Society*, *34*(2), 505-514. https://doi.org/10.1016/j.jeurceramsoc.2013.08.032

Maji, K., & Dasgupta, S. (2017). Effect of β-tricalcium phosphate nanoparticles additions on the properties of gelatin-chitosan scaffolds. *Bioceramics Development and Applications*, *7*(2), Article 10000103. https://doi.org/10.4172/2090-5025.1000103

Mikos, A. G., & Temenoff, J. S. (2000). Formation of highly porous biodegradable scaffolds for tissue engineering. *Electronic Journal of Biotechnology, 3*(2), 1995-2000.

Murphy, C. M., & O'Brien, J. F. (2010). Understanding the effect of mean pore size on cell activity in collagen-glycosaminoglycan scaffolds. *Cell Adhesion & Migration, 4*(3), 377-381. https://doi.org/10.4161/cam.4.3.11747

Murphy, C. M., Haugh, M. G., & Brien, F. J. O. (2010). The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. *Biomaterials*, *31*(3), 461-466. https://doi.org/10.1016/j.biomaterials.2009.09.063

Nam, Y. S., Yoon, J. J., & Park, T. G. (2000). A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive. *Journal of Biomedical Materials Research*, *53*(1), 1-7. https://doi.org/10.1002/(SICI)1097-4636(2000)53:1<1::AID-JBM1>3.0.CO;2-R

Patel, M., & Fisher, J. P. (2008). *Biodegradable materials for tissue engineering*. McGraw-Hill Global Education Holdings, LLC. https://doi.org/10.1036/1097-8542.YB080510

Philippart, A., Boccaccini, A. R., Fleck, C., Schubert, D. W., & Roether, J. A. (2015). Toughening and functionalization of bioactive ceramic and glass bone scaffolds by biopolymer coatings and infiltration: A review of the last 5 years. *Expert Review of Medical Devices, 12*(1), 93-111. https://doi.org/10.1586/17434440.2015.958075

Polak, S. J., Lan, S. K., Wheeler, M. B., Maki, A. J., Clark, S. G., & Wagoner, A. J. (2011). Analysis of the roles of microporosity and BMP-2 on multiple measures of bone regeneration and healing in calcium phosphate scaffolds. *Acta Biomaterialia*, *7*(4), 1760-1771. https://doi.org/10.1016/j.actbio.2010.12.030

Prasadh, S., & Wong, R. C. W. (2018). Unraveling the mechanical strength of biomaterials used as a bone scaffold in oral and maxillofacial defects. *Oral Science International*, *15*(2), 48-55. https://doi.org/10.1016/S1348-8643(18)30005-3

Qu, H., Fu, H., Han, Z., & Sun, Y. (2019). Biomaterials for bone tissue engineering scaffolds: A review. *RSC Advances*, *9*, 26252-26262. https://doi.org/10.1039/c9ra05214c

Qu, Z. H., Wang, H. J., Tang, T. T., Zhang, X. L., Wang, J. Y., & Dai, K. R. (2008). Evaluation of the zein/inorganics composite on biocompatibility and osteoblastic differentiation. *Acta Biomaterialia*, *4*(5), 1360-1368. https://doi.org/10.1016/j.actbio.2008.03.006

Reddy, N., & Yang, Y. (2011). Potential of plant proteins for medical applications. *Trends in Biotechnology*, *29*(10), 490-498. https://doi.org/10.1016/j.tibtech.2011.05.003

Reddy, N., & Yang, Y. (2013). Thermoplastic films from plant proteins. *Journal of Applied Polymer Science*, *130*(2), 729-738. https://doi.org/10.1002/app.39481

Reinwald, Y., Johal, R. K., Ghaemmaghami, A. M., Rose, F. R. A. J., Howdle, S. M., & Shakesheff, K. M. (2014). Interconnectivity and permeability of supercritical fl uid-foamed scaffolds and the effect of their structural properties on cell distribution. *Polymer*, *55*(1), 435-444. https://doi.org/10.1016/j.polymer.2013.09.041

Rezwan, K., Chen, Q. Z., Blaker, J. J., & Roberto, A. (2006). Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials, 27*(18), 3413-3431. https://doi.org/10.1016/j.biomaterials.2006.01.039

Rosa, D. S., Lopes, D. R., & Calil, M. R. (2005). Thermal properties and enzymatic degradation of blends of poly (ε-caprolactone) with starches. *Polymer Testing, 24*(6), 756-761. https://doi.org/10.1016/j.polymertesting.2005.03.014

Roseti, L., Parisi, V., Petretta, M., Cavallo, C., Desando, G., Bartolotti, I., & Grigolo, B. (2017). Scaffolds for bone tissue engineering: State of the art and new perspectives. *Materials Science and Engineering: C*, *78*, 1246-1262. https://doi.org/10.1016/j.msec.2017.05.017

Ru, J., Wei, Q., Yang, L., Qin, J., Tang, L., Wei, J., Guo, L., & Niu, Y. (2018). Zein regulating apatite mineralization, degradability, *in vitro* cells responses and in vivo osteogenesis of 3D-printed scaffold of n-MS/ZN/PCL ternary composite. *RSC Advances*, *8*, 18745-18756. https://doi.org/10.1039/c8ra02595a

Sabudin, S., Marzuke, M. A., & Hussin, Z. (2019). Effect of mechanical properties on porous calcium phosphate scaffold. *Materials Today: Proceedings*, *16*(Part 4), 1680-1685. https://doi.org/10.1016/j.matpr.2019.06.036

Sah, M. K., & Pramanik, K. (2011). Computational approaches in tissue engineering. *International Journal of Computer Applications, 27*(4), 0975-8887.

Salerno, A., Di Maio, E., Iannace, S., & Netti, P. A. (2011). Tuning the microstructure and biodegradation of three-phase scaffolds for bone regeneration made of PCL, Zein, and HA. *Journal of Cellular Plastics*, *47*(3), 245-260. https://doi.org/10.1177/0021955X11404832

Salerno, A., Oliviero, M., Di Maio, E., Netti, P. A., Rofani, C., Colosimo, A., Guida, V., Dallapiccola, B., Palma, P., Procaccini, E., Berardi, A. C., Velardi, F., Teti, A., & Iannace, S. (2010a). Design of novel three-phase PCL/TZ-HA biomaterials for use in bone regeneration applications. *Journal of Materials Science: Materials in Medicine*, *21*, 2569-2581. https://doi.org/10.1007/s10856-010-4119-0

Salerno, A., Zeppetelli, S., Di Maio, E., Iannace, S., & Netti A., P. (2010b). Novel 3D porous multi-phase composite scaffolds based on PCL, thermoplastic zein and ha prepared via supercritical CO_2 foaming for bone regeneration. *Composites Science and Technology*, *70*(13), 1838-1846. https://doi.org/10.1016/j.compscitech.2010.06.014

Salerno, A., Oliviero, M., Maio, E. Di, & Iannace, S. (2006). Thermoplastic foams from zein and gelatin. *International Polymer Processing, 22*(5), 480-488. https://doi.org/10.3139/217.2065

Shahbazarab, Z., Teimouri, A., Chermahini, A. N., & Azadi, M. (2018). Fabrication and characterization of nanobiocomposite scaffold of zein/chitosan/nanohydroxyapatite prepared by freeze-drying method for bone tissue engineering. *International Journal of Biological Macromolecules*, *108*, 1017-1027. https://doi.org/10.1016/j.ijbiomac.2017.11.017

Shamaz, M., & Halima, S. B. (2015). Bone Tissue Engineering and Bony Scaffolds. *International Journal of Dentistry & Oral Health, 1*(1), 15-20. https://doi.org/10.25141/2471-657X-2015-1.0001

She, H., Xiao, E. X., & Liu, E. R. (2007). Preparation and characterization of polycaprolactone-chitosan composites for tissue engineering applications. *Journal of Material Science, 42*, 8113-8119. https://doi.org/10.1007/s10853-007-1706-7

Shukla, R., & Cheryan, M. (2001). Zein: The industrial protein from corn. *Industrial Crops and Products*, *13*(3), 171-192. https://doi.org/10.1016/S0926-6690(00)00064-9

Subuki, I., Adnan, N., & Sharudin, R. W. (2018). Biodegradable scaffold of natural polymer and hydroxyapatite for bone tissue engineering: A short review. *AIP Conference Proceedings*, *2031*(1), 1-5. https://doi.org/10.1063/1.5066975

Subuki, I., Akhbar, S., & Nor Wahid, F. K. (2020). Influence of thermoplastic PEG, GLY and Zein in PCL/TZ and HAp bio composite via solid state supercritical CO_2 foaming. *Scientific Research Journal*, *17*(2), 177-190. https://doi.org/10.24191/srj.v17i2.9534

Tariverdian, T., Sefat, F., Gelinsky, M., & Mozafari, M. (2019). Scaffold for bone tissue engineering. In M. Mozafari, F. Sefat & A. Atala (Eds.), *Handbook of Tissue Engineering Scaffolds: Volume One* (pp. 189-209). Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102563-5.00010-1

Thavornyutikarn, B., Chantarapanich, N., & Chen, Q. (2014a). Bone tissue engineering scaffolding: Computer-aided scaffolding techniques. *Progress in Biomaterials, 3*, 61-102. https://doi.org/10.1007/s40204-014-0026-7

Thavornyutikarn, B., Chantarapanich, N., Sitthiseripratip, K., Thouas, G. A., & Chen, Q. (2014b). Bone tissue engineering scaffolding: computer-aided scaffolding techniques. In *Progress in Biomaterials* (pp. 61-102). Springer. https://doi.org/10.1007/s40204-014-0026-7

Tong, W., Suihuai, Y., Dengkai, C., & Yanen, W. (2017). Bionic design, materials and performance of bone tissue scaffolds. *Materials, 10*(10), Article 1187. https://doi.org/10.3390/ma10101187

Torabi, K., Farjood, E., & Hamedani, S. (2015). Rapid prototyping technologies and their applications in prosthodontics, a Review of Literature. *Journal of Dentistry, 16*(1)*,* 1-9.

Tortorella, S., Maturi, M., Vetri Buratti, V., Vozzolo, G., Locatelli, E., Sambri, L., & Franchini, M. C. (2021). Zein as a versatile biopolymer: Different shapes for different biomedical applications. *RSC Advances, 11*, 39004-39026. https://doi.org/10.1039/d1ra07424e

Wahid, F., Khan, T., Hussain, Z., & Ullah, H. (2018). Nanocomposite scaffolds for tissue engineering; properties, preparation and applications. In A. M. A. Inamudin & A. Mohammad (Eds.), *Applications of Nanocomposite Materials in Drug Delivery* (pp. 701-735). Elsevier. https://doi.org/10.1016/B978-0-12-813741-3.00031-5

Wang, C., Huang, W., Zhou, Y., He, L., He, Z., Chen, Z., He, X., Tian, S., Liao, J., Lu, B., Wei, Y., & Wang, M. (2020). 3D printing of bone tissue engineering scaffolds. *Bioactive Materials, 5*(1), 82-91. https://doi.org/10.1016/j.bioactmat.2020.01.004

Wang, H. J., Gong, S. J., & Wang, J. Y. (2008). Mechanical improvement of zein protein as scaffold for bone tissue engineering. *Materials Science and Technology*, *24*(9), 1045-1052. https://doi.org/10.1179/174328408X341735

Wu, F., Wei, J., Liu, C., O'Neill, B., & Ngothai, Y. (2012). Fabrication and properties of porous scaffold of zein/PCL biocomposite for bone tissue engineering. *Composites Part B: Engineering*, *43*(5), 2192-2197. https://doi.org/10.1016/j.compositesb.2012.02.040

Xiaohao, L., & Peter, X. M. (2004). Polymeric scaffolds for bone tissue engineering. *Annals of Biomedical Engineering, 32*(3), 477-486.

Yao, Q., Nooeaid, P., Roether, J. A., Dong, Y., Zhang, Q., & Boccaccini, A. R. (2013). Bioglass® -based scaffolds incorporating polycaprolactone and chitosan coatings for controlled vancomycin delivery. *Ceramics International*, *39*(7), 7517-7522. https://doi.org/10.1016/j.ceramint.2013.03.002

Yoshimoto, H., Shin, Y. M., Terai, H., & Vacanti, J. P. (2003). A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. *Biomaterials*, *24*(12), 2077-2082. https://doi.org/10.1016/S0142-9612(02)00635-X

Zhao, H., Li, L., Ding, S., Liu, C., & Ai, J. (2018). Effect of porous structure and pore size on mechanical strength of 3D-printed comby scaffolds. *Materials Letters*, *223*, 21-24. https://doi.org/10.1016/j.matlet.2018.03.205