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Biodegradable PLA-ZnO nanocomposite biomaterials with antibacterial properties, tissue engineering viability, and enhanced biocompatibility

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ABSTRACT

Polylactic acid (PLA) is a well-known biomaterial on account of its biocompatibility and biodegradability. Zinc oxide (ZnO) nanofillers may endow PLA with advantageous antibacterial and tissue regenerative properties, but may also compromise the biocompatibility of PLA. Several strategies have been developed to improve the biomedical practicality of such composites. The importance of surface properties on amplifying the therapeutic properties and safety of a material enables two potential strategies: (i) surface modification of ZnO nanoparticles, and (ii) surface engineering of the PLA/ZnO composites. Moreover, the controllable biodegradation of PLA allows a third possible strategy: (iii) biodegradation-controlled release of ZnO. The first part of this review introduces the controllable degradation of PLA and the mechanisms of therapeutic properties and cytotoxicity of ZnO. Following this, the paper highlights current research trends regarding the biomedical application of PLA-based ZnO nanocomposites. The final section of this review discusses the potential use of ZnO in tuning the degradation rate of PLA, and the possibility of manipulating the surface properties of ZnO nanoparticles and PLA/ZnO composites in order to optimize the therapeutic properties and safe usage of PLA/ZnO composites in the biomedical field.

1. Introduction

Polymer composites with a polylactic acid (PLA) matrix functionalized with zinc oxide (ZnO) nanofillers are gradually emerging in the literature as a promising class of materials suited for biomedical applications. One of the main strengths of PLA-matrix composites is the biodegradability of PLA, which avoids the need for secondary surgery and the risks associated with such procedures [1]. Besides being biodegradable and biocompatible, PLA is easily processable [2]. In recent years, the tailorable physicochemical and degradation properties of PLA have attracted the attention of researchers working in novel biomedical technologies such as tissue engineering and drug delivery. For example, the degradation rate of PLA-based resorbable tissue engineering scaffolds can be adjusted to match the healing rate of natural tissues. Biodegradable scaffolds provide mechanical support during cell/tissue growth, and gradually degrade into non-toxic products within the time frame required for complete regeneration [3]. Likewise, the degradation rate of drug delivery vehicles can be controlled to release drugs at desired rates to maintain optimum drug levels in the body within the therapeutic window [4]. The functionalization of PLA with ZnO nanofillers could further improve the therapeutic benefits of the polymer, with a well-known advantage of providing antibacterial properties to the polymer to prevent bacterial adhesion [5,6]. Aside from their excellent antibacterial properties, ZnO nanofillers are also gaining increasing recognition for their ability to enhance the regeneration process of several types of cells/tissues [7]. As an additional advantage, ZnO nanofillers play an important role in tuning the degradation rate of PLA, thus paving the way for smart materials whose therapeutic response can

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be tuned over time according to specific treatment requirements.

PLA/ZnO composites harness the desirable properties of the individual constituent materials and can easily be processed into various composite forms to target a wide spectrum of biomedical applications [8–11]. The greatest part of the literature is focused on wound and bone healing applications, where PLA/ZnO composites are employed in the form of wound dressings, tissue engineering scaffolds, and coatings for medical devices and implants. Moreover, PLA/ZnO composites are becoming increasingly popular in the field of 3D printing [9,12–14], as they enable cost-effective additive manufacturing of antibacterial constructs with complex and customized geometries [15].

Despite the substantial advantages that PLA/ZnO composites may offer in the biomedical field, there are still limitations hindering their adoption for medical treatment of patients in clinical settings. One critical issue is the potential cytotoxicity of ZnO nanoparticles [16]. Generally, the risk of toxicological effects increases with the exposure to ZnO, and has been indicated to be highly concentration-dependent [17–20]. Reducing the exposure concentration may alleviate such adverse effects, however it may compromise the therapeutic properties of ZnO-filled composites to some extent. For instance, this approach may diminish the antibacterial efficacy of ZnO-functionalised composites which is usually proportional to the concentration of ZnO [21–23]. A new and more profitable approach is thus needed to enable the safe usage of PLA/ZnO composites in the biomedical field. As discussed in the following sections, it is envisaged that maximizing the therapeutic effectiveness of PLA/ZnO composites, rather than restraining the action of ZnO nanoparticles, would be the way forward for safe biomedical applications. According to this strategy, the potential of ZnO nanoparticles can be fulfilled with the minimum filler loading. This is key to leveraging the multifunctionality of ZnO nanoparticles while avoiding overexposure, which is the main cause of harm to human health.

This review aims to provide information on the current development of PLA/ZnO composites in the biomedical field and the associated challenges. In particular, the insightful analysis of the potential tactics that can be deployed to optimize the therapeutic properties associated with ZnO fillers will contribute to direct future research and, ultimately, to prompt the wider uptake of PLA/ZnO in biomedical applications.

2. Characteristics and properties of PLA and ZnO

2.1. PLA

PLA has been approved by the Food and Drugs Administration (FDA) for direct contact with biological fluids [24]. In biological media, PLA degrades naturally through hydrolytic mechanisms to form non-toxic substances such as carbon dioxide, water, and lactic acid. All of them can be metabolized by the body or excreted through normal cellular activities. During hydrolysis, the diffusion of water molecules cleaves the ester bonds of the main polymer chain of PLA to form smaller chains of lactic acid (LA) monomers and oligomers, leading to PLA with lower molecular weight (MW) (Fig. 1) [25]. This reaction leads to the formation of acidic groups and degradation products that reduce the pH of the surrounding medium, subsequently accelerating the degradation of PLA [26]. Alternatively, PLA may also degrade enzymatically in the event of an infection. Enzymes such as *proteinase K* and *pronase* secreted by bacteria, and *acid phosphatase* and *lactate dehydrogenase* produced by immune cells when they generate inflammatory responses to combat infections, have also shown to enhance the degradation rates of PLA [27].

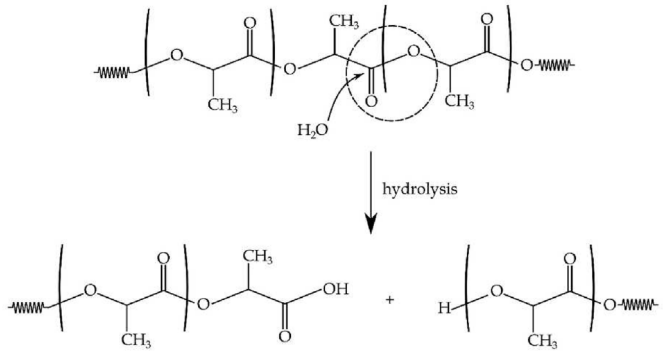

Fig. 1. Hydrolysis of PLA. Reproduced from Ref. [28] under the terms and conditions of Creative Common Attribution (CC BY) license.

LA, which is the monomer of PLA, is a chiral molecule with two enantiomers known as L-lactic acid and D-lactic acid [29]. As shown in Fig. 2, the synthesis of PLA starting from either enantiomer leads to the production of poly-L-lactic acid (PLLA) and of poly-D-lactic acid (PDLA), respectively; a combination of both L- and D-lactic acid isomers results in poly-D, L-lactic acid (PDLLA).

Accordingly, the stereochemistry and MW of PLA largely influence the crystallinity of PLA, and hence, its material properties [29]. For instance, amorphous PLA with low MW (<50,000 Da) has a nominal melting temperature (T_m) around 130–150 °C [30]. On the other hand, semicrystalline PLLA and PDLA with high MW (>50,000 Da) possess a higher T_m around 170–180 °C [30]. Typical values of the thermal and mechanical properties of PLA in its different forms have been outlined in Table 1 according to the data discussed by Farah et al. [32]. Furthermore, being a semicrystalline polymer, the degradation rate of PLA varies according to its degree of crystallinity. The incorporation of higher L-lactic acid content enhances the crystallinity of PLA and results in slower degradation rates and vice versa [27]. In addition, PLA with high MW also contributes to slower degradation rates. An increase in MW decreases the propensity for water uptake and the relative number of carboxyl end groups of PLA, consequently reducing hydrolytic degradation [29]. Other than the intrinsic properties of PLA, environmental factors such as pH and temperature have also shown to affect the degradation rate of PLA [26].

Commonly, desirable degradation kinetics can be obtained by methods such as copolymerisation or blending of PLA with other polymers having either higher or lower degradation rate [33]. Moreover, additives can be used to regulate the degradation rate of PLA such that, if required, acidic components can be added to catalyse and accelerate the degradation process [34]. Futhemore, it was reported that electron beam irradiation produces a geometry-related variation in MW through a PLA bulk, meaning that irradiated polymer surfaces (which are directly exposed to irradiation) possess lower MW compared to the core [34]. As a result, accelerated degradation will occur on the surface as compared to the bulk of PLA.

The controllable degradation rate of PLA and PLA-based materials has been extensively investigated for and leveraged in various biomedical applications [27]. However, as a downside, PLA is susceptible to bacterial contamination and can therefore act as a potential source of infection [35]. Moreover, PLA is biologically inert and hydrophobic [36]. These characteristics hinder the interaction with human cells, thus limiting the efficiency of PLA in tissue engineering applications [36].

To overcome these limitations, the biological properties can be strengthened via blending or copolymerizing PLA with natural polymers that are bioactive (i.e., chitosan, silk fibroin (SF), starch, gelatin, and keratin) [37–40]. On the other hand, desirable mechanical and degradation properties can be obtained by copolymerizing PLA with synthetic polymers having predictable and reproducible material properties [41,42]. One popular example used in many biomedical applications is poly (lactic acid-*co*-glycolic acid) (PLGA) [43]. PLGA is a copolymer of PLA and polyglycolic acid (PGA) whose material properties can be tuned by varying the relative amount of its constituent monomers [42]. As a result, copolymers and PLA-based blends are often preferred to neat PLA for biomedical applications. Over the years, a variety of fillers have also been added to PLA and PLA-based materials to target a wide range of biomedical applications [36]. Among the different types of fillers, ZnO stands out as it imparts antibacterial and tissue regenerative properties to

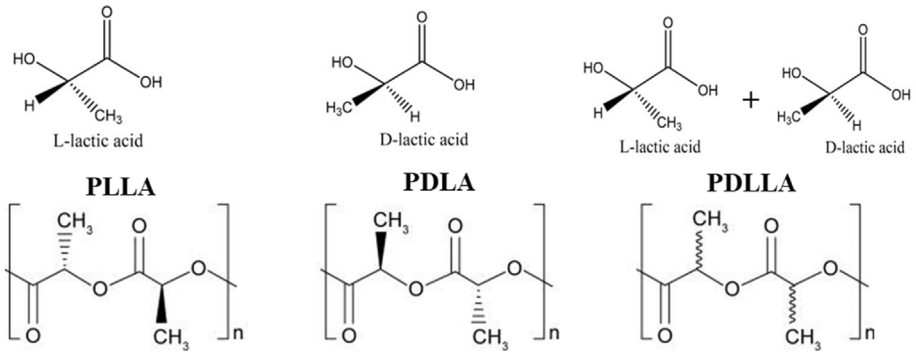

Fig. 2. Enantiomers of lactic acid and the three forms of PLA, namely PLLA, PDLA, and PDLLA. Adapted from Refs. [30,31] under the terms and conditions of Creative Common Attribution (CC BY) license.

Table 1
Typical range for the thermal and mechanical properties of different forms of PLA.

Material Properties	PLA	PLLA	PDLLA	Ref.
Melting temperature, T_m (°C)	150–162	170–200	No melting point (amorphous)	[32]
Glass transition temperature, T_g (°C)	45–60	55–65	50–60	
Tensile strength, σ (MPa)	21–60	15.5–150	27.6–50	
Elastic modulus, E (GPa)	0.35–0.5	2.7–4.14	1–3.45	

PLA, ultimately leading to a superior material for biomedical applications.

2.2. ZnO

2.2.1. Tissue regenerative properties

ZnO can promote the growth, proliferation, and differentiation of several cell lines, which is paramount to assist in tissue regeneration [7]. This has led to the development of various ZnO-functionalized bio--composites with demonstrated results for skin and bone tissue engineering applications [7,44–46]. The tissue regenerative properties of ZnO have been linked to several reasons, including: (i) the function of zinc (Zn) as an essential micronutrient in the human body, (ii) the formation of reactive oxygen species (ROS), and (iii) the electrical stimulation of cells.

2.2.1.1. Zn as an essential micronutrient in the body. Zn is an essential micronutrient in the body that takes part in various biological processes [47,48]. According to Maret [49], the term 'Zn' when used to describe biological functions refers to the oxidized form of Zn, which is Zn^{2+}, as it is the only valence state of Zn applicable in biology. The mass of Zn^{2+} present in the body is around 0.8–3 g, and it is mostly distributed in muscles and bone tissues (85% of the whole body), skin (5%), liver (5%), and the remaining 5% is present in other organs, such as the brain, pancreas, and kidneys [50]. Zn is a vital cofactor for the function of more than 10% of the proteins encoded by the human genome [47], including Zn-dependent proteins/enzymes such as matrix metalloproteinases (MMPs) and alkaline phosphatase (ALP), which are crucial in the stimulation of wound healing and bone regeneration processes, respectively [47,48]. As a result, Zn deficiency has been linked to impaired skeletal and bone development [51,52], and to delayed wound healing [53,54]. The therapeutic administration of ZnO is a viable strategy to increase and prolong Zn supply in Zn-deficient patients. For example, topical ZnO is widely used to enhance wound healing in Zn-deficient patients and has been associated with accelerated epithelialization of wounds [55].

2.2.1.2. Production of ROS. The ability of ZnO to form ROS is crucial for angiogenesis [56], which is the process that induces the development of new blood vessels [57]. In engineered tissue constructs, the formation of new blood vessels enables the supply of oxygen and nutrients to the cells that are colonizing the scaffold, and this is vital for tissue regeneration [57]. Angiogenesis is initiated by vascular endothelial cells (EC), involving the orderly proliferation, migration, and morphogenesis of EC into new a capillary network [58]. ROS in appropriate levels stimulate the signaling of multiple angiogenic growth factors, including the primary growth factor known as vascular endothelial growth factor (VEGF) [59]. VEGF promotes angiogenesis by potentiating EC migration, proliferation, and capillary tube formation, mainly via the VEGF receptor type 2 (VEGFR2) [60]. It was shown that endogenous ROS increased VEGF or VEGFR2 expression and stimulated EC proliferation and migration [61].

Both *in vitro* and *in vivo* experiments confirmed enhanced attachment and proliferation of fibroblasts (epidermal cells associated with wound healing) in electro-spun polycaprolactone (PCL) non-woven membranes combined with ZnO nanoparticles (NPs) [43,62]. It was hypothesized that optimal levels of ROS such as hydrogen peroxide generated by ZnO NPs increased fibroblast proliferation through mitogen activated protein kinases (MAPK), Jun-N-terminal kinase (JNK), and p38 MAPK activation [62], all of which are important cell signaling pathways that amplify, relay, and integrate signals from a range of stimuli and elicit appropriate physiological response including proliferation, differentiation, and growth of mammalian cells [63].

2.2.1.3. Electrical stimulation of cells. Endogenous electric fields play a major role in the organisation and development of various tissues, as well as in their regeneration after an injury. Accordingly, electrical stimulation can activate many intracellular signaling pathways to induce cell migration, proliferation, and differentiation [64,65], which are important processes of tissue regeneration. For example, intact skin has an endogenous electrical potential and a transcutaneous current potential of 20–50 mV resulting from the transport pathways of sodium ions through the Na^+/K^+ ATPase pumps in the epidermis (Fig. 3) [66]. After an injury,

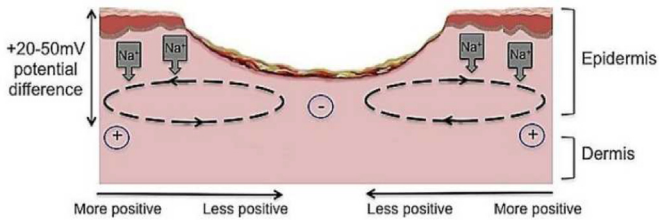

Fig. 3. Illustration showing the potential difference and the ion flow between intact skin and wound area. Reproduced from Ref. [71] under the terms and conditions of Creative Common Attribution (CC BY) license.

epithelial disruption induces an electric current flow towards the wound site, generating a voltage difference of about 100–150 mV/mm between the intact skin and the wound area [66]. These endogenous electric fields prompt cellular migration towards the injury site to facilitate re-epithelialization and accelerate wound healing [67]. As for bones, the mechanical stresses applied to bones trigger electrical potentials. Bones under compression generate an electronegative potential which causes bone resorption, whereas bones in tension generate an electropositive potential which causes bone formation [68]. A well-coordinated action of bone resorption and bone formation is necessary for repairing damaged bones [69], and electro-stimulation has been regarded as an effective method for inducing bone healing [70].

The piezoelectricity of ZnO has been leveraged to provide electrical stimulation to enhance tissue renegeneration processes [37,65,72,73]. For example, the piezoelectricity of ZnO was exploited for *in situ* electrical stimulation of human cells. Murillo et al. [73] demonstrated improved electromechanical interactions between two types of human cell lines (macrophages and Saos-2 osteoblast-like cells), and piezoelectric nanogenerators developed from a network of ZnO nanosheets. These cells exerted an instantaneous force over the nanogenerators, causing them to deflect. Due to the piezoelectric effect of ZnO, the exerted mechanical force was translated into an electrical field near the cell plasma membrane, and this subsequently led to enhanced cell activity by stimulating the motility of macrophages and triggering the opening of ion channels present in the plasma membrane of osteoblast-like cells [73].

2.2.2. Antibacterial properties

ZnO particles have an excellent antibacterial activity against a broad spectrum of gram-positive and gram-negative bacteria [74], as well as a strong efficacy in inhibiting biofilm formation [75]. The antibacterial mechanisms of ZnO are multifaceted and include: (i) the disruption of cell membranes, (ii) the release of ROS, and (iii) the release of Zn^{2+} [76]. Although the exact principles that govern the bactericidal effect of ZnO are still the subject of debate, it is believed that a connection exists between different mechanisms that enable the simultaneous delivery of more than one antibacterial action [77].

2.2.2.1. Disruption of cell membranes. The disruption of bacterial cell membranes is usually mediated by electrostatic interactions between ZnO, or the dissociated Zn^{2+} form, with bacterial cell walls. It is still unclear whether the antibacterial activity is mediated through an independent or a dual mode of action of the NPs and the released metal ions. Generally, Zn^{2+} and wurtzite type ZnO NPs are positively charged [78], whereas bacteria are negatively charged [76]. This produces a surface potential difference which induces ZnO or Zn^{2+} to be electrostatically attracted to bacterial cells, permeating through the membranes, and affecting bacterial structural integrity. The damaged bacterial cell walls then increase membrane permeability, leading to the internalization of ZnO NPs, or in other words, to the uptake of ZnO by bacterial cells, and subsequently to the generation of excessive ROS that can compromise bacterial metabolic activity [76].

2.2.2.2. Production of ROS. ROS in low concentrations are essential for regulating physiological cellular functions through redox signaling [79]. However, the high photocatalytic activity and electron mobility of ZnO NPs induce excessive ROS generation. Under visible light [80] and UV irradiation [81], electrons (e^-) are excited and transferred from the valence band to the conduction band, leaving positive holes (h^+) in the valence band. The e^- and h^+ pair reacts with oxygen and water to generate high concentrations of ROS, leading to oxidative stress that damages bacterial cells, proteins, and DNA. In some instances, ROS can be generated even in dark conditions due to surface defects of ZnO [82].

2.2.2.3. Release of Zn^{2+}. As mentioned in Section 2.2.1, Zn is an essential micronutrient involved in various cellular reactions at low concentrations. Zn concentration under physiological conditions is controlled by several Zn transporters, so that Zn remains non-toxic to cells in higher organisms [83]. Bacteria, like all living systems, need Zn for their growth [84]. Depending on the strain, bacteria typically need a Zn concentration between 10^{-10} M to 10^{-7} M for growth, and between 10^{-7} M to 10^{-5} M for optimal growth [84]. Beyond this threshold, excessive Zn levels disrupt the homeostatic processes that are responsible for regulating Zn uptake by cells, and this allows more Zn to enter inside cells [85]. Pasquet et al. [85] described that the dissolution of ZnO in aqueous suspension produces Zn^{2+} that participate in the antimicrobial activity of ZnO. Upon reaching concentrations above 10^{-4} M [85], Zn^{2+} becomes cytotoxic to bacterial cells. This is because excess Zn^{2+} may compete with other metals to bind with proteins and provoke mismatch binding, resulting in protein malfunction, inactivation, and denaturation [86].

2.2.3. Potential health hazards

The mechanisms that govern the antibacterial properties of ZnO, such as the release of Zn^{2+} and ROS formation, are also responsible for potential cytotoxic effects and subsequent health hazards. Firstly, the release of intracellular free Zn^{2+} ions into the body can potentially disrupt cellular Zn homeostasis, resulting in cellular damage and mitochondrial dysfunction [87]. Secondly, cellular internalization of ZnO prompts excessive ROS generation that surpasses the antioxidative protective limit, leading to oxidative stress and inflammatory responses within the cells [88]. Thirdly, the direct interaction between ZnO and cell surfaces can cause serious harm, such as alteration in cell morphology, disruption of cell membranes, and subsequent leakage of important intracellular components [87].

Aside from cytotoxicity, several *in vitro* and *in vivo* studies have also suggested that ZnO may have genotoxic effects [89–91]. However, the genotoxicological study of ZnO remains a controversial subject, as there were instances that showed no evidence of DNA damage upon ZnO exposure [92]. At present, there is a limited understanding on the genotoxic effects of ZnO, since more research has been focused so far on evaluating the cytotoxicity rather than the genotoxicity of ZnO when assessing its potential health effects [93]. Thus, more work will be required in future to properly assess the potential risk of ZnO in causing DNA damages.

NPs possess a higher surface-to-volume ratio compared to microparticles and bulk materials, and therefore enable enhanced interactions with the surrounding materials to obtain improved functionality. Also, NPs can be engineered and modified at the atomic and molecular scales to achieve better specificity [94]. Despite these benefits, there are open questions regarding the potential hazards of nano-scale materials towards human health. Although ZnO in its bulk form has been approved by the FDA as “generally recognized as safe (GRAS)” [95], ZnO particles in the nano-range (below 100 nm) have properties very different from their larger-scale counterparts, and hence different toxicity levels. To some extent, smaller ZnO particles may cause higher toxicity due to their higher dissolution rates that result in higher Zn^{2+} concentration in the cell culture medium [96]. Also, cells can easily internalize smaller particles, with the predicted uptake efficiency of NPs being about 10–15 times higher than that of bulk materials [20]. Also, the internalization of particles smaller than 100 nm through endocytosis is an energy consuming process, hence, it is expected that smaller particles consume less energy to diffuse into the cells [97]. In addition, the increased surface-to-volume ratio increases the number of active sites that can generate excessive ROS and the subsequent oxidative stress leading to toxicity [98].

Some researchers argue that a correlation exists between the size-dependent toxicity of ZnO and its various modes of action [96]. For instance, it was reported that smaller ZnO particles produce worse cellular damage as they induce higher release of Zn^{2+} and easier internalization of ZnO. However, the difference in particle size did not impact the intensity of cell death induced by ROS. This was linked to the

propensity for smaller particles to agglomerate. As a result, ZnO NPs of different sizes (26 nm, 78 nm, and 147 nm) formed agglomerates with comparable hydrodynamic sizes, which subsequently led to the production of similar ROS levels [96]. On the other hand, some researchers have disregarded the effect of ZnO particle size on its toxicity [99,100]. Instead, they have associated ZnO cytotoxicity with other factors such as time, dosage, and the type of cells. For instance, ZnO particles with sizes 70 nm and 420 nm caused similar extents of cellular membrane leakage and oxidative stress in human bronchoalveolar carcinoma-derived cells (A549). However, the cell viability of A549 reduced in a time- and dosage-dependent manner [99]. In another study [100], the cytotoxic effects of ZnO on two types of cells (human lung epithelial cells (L-132) and human monocytes (THP-1)) were both ZnO concentration-dependent. Nonetheless, only THP-1 cells exhibited different toxic response when being exposed to ZnO of different sizes [100]. There is clearly a lack of consensus regarding the size-related toxicity of ZnO on account of the controversial data presented in the literature.

Exposure to ZnO NPs via inhalation, ingestion, injection, and dermal penetration may pose an important hazard to human health. Among all, inhalation represents the primary exposure pathway starting from the synthesis to the disposal of such NPs [87], owing to their small size which makes them easily suspended in the air for a long time and capable of travelling a long distance. The accumulation of ZnO NPs in organs such as the liver, spleen, lungs, and kidney may lead to adverse health effects induced by the non-selective toxicity mechanisms of ZnO [101]. For example, systemic inflammatory effects caused by inhaled ZnO NPs have been reported at concentrations above 1 mg/m^3, which is five times below the recommended occupational exposure limit of 5 mg/m^3 [102]. Aside from inhalation, dermal absorption is another common exposure pathway. This is due to the frequent employment of ZnO NPs as one of the ingredients in cosmetic and sunscreen products. Many researchers have confirmed the limited dermal absorption of ZnO and therefore justified the safe usage of ZnO NPs in such products [103–105]. However, higher dermal absorption was detected in sunscreens functionalized with nano-sized particles (19 nm) as compared to sunscreens containing bulk particles (above 100 nm) [106]. ZnO in the nano-range has also shown to affect skin cells *in-vitro*, leading to cell apoptosis after short-term exposure, causing excessive ROS generation, and ultimately inducing cell death after long-term exposure [107].

The current knowledge on the health hazards of ZnO is incomplete. This is due to the involvement of multiple parameters in the assessment of ZnO toxicity [18,19,97,108–112]. As a result, this poses substantial challenges in the establishment of a standard of practice, and new systematic methods are certainly needed to accurately verify the toxicity of ZnO.

3. Biomedical applications of PLA-based ZnO composites

The greatest part of the literature is currently exploring the efficacy of PLA-based ZnO composites in wound and bone healing applications. Several examples are summarized in Table 2 and discussed in the following sections.

3.1. Wound healing

Modern wound dressings are not only a passive appendage to wound healing. This means that, ideally, in addition to covering the wound area and containing exudate, they should also possess characteristics such as mechanical stability, oxygen permeability, moisture regulation, secretion management, antibacterial properties, non-toxicity, biodegradability, easy removal after complete skin regeneration, and cost-effectiveness [127]. PLA-based/ZnO composite wound dressings can address several of these requirements, such as providing adequate mechanical properties, excellent antibacterial activity, and a great potential in accelerating skin tissue regeneration [37–41].

3.1.1. Mechanical and moisture absorbance properties

PLA-based/ZnO wound dressings have demonstrated compatible mechanical properties with the human skin. According to the literature, the tensile strength of human skin ranges between 1 MPa and 32 MPa, and its elongation at break ranges between 17% and 207%, depending on factors such as age, skin color, and genetics [128]. The tensile strength and elongation at break reported for PLA-based/ZnO wound dressings perfectly fit within this range of values [38–40,115,118], indicating comparable mechanical properties to human skin.

Apart from having appropriate mechanical properties, wound dressings should also have sufficient absorptive capacity to absorb wound exudates and to balance the moisture level around the wound [129]. Generally, a moist environment accelerates wound healing and promotes the growth of new tissues. However, moisture may also facilitate bacterial proliferation [130]. Thus, it is essential for wound dressings to maintain the right balance of moisture in wound environments to promote fast wound healing. For example, it was reported that a moisture-balanced wound dressing significantly enhanced the rate of re-epithelialization by 50% [130].

The water uptake capacity of PLA/chitosan/starch/ZnO wound dressings has been evaluated through an *in vitro* immersion test with Phosphate Buffer Saline (PBS) solution of pH 7.4 at 37 °C [38]. While chitosan and starch both increased the water uptake of PLA, the greatest improvement was achieved from the addition of ZnO. ZnO increased the water uptake capacity by approximately seven times over neat PLA, demonstrating a superior ability to maintain a wet environment for wound healing. Mao et al. [117] compared the water absorption and air permeability of PLA/gelatin/ZnO in the form of electrospun membranes and freeze-dried aerogel scaffolds. Whilst the membranes had a two-dimensional structure, the aerogel scaffolds possessed a three-dimensional macro-porous structure. Due to their structural advantage, the aerogel scaffolds achieved better water absorption and gaseous exchange properties as compared to the membranes. The hydrophilicity of the scaffolds was also reported to increase with the concentration of ZnO [117].

Nevertheless, the incorporation of ZnO may not necessarily lead to higher absorptive properties. For example, it was hypothesized that the addition of ZnO NPs reduced the water absorption properties of PLGA/SF wound healing scaffolds [40]. In this case SF was considered the key ingredient to improve the hydrophilicity of the wound dressing. This was related to SF being a natural polymer with many hydrophilic groups like amine, hydroxyl, and carbonyl groups [40]. In a separate study, Radwan-Pragłowska et al. [37] investigated the water vapor permeability of a wound healing scaffold based on a PLA/chitosan blend modified with ZnO NPs. Due to the low moisture permeability, the scaffold was suitable to treat shallow wounds, but it was insufficient to treat full-thickness skin injuries [37].

From the abovementioned studies, contradictory results have been reported regarding the effect of ZnO on the moisture absorption properties of wound healing scaffolds. This was very likely influenced by the differences in experimental parameters such as the type of polymer matrix and the structure of the wound healing scaffolds. Nonetheless, Rahman et al. [131] showed potential in adjusting the moisture levels of PLA-based ZnO wound dressings. In this case, the water absorption properties of PLA/chitosan/ZnO nanocomposites were tuned according to the concentration of ZnO [131]. Ultimately, this paves the way for designing new wound dressings with tailorable moisture levels to target different wound characteristics.

3.1.2. Antibacterial properties

Acute wounds take only a few weeks to heal [132], where healing progresses through four stages: homeostasis, inflammatory, proliferative, and remodeling stages [133]. However, the potential formation of a biofilm may interfere with these processes as it prolongs the inflammatory phase of wound healing [134]. Consequently, acute wounds are transformed into chronic wounds that take up to a few months to heal

Table 2
PLA-based ZnO composites in wound and bone healing applications.

Composite materials	Composite form	Biological characterizations	Summary	Ref.
Matrix: PLLA **Filler: ZnO NPs *(1 mg/cm^2)***	Electrospun wound healing scaffolds	Antibacterial properties (*Staphylococcus aureus (S. aureus), Escherichia coli (E. coli)*)	The antibacterial properties of ZnO prepared by nanosecond pulsed laser through ablation of metallic Zn target either in air or water, were compared when incorporated in PLLA wound healing scaffolds. The former method presented higher antibacterial efficacy.	[113]
Matrix: PLA-co-polycaprolactone (PLACL) and **PLACL/SF** **Filler: ZnO NPs *(6.25 and6.7%)*, (for comparison)** **ZnO + hydroxyapatite (HAp) NPs *(6.7%)*,** **ZnO NPs doped with HAp (ZnO (HAp)) *(13.3%)***	Electrospun nanofibrous scaffolds for bone tissue engineering	Cell viability *(osteoblast cells)* Bone-forming ability *(ALP activity and mineralization of osteoblast)*	The presence of SF and the incorporation of HAp improved the biocompatibility of the scaffolds. PLACL/SF/ZnO (HAp) increased osteoblast cell proliferation, ALP activity, and bone minerals secretion to aid with bone formation, but lacked in mechanical strength due to the crystallinity of HAp.	[114]
Matrix: PLLA **Filler: ZnO NPs *(5, 10, 20, and 40 wt%)***	Electrospun wound healing membranes	Antibacterial properties *(S. aureus, E. coli)*	Antibacterial activity increased with increasing ZnO concentration (5, 10, 20 & 40 wt%). Bactericidal effect against *S. aureus* was detected at 40 wt% ZnO concentration. Mechanical properties were compromised after 10 wt%	[115]
Matrix: PLA/PCL/ Thermoplastic starch (TPS) **Filler: ZnO NPs *(3 and 5 wt%)*, (for comparison)** **Thymol *(9 and 12 wt%)*,** **ZnO *(3 and 5 wt%)* + Thymol *(9 and 12 wt%)***	Hot-pressed wound healing films	Antibacterial properties *(S. aureus, E. coli)* Cytotoxicity *(L929 fibroblast cells)*	Composites with ZnO NPs (3 & 5 wt%) reduced the survival of L929 fibroblast cells (<30%). The addition of 12 wt% thymol enhanced the cell survival (>80%) and improved antibacterial properties.	[116]
Matrix: PLGA/SF **Filler: ZnO NPs *(1, 2, and 3 v/v %)***	Electrospun wound healing nanofibrous membranes	Antibacterial properties *(S. aureus, E. coli)* Antioxidant activity Cytotoxicity *(L929 fibroblast cells)* In vivo wound healing *(rats)*	Composites with 3 wt% ZnO NPs demonstrated effective antibacterial activity, wound contraction, re-epithelialization, cellular migration, collagen deposition, and capillary network formation, while remained biocompatible. SF contributed to mild antioxidant activity which enhanced wound healing and biocompatibility.	[40]
Matrix: PLA/Gelatin **Filler: ZnO NPs *(0.5, 1, and 1.5 wt%)***	Electrospun + freeze-dried 3D aerogel wound healing scaffolds	Antibacterial properties *(S. aureus, E. coli)* ROS generation Cytotoxicity *(L929 mouse fibroblast cells)* Angiogenesis *(chick embryo allantoic chorion)* In vivo wound healing (*mice)*	The 3D structure of the scaffold provided an effective physical barrier against wound infection and was efficient in absorbing wound exudate and gaseous exchange. Scaffolds incorporated with 1.5 wt% ZnO demonstrated effective antibacterial properties, reduced inflammatory responses, and promoted angiogenesis to assist with wound healing.	[117]
Matrix: PLLA **Filler: ZnO *NPs (0.5, 1, and 2 wt %)* +** **Tranexamic acid (TXA) *(2 wt%)***	Electrospun wound healing nanofibers	Antibacterial properties *(S. aureus, E. coli)* Cytotoxicity *(human skin fibroblast and mesenchymal stem cell)* In vivo wound healing *(mice)*	Composites with 2 wt% of ZnO reduced 98% of *S. aureus* and 75% of *E. coli* bacterial population. The composites improved the dermal and epidermal tissue regeneration and reduced wound leakages and infections. TXA demonstrated good blood clotting effect for effective blood loss control. Only weak cytotoxicity was induced by ZnO.	[118]
Matrix: PLLA **Filler: ZnO NPs *(0.5, 1, and 2%)* +** **TXA *(2%)*, (for comparison)** **(Copper Oxide) CuO *(0.5, 1, and 2%)* + TXA *(2%)***	Electrospun nanofibrous nanocomposites coated on sterile wound healing cotton gauze	Antibacterial properties *(S. aureus, E. coli)* Cytotoxicity *(L929 fibroblast cells)*	Antibacterial properties of the coated sterile cotton gauze increased with increasing ZnO and CuO nanoparticle loadings up to 2 wt%. ZnO demonstrated higher bacterial growth inhibition effects than CuO. Cell adhesion and expansion were observed on the vicinity and surface of the sample which indicated material biocompatibility.	[119]
Matrix: PLA **Filler: ZnO NPs *(5 and 10 wt%)*** **Substrate: (Magnesium) Mg AZ31 alloy**	Nanocomposite membrane layer dip coated onto Mg alloy-based bone implants	Antibacterial properties *(E. coli)* Biocorrosion *(Hanks's balanced salt solution* pH *7.4, 37 °C)* Cytotoxicity *(MC3T3-E1 osteoblast-like cells)* In vivo biodegradation *(ex-ova chick embryo)*	The nanocomposite membrane layer strongly adhered to the surface of the Mg substrate and contributed to tailorable implant degradation rates, anti-corrosion, antibacterial properties, and enhanced osteoblast like cell proliferation without negatively affecting cytocompatibility at 5 wt% ZnO.	[120]
Matrix: PLA **Filler: ZnO nanofibers *(1 wt%)***	Solvent-cast 3D printed nanocomposite films for bone implants	Antibacterial properties *(E. coli)* Mechanical fatigue resistance at human body temperature	The composites withstood more than 3600 cycles of tensile cyclic loading and remained intact at the end of fatigue test (after 1 h) simulated at human body temperature. The composites reduced *E. coli* population by 10 times after 7 days.	[121]
Matrix: PDLLA, (for comparison) polyhydroxybutyrate (PHB) **Filler: ZnO NPs *(1, 3, and 5 wt %)***	Centrifugal spun nanofibrous scaffolds for bone tissue engineering	Antibacterial properties *(S. aureus, E. coli)* Cytotoxicity *(MC3T3-E1 mouse osteoblastic cells)*	ZnO demonstrated greater interactions with PDLLA than PHB matrices. This resulted in PDLLA fibers having a higher number of embedded NPs, leading to weaker antibacterial properties but higher osteoblast cell viability.	[122]
Matrix (top layer): PLA (bottom layer): acylated chitosan **Filler (top layer): ZnO NPs**	Bi-layer skin tissue engineering scaffolds (top): electrospun PLA/	In vivo biodegradation *(enzymatic hydrolysis in human lysozyme)* Cytotoxicity *(Human dermal fibroblast and L929 fibroblast cells)*	All composites were biocompatible and demonstrated biodegradability rates that are compatible with deep thermal burn injuries. However, the water permeability of scaffolds with ZnO were relatively low and only suited to	[37]

(*continued on next page*)

Table 2 (*continued*)

Composite materials	Composite form	Biological characterizations	Summary	Ref.
(1%)*,** **(for comparison)** **Iron (II, III) oxide (Fe_3O_4) NPs *(1%)*,** **Gold (Au) NPs *(1%)	nanofillers nanofibers (bottom): acylated chitosan		use for shallower wounds. The nanofillers improved material conductivity and promoted electrostimulation wound healing.	
Matrix: PLA/Polyvinyl alcohol (PVA)/ Keratin **Filler: Nanofibrillated chitosan (CHNF)/ZnO NPs *(10 wt%)***	Electrospun wound healing nanofibers	Antibacterial properties *(S. aureus, E. coli)* Cytotoxicity *(L929 fibroblast cells)*	Hybrid CHNF/ZnO nanofillers, due to the antioxidant properties of chitosan reduced the cytotoxic effects of ZnO NPs. The presence of CHNF also enhanced the antibacterial properties of ZnO.	[39]
Matrix: PLA **Fillers: ZnO NPs *(1, 3, and 5 wt %)***	Electrospun nanofibrous mats, (for comparison) Electrospun + electrosprayed nanofibrous mats for wound dressings	Antibacterial properties *(S. aureus, E. coli)*	The antibacterial activity of the mats produced through a combination of electrospinning and electrospraying demonstrated higher antibacterial efficacy than the mats produced via electrospinning only. This was due to the deposition of NPs on the surface of the mats from electrospraying which increased the direct interaction of the NPs with bacterial cells.	[123]
Matrix: PLA **Filler: ZnO NPs *(1, 3, and 5 wt %)*,** **(for comparison) ZnO-graft PLA NPs *(1, 3, and 5 wt%)***	Electrospun wound healing nanofibrous mats	Antibacterial properties (*S. aureus, E. coli)*	PLA grafts on the surface of ZnO NPs improved the NPs dispersion within PLA. This significantly enhanced the antibacterial properties (3 and 5 wt% NP loadings demonstrated bactericidal effects against *E. coli*) and the mechanical properties of the composites.	[124]
Matrix: PLA **Filler: ZnO NPs *(0.5, 1, and 1.5 wt%)*,** **(for comparison) silicon dioxide (SiO_2) NPs *(0.5, 1, and 1.5 wt%)*,** **ZnO + SiO_2 NPs *(0.5, 1, and 1.5 wt%)*** **Substrate: Titanium (Ti) pedicle screw**	Dip-coated nanocomposite films on Ti pedicle screws	Antibacterial properties *(S. aureus)* Cell viability *(L929 mouse fibroblast cells)*	PLA incorporated with 1 wt% ZnO demonstrated bactericidal effect (1 log reduction in bacterial cells). SiO_2 NPs decreased the antibacterial actions of ZnO, but improved the cell viability of the coatings. PLA coatings with 1.5 wt% ZnO and SiO_2 impaired bacterial cell adhesion and provided bacteriostatic effects to the screws without inducing any cytotoxic effects.	[125]
Matrix: Polyethylene glycol (PEG)-b-PCL and PLLA **Filler: ZnO NPs (doped) *(0.33, 3.23, and 25 wt%)*** **Substrate: glass**	Spin-coated nanofilms on glass	Degradation Cell viability *(human dermal fibroblasts, MG-63 osteoblasts, C2C12 myoblasts, human chondrocytes)*	The degradation rates of the films were inversely proportional to ZnO concentration, showing enhanced resistance to material erosion and improved biomaterial integrity. All cell types showed good adhesion and cell viability. Higher fibroblast, myoblast, and chondrocytes cell metabolic activities, and higher osteogenic and myogenic differentiation were observed with increasing ZnO concentration.	[41]
Matrix: PLGA **Filler: Silver (Ag) NPs *(1, 3, and 6 wt%)*** **Substrate: Ti disks with hydrothermally grown ZnO nanorods**	Spin-coated nanocomposite films on Ti implants	Antibacterial properties *(S. aureus, E. coli)* Cell viability *(MC3T3-E1 mouse calvarial cells)* In vitro osteogenic differentiation *(ALP activity, initial cell adhesion and spreading activity)*	The antibacterial activity of the coated Ti implants lasted up to 100 days and was highly dependent on the concentration of Ag NPs. Ag concentration above 3 wt% demonstrated toxicity against MC3T3-E1. PLGA/ZnO coatings incorporated with 3 wt% Ag NPs demonstrated sufficient antibacterial activity and minimal toxicity.	[126]
Matrix: PLA/Chitosan/Starch **Filler: ZnO NPs *(1 wt%)***	Electrospun nanofibrous wound healing scaffolds	Cytotoxicity *(primary dermal fibroblast cells)*	The nanofibrous scaffold with 1 wt% ZnO exhibited a time-dependent cytotoxic effects. The cell viability decreased from 107% (24 h), to 101% (48 h) and finally, to 94% after 72 h of incubation.	[38]

completely [132]. Therapeutic procedures through mechanical or sharp debridement (a procedure to remove damaged or dead tissues and bacteria from wound) can be used to clean off biofilms. However, such procedures cause pain and discomfort in patients. Also, there is a possibility for biofilms to gain antibiotic resistance 3 days after the procedure [135]. To support quick and effective wound healing, it is the minimum requirement for wound dressings to inhibit biofilm formation.

Biofilms are developed from bacteria attached on surfaces that grow into microcolonies [136]. Therefore, antibacterial wound dressings that can inhibit and prevent the growth of bacteria may prevent the development of biofilms. In this regard, PLA-based/ZnO wound dressings have been widely explored against two of the most common biofilm-forming bacterial species [39,40,113,115,116,118,123,124] such as *S. aureus* (gram-positive bacteria) and *E. coli* (gram-negative bacteria) [137]. Higher antibacterial efficacy has been demonstrated against *S. aureus*, very likely due to the differences in bacterial cell wall structures. As seen in Fig. 4, gram-negative bacteria possess an additional outer membrane

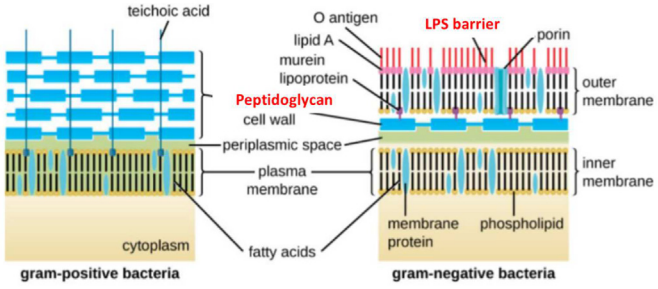

Fig. 4. Cell wall structures of gram-positive bacteria (left) and gram-negative bacteria (right). Adapted from Ref. [138] under the terms and conditions of Creative Common Attribution (CC BY) license.

containing lipopolysaccharides (LPS) and a thinner peptidoglycan layer. The LPS layer acts as a permeability barrier which increases the resistance of gram-negative bacteria towards antibacterial agents like ZnO [76,77]. However, there is a counterargument that gram-negative bacteria are more susceptible to the antibacterial actions of ZnO as compared to gram-positive bacteria as a consequence of their thinner peptidoglycan layer [10].

Generally, provided that the NPs do not agglomerate, the antibacterial properties of ZnO-functionalized wound dressings increase with the concentration of ZnO [40,116,124]. For instance, Rodríguez-Tobías et al. [124] reported no significant improvements in the antibacterial efficacy of PLA-based/ZnO wound dressings when the concentration of ZnO exceeded 3 wt% and diffused agglomeration occurred. This was resolved by grafting PLA onto the surface of ZnO, or, in other words, attaching PLA chains onto the surface of ZnO to form ZnO-graft-PLA NPs. These NPs were produced via one-pot microwave assisted ring-open-polymerization of LA in the presence of ZnO NPs [139]. The presence of PLA grafts on the surface of ZnO improved the dispersion of the nanofillers within the PLA matrix, and subsequently enhanced the antibacterial properties of the material [124].

Despite having stronger antibacterial effect, high ZnO concentrations increase the risk of cytotoxicity [40]. The use of antioxidant agents has shown potential in mitigating the cytotoxicity of ZnO while preserving the antibacterial efficacy. In this regard, Kazemi-Paservi et al. [116] used thymol (a major constituent of essential oils of thyme [140]) as the antioxidant agent to reduce the cytotoxicity of ZnO in PLA/PCL/thermoplastic starch (TPS) wound healing films. Interestingly, the addition of thymol successfully neutralized excess ROS and oxidative stress, and simultaneously increased the antibacterial properties of the material. Another study [39] demonstrated that PLA/keratin/polyvinyl alcohol (PVA) wound healing scaffolds filled with nanofibrillated chitosan (CHNF)/ZnO demonstrated higher antibacterial properties and cell viability as compared to neat ZnO NPs. The improvement in biocompatibility was related to the antioxidant properties of chitosan, thanks to the presence of amino and hydroxyl groups that were capable of scavenging ROS.

Based on the available knowledge, it still remains unclear how long the antibacterial effects of ZnO NPs will survive in wound dressings. Long-lasting antibacterial properties are especially crucial for chronic wound dressings as they should deliver their antibacterial properties throughout the duration of chronic wound healing [141]. Most studies [39,40,113,115,116,118,124] have only considered the antibacterial activity over a short period of time (24–72 h), which may be insufficient to accurately represent the antibacterial response throughout the whole wound healing process. Hence, future studies should also consider the antibacterial functionality of PLA-based/ZnO composites in the long run to ensure effective antibacterial properties during wound healing.

3.1.3. Skin tissue regenerative properties

Wounds can be classified into different categories based on wound depths, as seen in Fig. 5. Superficial wounds (shallow wounds) such as sun burns, light scalds, and grazing do not require surgical treatment as only the epidermis layer is affected [142]. Usually, these wounds only require protection against external stress and infection while they self-repair in the presence of stem cells [143]. In contrast, partial or full thickness wounds (deep wounds) such as thermal injuries extend beyond the epidermis and cannot re-epithelialize on their own [142]. Any loss of full thickness skin more than 4 cm diameter requires skin grafting treatments to assist with wound recovery [143]. However, the transplantation of autologous tissue is often limited by the availability of donor sites. On the other end, the use of foreign tissues as substitutes may trigger immune rejection [143]. Skin tissue engineering explores new approaches to solve these problems, via the implantation of tissue-engineered scaffolds into injured sites to deliver antibiotics, cells, and growth factors to reconstruct functional components of the skin [144]. ZnO NPs may boost the efficacy of skin tissue engineering, as they are well known for their ability to promote the growth, proliferation, and differentiation of skin cells such as keratinocytes [145] and fibroblasts [40,45], which are important for skin regeneration. Moreover, topical administration of ZnO facilitates the participation of Zn-dependent proteins in DNA repair and in other metabolic activities that can stimulate re-epithelialization (i.e., resurfacing of wound with new epithelium [146]), angiogenesis, and scar formation [47].

Several findings have suggested that PLA-based/ZnO composites can promote wound repair and skin regeneration activities [37,38,40,118]. For instance, Khan et al. [40] confirmed the ability of PLGA/SF functionalized with ZnO to favor wound healing *in vivo* . The presence of SF improved the biocompatibility of the material as it neutralized the harmful effects of ROS [40]. Fig. 6 compares the progress of spontaneous healing of untreated wounds to the recovery of wounds that have been treated with PLGA/SF dressings without and with ZnO, respectively.

As seen in Fig. 6, the wound dressing containing both SF and ZnO (PSZ3) induced the most effective wound healing after 10 days. The wound contraction of PSZ3 was about 14.6% and 21.4% higher than that

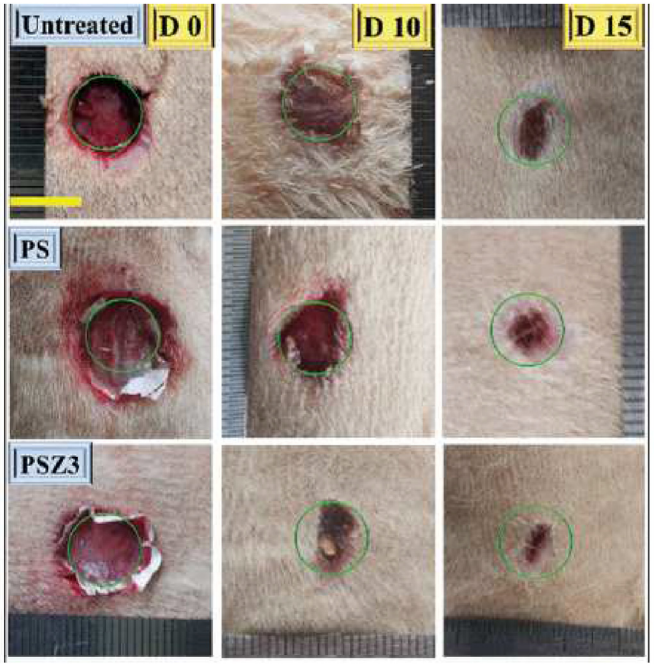

Fig. 6. Comparison showing untreated, PLGA/SF treated (PS), and PLGA/SF/ZnO treated (PSZ3) wounds in rats at day 0 (D 0), day 10 (D 10), and day 15 (D 15) of observation. Reproduced from Ref. [40] with permission from Copyright Clearance Center on behalf of Royal Society of Chemistry.

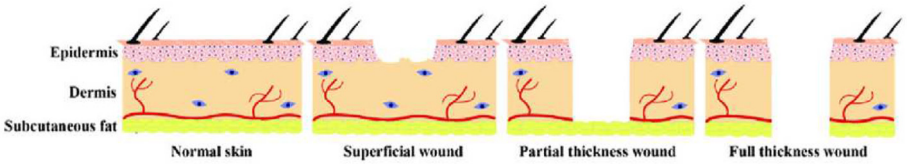

Fig. 5. Different wound classifications based on depth. Adapted from Ref. [147] under the terms and conditions of Creative Common Attribution (CC BY) license.

observed for the PS and untreated groups, respectively. Not only did ZnO accelerate wound contraction, but also favored re-epithelization, fibroblast and keratinocytes migration, collagen deposition and capillary network formation [40]. Moreover, the wound contraction of PSZ3 was related to epidermis formation and did not result in scar tissue formation [40].

Similar results were obtained within another *in vivo* wound healing assessment undertaken by Molapour Rashedi et al. [118]. In this study, PLA/ZnO nanofibrous wound dressings were loaded with an additional drug known as tranexamic acid (TXA), which is commonly used in chronic wound medication to treat or prevent excessive bleeding. The composite demonstrated good biocompatibility with human skin fibroblast cells. In addition, TXA effectively reduced blood loss in chronic wounds. When tested in mice, wounds that had been treated with PLA/ZnO/TXA achieved a 25% higher wound closure rate over the control group. Additionally, the wounds were completely re-epithelialized, demonstrating enhanced infiltration of fibroblasts to the wound area [118].

Recently, Mao et al. [117] also confirmed the biocompatibility and wound healing capabilities of PLA/gelatin/ZnO aerogel scaffolds. The presence of ZnO (0.5–1.5 wt%) enhanced the *in vivo* wound healing efficacy (tests conducted on mice). Notably, the reduction in inflammatory responses, the regeneration of collagen fibers, and diffused formation of hair follicle, all improved with increasing ZnO concentration. Furthermore, as seen in Fig. 7, the scaffold filled with the highest ZnO concentration of 1.5 wt% formed the densest blood vessel network, demonstrating the strongest angiogenic effect [117]. This outcome was predictable on account of the highest amount of ROS generated in the scaffold functionalized with 1.5 wt% of ZnO, due to the role of ROS in initiating angiogenesis, as already discussed in Section 2.2.1.

The potential of PLA/ZnO composites in skin tissue engineering applications is also corroborated by the compatibility of PLA/ZnO and PLA/ZnO-lignin nanocomposites with human bone marrow-mesenchymal stem cells (hBM-MSCs) and human adult adipose stem cells (hASCs) [148]. Both types of stem cells are capable of self-renewing and differentiating into epithelial cells to generate human skin substitutes [149]. Cell viability tests showed that both stem cells had similar cell viability and proliferation rates when cultured on pure PLA and on PLA nanocomposite films [148]. In addition to the absence of any toxic effects, the biocompatibility of the composite materials was further confirmed by the ability of both kinds of stem cells to interact with the surface of the nanocomposites through vinculin focal adhesion spots [148]. Vinculin is an extremely important protein that contributes to the ability of cells to tightly adhere to one another and to the extracellular matrix [150]. It also regulates the focal adhesion complexes to mediate cell migration [151].

Additional evidence of the supportive role of PLA-based/ZnO composites in skin tissue engineering comes from the successful usage of these materials in electrostimulation therapy. In this regard, Radwan-Pragłowska et al. [37] evaluated the potential of a double-layered scaffold, with PLA and ZnO at the top and acylated chitosan at the bottom (Fig. 8), to stimulate cell proliferation through direct current. The increased conductivity of the bi-layered system due to the addition of ZnO led to the enhancement of cell proliferation of L929 mouse fibroblasts and human dermal fibroblasts by approximately 20% under direct current electrical stimulation [37].

PLA/ZnO wound dressings exhibit remarkable wound healing properties. However, it is important to keep in mind that identifying the optimal concentration of ZnO is crucial to achieving positive therapeutic outcomes without imparting toxicity. This is still a major challenge that requires further attention to broaden the usage of ZnO-filled composites in the field of skin healing.

3.2. Bone healing

Compared to wound healing applications, PLA/ZnO composites are less explored in the field of orthopedics. Nonetheless, a thorough search in the literature reveals potential applications in two main areas, which are (i) coatings for load-bearing implants and (ii) bone tissue engineering scaffolds.

3.2.1. Coatings for implants

For advanced load-bearing applications, PLA is more commonly used as a functional coating rather than as the main structural material as it lacks the required mechanical strength and reliability [152]. Conventional load-bearing implants largely exploit metal-based materials (for example, stainless steel, cobalt chrome, titanium and its alloys) owing to their excellent mechanical properties (high tensile strength, fracture toughness, hardness, fatigue resistance), and good biocompatibility [153, 154]. However, metallic implants have serious limitations. Several metals are likely to favor infection and may suffer corrosion in biological environments. Most of all, the use of metals is impaired by the high risk of implant loosening due to poor osseointegration (weak bone-implant connection) [155]), and by the insufficient ability to induce osteogenesis (the process of bone formation) [156]. Aseptic loosening and infection are generally recognized as the two main barriers that jeopardize the extended adoption of metals for orthopedic implants [157].

Fig. 9 shows the concept of "race for surface" introduced by Gristina et al. [158], where bacterial cells compete against tissue cells to colonize the surface of the implant. This implies that successful osseointegration

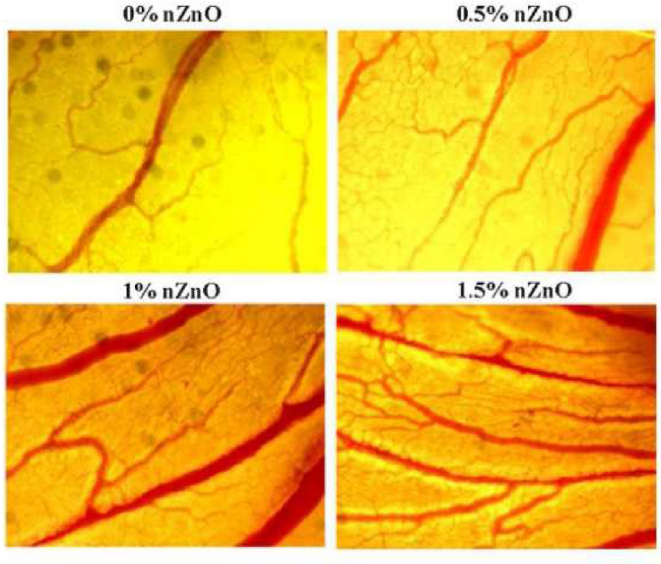

Fig. 7. Formation of blood vessels (angiogenesis) on chick embryos placed with PLA/gelatin/ZnO scaffolds at different concentrations of ZnO nanoparticles (nZnO). Reproduced from Ref. [117] with permission from Copyright Clearance Center on behalf of Elsevier.

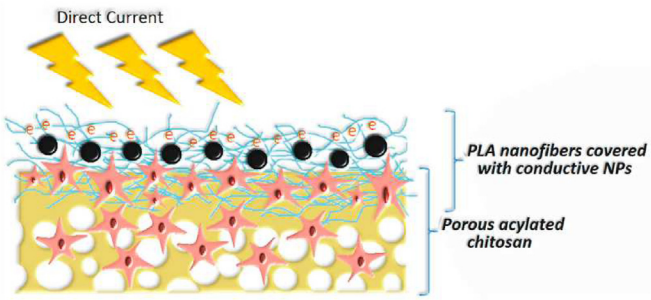

Fig. 8. Illustration of hybrid bilayer PLA/chitosan nanofibrous scaffolds doped with NPs for skin tissue engineering. Reproduced from Ref. [37] under the terms and conditions of Creative Common Attribution (CC BY) license.

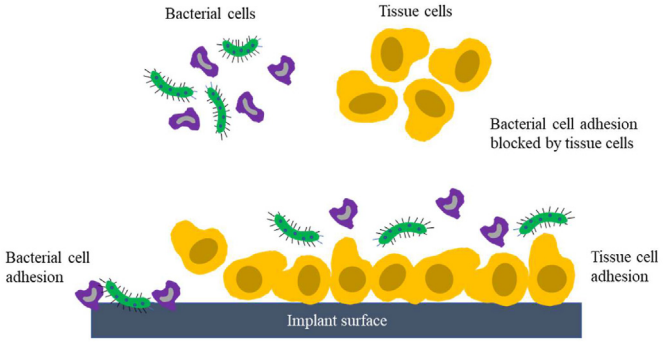

Fig. 9. Illustration of "race for surface" concept.

may only occur if bone tissue cells overtake bacterial cells in colonizing the implant surface to prevent microbial attachment. For this reason, surface modification is an important strategy to simultaneously improve the antibacterial properties and the osteogenic functionalities of metal-based bone implants. Over the years, a multitude of implant coatings have been developed to this aim [156,159]. Interestingly, the quest for bone implant coatings also includes PLA-based/ZnO composites.

Recently, Şen Karaman et al. [125] evaluated the potential of PLA/ZnO and PLA/ZnO/Silicon dioxide (SiO_2) coatings on titanium (Ti) pedicle screws to prevent bacterial infections in spinal surgery. PLA coatings filled with 1 wt% of ZnO provided the screws with bactericidal properties. This was indicated by the ability of the screws to produce one-log (>90%) reduction in bacterial cell population when immersed in a bacterial cell suspension. The subsequent introduction of 1 wt% of SiO_2 into the coatings decreased the antibacterial effects of ZnO, rendering the surface bacteriostatic instead of bactericidal. This was evident as the coatings did not kill bacteria, but prevented bacterial growth by keeping bacteria in their stationary phase of growth [160]. Nonetheless, the antibacterial properties of the coatings were improved when the concentration of both ZnO and SiO_2 was increased to 1.5 wt%. This was proven by the more efficient bacterial growth inhibition, and by the reduction in bacterial cell adhesion and viability. While all the coatings in this study showed no cytotoxic effects on L929 cells, the presence of SiO_2 significantly enhanced the biocompatibility of the material by promoting the proliferation of fibroblasts [125].

Another example of the viability of PLA-based/ZnO coatings for bone implants is provided by the contribution of Vannozzi et al. [41]. Thin films composed of a blend of poly(ethylene glycol)-*block*-poly(ε-caprolactone) methyl ether (PEG-*b*-PCL) and PLLA doped with ZnO NPs, and were spin-coated over a glass substrate. An *in vitro* cell study was performed with numerous cell models in the musculoskeletal system to assess the biocompatibility and bioactivity of the films [41]. The chosen cell models included fibroblasts (primary producer of extracellular matrix (ECM) in skeletal muscles [161]), myoblasts (muscle cells), osteoblasts (bone forming cells), and chondrocytes (cartilage--forming cells). All cell types demonstrated good cell adhesion and viability on the nanocomposite films of different ZnO concentrations. Increasing the ZnO loading (0.33, 3.23, and 25 wt%) enhanced the cellular metabolic activity of fibroblasts, myoblasts, and chondrocytes, and promoted osteogenic and myogenic differentiation. The presence of ZnO also increased the surface roughness of the films which facilitated bone formation and mineralization. Moreover, ZnO increased the piezoelectricity of the material, which is thought to enhance the maturation of bone-like tissues [162].

Xiang et al. [126] showed the ability of PLGA/silver (Ag)/ZnO coatings to provide long term antibacterial properties and osteogenic functionalities when coated on Ti substrates. ZnO and Ag NPs enwrapped in PLGA ensured over 100 days of Zn^{2+} and Ag^{+} release to provide long-lasting antibacterial effect and good biocompatibility. The nanocomposite coatings were immersed in PBS at 37 °C to analyze the release of Zn^{2+}. It was revealed that the hydrolysis of PLGA produced acidic degradation products that increased the acidity of the surrounding medium. As a result, the hydrogen ions (H^{+}) in the acidic medium subsequently reacted with ZnO, leading to the release of Zn^{2+}. Despite that, without the incorporation of Ag NPs, the antibacterial action of ZnO was inadequate to provide effective antibacterial properties. This was attributed to PLGA covering the ZnO NPs and significantly masking their antibacterial efficacy. Moreover, the maximum concentration of Zn released throughout the experiment was below 6 mg/L, which has been reported to be inadequate for inhibiting the growth of *E. coli* and *S. aureus* [163,164]. In terms of osteogenic properties, the coatings released Zn^{2+} concentrations that satisfied the minimum requirement (6.5×10^{-4} mg/L) reported to promote bone formation, but did not exceed the critical concentration of 6 mg/L that is responsible for cytotoxic effects *in vitro* [126].

Aside from improving osseointegration and antibacterial properties of metal implants, PLA-based/ZnO coatings can potentially be used to minimize the corrosion and uncontrollable degradation associated with magnesium (Mg) alloy implants. This functionality was investigated by Mousa et al. [120], using Mg alloy AZ31 substrates coated with PLA/ZnO composites. The coatings with increasing ZnO concentration featured controllable layer thickness, enhanced wettability, and higher coating-substrate adhesion strength. Moreover, Mg substrates coated with the PLA/ZnO composite experienced the slowest degradation rates compared to bare implants and to neat PLA-coated samples. Similarly, electrochemical corrosion tests demonstrated that remarkable anticorrosion properties were achieved with the inclusion of ZnO NPs. In addition to corrosion resistance, nanocomposite coatings with 5 wt% of ZnO rendered the surface antibacterial and promoted the proliferation of osteoblast-like cells.

3.2.2. *Bone tissue engineering scaffolds*

Most bone fractures are capable of self-repairing, through either primary or secondary healing processes. The most common process is secondary healing, which involves four main phases known as: hematoma formation, fibrocartilaginous formation, bony callus formation, and bone remodeling [165]. Alternatively, primary bone healing occurs when there is a direct fixation between the fracture surfaces, since bone heals without the formation of callus [166]. The natural healing ability of bones is hampered when the size of the damage exceeds the "critical size defect". Currently, the definition of a critical size has not been standardised as it is largely influenced by the anatomic location and the condition of the tissues surrounding the fracture site [167]. Nonetheless, as a general guideline, indicative values reported in the literature are 3 cm in the radius and ulna, 5 cm in femur and tibia, and 6 cm in the humerus [168]. Some authors stated that the critical size is in the order of 2.5 cm, or that a critical-size defect causes a 50% loss in the original circumference of the bone [167]. Any fractures or defects that are larger than the critical size or are extremely complex have poor spontaneous healing characteristics and require surgical intervention [167]. As already seen for wound healing, conventional options involve transplantation of bone tissue from another part of a person's body (autograft), or from another person (allograft). However, autografting has limitations such as the need for a second operation at the site of tissue harvest, donor site morbidity, and limited bone availability [169]. On the other hand, allografting poses a high risk of rejection [169]. Ultimately, these shortcomings have led to the development of new methods through bone tissue engineering. By this means, implantable bone substitutes are engineered in the form of three-dimensional scaffolds that mimic the ECM. In other words, bone scaffolds are designed to reproduce the natural structure of bone tissue that is responsible for directing cell adhesion and migration, as well as for regulating cellular growth, metabolism, and differentiation signals required for tissue regeneration processes [170]. As a result, bone scaffolds enable the attachment and proliferation of bone cells such as osteoblasts and osteoclasts, growth factors, and other

bioactive materials to induce osteogenesis.

Nonato et al. [121] investigated the usability of PLA/ZnO composites as bone implants. The composites were fabricated via solvent-cast 3D printing of PLA and ZnO nanofibers and tested for their mechanical fatigue resistance and antibacterial activity. The fatigue test was performed by cyclically loading the composites under tensile strain (frequency of 1 Hz and amplitude of 10 μm) at isothermal conditions of 36.5 °C to simulate the temperature of the human body. Whilst the composites were still intact after 1 h of cyclic loading (corresponding to 3600 cycles), neat PLA failed completely after 1768 cycles. The high fatigue resistance of the composites was associated with their high storage modulus, which was the result of the strong anchoring of PLA chains to ZnO nanofibers [121]. Additionally, ZnO nanofibers may have acted as a physical barrier that prevented cracks from propagating through the material [121]. On top of that, the nanocomposites also showed good antibacterial properties. A substantial reduction (by 10 times) of *E. coli* population on the nanocomposites was achieved after 7 days.

Gnaneshwar et al. [114] investigated the potential of PLA-co-polycaprolactone (PLACL)/SF nanofibrous scaffolds loaded with ZnO NPs doped with hydroxyapatite (HAp). Plasma treatment was used to convert the composites from hydrophobic to hydrophilic to improve cell adhesion and proliferation. The composites were able to mimic the native environment of ECM due to their porous structure and favorable bioactivity. The nanocomposite scaffold supported the proliferation of osteoblast cells, with a 98% increase in cell growth after 15 days of cell incubation. Moreover, the scaffolds also triggered osteoblast cells to produce higher levels of bone minerals that are essential for bone formation. In this case, it is important to highlight the substantial role of HAp in enhancing the bioactivity of ZnO. In the absence of HAp, the ZnO-filled scaffolds resulted in less bone mineral secretion and cell proliferation, and an abnormal cell morphology.

Padilla-Gainza et al. [122] compared PDLLA/ZnO and polyhydroxybutyrate (PHB)/ZnO composite fibrous mats for bone regeneration scaffolds. Comparatively, the composites based on PDLLA demonstrated poorer antibacterial performance. This was attributed to the stronger interaction occurring between ZnO NPs and PDLLA, causing the NPs to be completely and firmly embedded within the polymer, and consequently reducing their antibacterial efficacy. On the contrary, PDLLA composites exhibited higher cell viability than their PHB counterparts. This was related to the larger surface area and adequate porosity found in PDLLA scaffolds. It was reported that all PDLLA/ZnO scaffolds had porosity levels above 90%, which satisfied the porosity criteria for supporting cellular migration [171]. The addition of ZnO into PDLLA did not affect osteoblast cell viability, leading to good cytocompatibility [122]. However, significant antibacterial activities (above 97%) was only observed at 5 wt% of ZnO. At this concentration, ZnO formed agglomerates that considerably reduced the mechanical properties of the composites. Thus, apart from material biocompatibility, more work will be required to tune the mechanical and antibacterial properties of the material to be implemented in bone regeneration applications.

Research on the viability of PLA-based/ZnO composites for bone scaffolds is still in its infancy. Despite the promising results reported so far, more effort is needed to effectively assess the suitability of these composites for bone regeneration, as there is insufficient experimental data in the literature to reach any sound conclusion regarding the ability of such composites to meet the functional criteria required of bone scaffolds.

4. Optimization of therapeutic properties of ZnO in PLA-based composites

To further extend the applicability of PLA-based/ZnO composites in the biomedical field, it is important to maximize the therapeutic efficacy and amplify the antibacterial properties of ZnO while minimizing the filler loading. The potential strategies to achieve this include: (i) regulating the release rate of therapeutic ZnO and Zn^{2+} through the controlled degradation of PLA matrices, (ii) modifying the surface properties of ZnO particles, and (iii) engineering the surface properties of PLA-ZnO composites. These points are discussed in the sections below.

4.1. Biodegradation-controlled release in PLA/ZnO systems

The entrapment or encapsulation of ZnO NPs in a biodegradable polymer matrix offers two key advantages. Firstly, embedding the nanoparticle in a polymer matrix reduces the direct contact between NPs and cells, thus mitigating potential cytotoxic effects. Secondly, the tunable degradation rate of the polymer matrix enables the controlled release of ZnO NPs to meet specific therapeutic requirements [172–174]. For instance, ZnO NPs loaded into biodegradable sodium alginate/gum acacia nanohydrogels gradually released up to 68% of ZnO NPs over a 2-week period. The sustained delivery instead of the burst release of ZnO from the polymeric hydrogel matrices halved the cytotoxicity of bare ZnO NPs [172].

Biodegradable polymers have a significant role in many drug delivery systems as the polymer degradation rate can be tuned to provide therapeutic agents at a controlled rate [175]. While not specific to the controlled release of ZnO, numerous studies have identified PLA as one of the most attractive biodegradable polyesters for the development of drug delivery systems due to its excellent biocompatibility and controllable biodegradability [175].

The incorporation of ZnO NPs into PLA matrices adds complexity to the degradation mechanisms of PLA. This is because ZnO NPs are known for catalyzing the hydrolytic degradation of PLA [176]. The adsorbed water molecules on the surface of ZnO NPs partially dissociate to form hydroxyl groups [177]. As seen in Fig. 10, the hydroxyl groups attached on ZnO interact with the acid end groups of PLA chains. Ultimately, this promotes the hydrolytic degradation mechanisms of PLA [177].

Anžlovar et al. [178] described two reaction mechanisms for the participation of ZnO NPs in PLA degradation. The first one is the reaction between the carboxylic end groups of the hydrolytic degradation products of PLA and the hydroxyl groups on the surface of ZnO to form Zn salts [178]. The second one is through the direct interaction between the hydroxyl groups attached on ZnO NPs and the carbonyl groups of PLA, resulting in smaller PLA chains and the formation of Zn carboxylic salts [178]. In both cases, the residues of Zn salts enhance PLA degradation [178]. Usually, these degradation processes intensify under high heat [176]. However, some studies in the literature have also described similar catalytic effects at lower temperatures, sometimes even below the glass transition temperature of the polymer [177,179]. This pro-degradant effect of ZnO NPs on PLA should be considered when designing controlled-release systems based on PLA/ZnO composites, as ZnO may speed up PLA degradation, leading to lower PLA MW which can negatively impact the material properties [11].

Several methods have been developed to control the catalytic effect of ZnO on the degradation rate of PLA. One common approach is the surface treatment of ZnO. For instance, ZnO NPs coated with silane coupling agents have shown to delay the degradation of PLA [11,176]. Silanes contain hydrophobic groups that can modify the surface of ZnO from their hydrophilic nature to hydrophobic, restricting water diffusion into PLA matrices and thus mitigating hydrolysis [176]. Other than silanes, Restrepo et al. [180] showed the potential of PVA coatings in minimizing the degradation effects of ZnO NPs on PLA. This was linked to the

Fig. 10. Hydroxylation of ZnO NPs. Reproduced from Ref. [177] with permission from Copyright Clearance Center on behalf of John Wiley and Sons.

intramolecular entanglement between PLA and PVA, causing PVA to act as stabilizing agent for ZnO NPs and ultimately reducing their catalytic action on PLA [180]. Moreover, Murariu et al. [11] reported that PLA with a higher number-average molar mass (M_n) of 88.5 k as compared to 55.5 k possessed better thermal stability and thus had more resistance against the degradation induced by ZnO [11]. This phenomenon was confirmed in a subsequent study by Murariu et al. [181]. In this case, the incorporation of 1 wt % chain extenders (CE) into PLA/ZnO nanocomposites nearly doubled their MW, and significantly enhanced their thermal stability. This reduced the thermal degradation of the nanocomposites by at least 43% as compared to the nanocomposites without CE. In another study [182], the addition of silver phosphate (Ag_3PO_4) NPs into PLA/ZnO nanocomposites limited the catalytic role of ZnO in the hydrolytic degradation of PLA. It was proposed that the tendency of Ag_3PO_4 NPs to migrate onto the surface of the nanocomposites created a barrier effect [182]. As a result, hydrolysis was restricted due to the lack of water penetration into the polymer bulk [182]. In contrast to this, the addition of ZnO and TiO_2 nanofillers acted synergistically to accelerate the degradation of PLA [183].

The degradation of PLA matrices in PLA/ZnO nanocomposites leads to the release of ZnO NPs and Zn^{2+} [184,185]. Thus, the catalytic degradation effects of ZnO on PLA can be regulated to release ZnO or Zn^{2+} at a suitable rate for the desired therapeutic activities. As seen in Fig. 11, this biodegradation release system provides advantages such as prolonging the release of nanomaterials to provide sustained antibacterial properties in long-term medical treatments. Furthermore, it minimizes the potential cytotoxic effects of ZnO and Zn^{2+} by preventing excessive exposure due to burst release from the matrix.

Trujillo et al. [186] demonstrated that the hydrolytic degradation of PLLA/ZnO films soaked in PBS at 37 °C gradually exposed ZnO NPs on the material surface. However, the NPs were not displaced from the nanocomposite films within the experimental timescale of 15 days, with only negligible amount of Zn being released into the culture medium. The release of ZnO and Zn^{2+} from PLA/ZnO nanocomposites can be adjusted by varying several parameters. One of the parameters is the type of culture medium used. Lu et al. [185] showed that the release of ZnO from PLA/ZnO nanocomposite films was higher when incubated in 10% [v/v] ethanol instead of 10% [v/v] isooctane. The migration of Zn^{2+} was subsequently analyzed. It was found that the concentration of ZnO nanofillers, the degradation time, and the type of food simulants all influenced the release of Zn^{2+} into the culture medium. A study by Heydari-Majd et al. [184] also supported the effect of the type of culture medium on Zn^{2+} migration from PLA/ZnO nanocomposites. In addition, the effect of temperature was also noted, as the release of Zn^{2+} increased with the temperature of the culture medium. One common observation in these studies was the low tendency for Zn^{2+} to migrate from PLA [184, 185] and PLLA [186] into the culture medium. The concentration of released Zn^{2+} in these studies was determined to be non-cytotoxic [184–186]. It is generally understood that material biocompatibility is favored by minimal release of metal ions. However, this may fall short of the minimum concentration of metal ions required for effective therapeutic treatments.

Another notable factor that affects the dissolution of PLA, as well as the antibacterial efficacy of ZnO NPs, is the location of ZnO NPs, as the effect is different if NPs are located into or onto the PLA matrix. For example, ZnO NPs encapsulated entirely within rather than protruding from the surface of PLA usually possess weaker antibacterial activity over the first 24 h [115,123,187]. This phenomenon is related to the lack of immediate interaction between the encapsulated NPs and bacteria. However, the progressive degradation of PLA gradually releases or exposes the antibacterial agents, which leads to an increase in the antibacterial effect over time. For example, Pantani et al. [10] showed that the concentration of antimicrobial agents (ROS and Zn^{2+}) released from PLA/ZnO nanocomposites in the first 24 h was insufficient to inhibit the growth of *E. coli* and *S. aureus*. After 7 days, however, the nanocomposites successfully reduced over 99% of both the bacterial populations [115].

Aside from the antibacterial properties, PLA/ZnO systems having controllable degradation rate may also be useful to tailor the tissue engineering properties of the material. For instance, the rate of C2C12 myoblast cell differentiation and proliferation was shown to increase with the availability of ZnO on the surface of PLLA/ZnO nanocomposites upon PLLA degradation [186]. This shows that PLLA/ZnO systems can be engineered to tune the degradation of PLLA to expose ZnO NPs at the time scale required to promote cell growth in tissue engineering applications [186].

Therapeutic responses vary according to different cell types and require the risk/benefit analysis to be performed on a case-by-case basis. As a result, it is difficult to generalize the outcomes from a single study, and therefore more research in this area is still needed to assess the potential of controlled-release strategy in PLA-based/ZnO composites for biomedical applications.

4.2. Surface modification of ZnO particles

A targeted approach to reducing the cytotoxicity of ZnO consists of modifying the surface of ZnO particles through the introduction of appropriate functional groups. This can be achieved by several methods such as silanization, grafting with biocompatible polymers, and the adsorption of surfactants [188–191]. These methods have shown to selectively alter the surface properties of ZnO that directly affect the cytotoxic mechanisms of ZnO.

Punnoose et al. [188] showed that ZnO nanocrystals synthesized from the same precursor using a forced hydrolysis process, but with two different reaction solvents (namely, diethylene glycol (ZnO–I) and denatured ethanol (ZnO-II)), led to significant differences in the chemical structure of the NPs' surface. It was proposed that the bonding of hydroxyl groups was stronger in ZnO–I than in ZnO-II, and this produced a more positively charged surface/higher zeta potential. The highly positively charged ZnO–I, in turn, led to higher cytotoxic effects as compared to ZnO-II. This was caused by the increased attraction with negatively

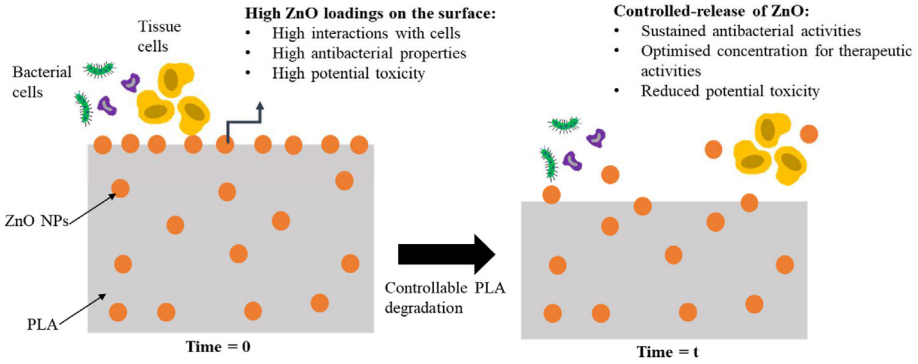

Fig. 11. Illustration of the biodegradation-controlled release PLA/ZnO systems.

charged cell membranes [188]. Furthermore, a higher zeta potential also contributed to a stronger catalytic activity of ZnO–I as compared to ZnO-II, which favored the production of ROS [188]. In this case, higher cytotoxicity was preferred to target Hut-78 cancer cells for more effective cancer treatments. This study demonstrates the potential of modifying the surface properties of ZnO to adjust their cytotoxicity to desirable levels, providing an approach to produce safer ZnO NPs. Furthermore, enhancing the preferential cytotoxicity of ZnO to cancer cells may indicate that less ZnO concentration is required to accomplish effective treatments, minimizing the risk of exerting cytotoxic side-effects on healthy cells. This has been demonstrated by an earlier study conducted by the same research group [192]. Thurber et al. [192] improved the selective cancer killing ability of ZnO NPs by doping them with iron (Fe) ions. The cytotoxic effects on Jurkat leukemic cancer cells and primary human immune cells depended on the Fe concentration. The cytotoxicity against cancerous cells increased with increasing concentration of Fe up to 7.5%, and then started to decrease above this concentration. It was hypothesized that low concentrations of Fe favor a fine and homogeneous dispersion of Fe ion active sites to act as a catalyst for ROS production [192]. Conversely, high concentrations of Fe may have saturated the surface of ZnO with Fe ions, thus limiting the formation of active sites [192]. The results of the research conducted by Thurber et al. [192] showed that cancerous cells treated with 24 μg/ml (0.3 mM) of ZnO doped with 2.5% Fe experienced a substantial reduction in cell viability. In contrast, the same treatment on non-cancerous cells had no detectable effect on cell viability, which remained comparable to that of untreated cells.

Chia et al. [193] demonstrated that silica coating changed the charge of ZnO from positive to negative. It was estimated that the silica coating, due to its innate acidic resistance, may limit the dissolution of ZnO into Zn^{2+} from 98% (bare ZnO) to 15% (coated ZnO) in acidic solution, resulting in lower cell cytotoxicity. Reasonably, this effect was only observed in acidic solution using synthetic gastric juice as the liquid medium, whilst the dissolution rates of coated and uncoated ZnO remained very similar in a neutral solution (synthetic saliva) [193]. These results suggest that the functionality of silica coatings cannot be generalized, since the dissolution rates of coated ZnO may be different in different biological media. More importantly, caution must be exercised when utilizing silica coatings for ZnO, as cytotoxicity was still observed when the dosage was 1250 μM.

Various contributions in the literature have reported that silane agents alone may be insufficient to reduce ZnO cytotoxicity [189,190,194]. For example, it was shown that 3-aminoppropyltriethoxysilane (APTES) treated- and untreated-ZnO exhibited similar cytotoxicity towards cells such as THP-1 immune cells [189], and cells from HeLa and NCI–H460 cell lines [194]. Another study [190] demonstrated that, in the short term, ZnO silanized with 3-glycidoxypropyl trimethoxysilane (GPTMS) had lower cytotoxic effects on L929 cells as compared to untreated ZnO. This was related to the presence of silane groups that are known to enhance the activity of fibroblast cells. Despite that, after 48 h, the survival fraction of cells treated with GPTMS-modified ZnO dropped by approximately 25–59%. This delayed cytotoxic outcome was overcome by the additional covalent bonding of GPTMS-modified ZnO NPS with chitosan. The presence of chitosan minimized the release of Zn^{2+}, and considerably enhanced the biocompatibility of the composite [190]. According to the study published by Esparza-González et al. [194], APTES- and dimethyl sulfoxide (DMSO)-silanized ZnO had comparable cytotoxicity to neat ZnO. However, the antibacterial activity of treated ZnO was higher than that of untreated ZnO. This was linked to the presence of Si–O and S–O bonds that increased the number of oxygen vacancies, leading to positively charged ZnO NPs with enhanced electrostatic interactions with bacterial cells [194]. This indicates that a lower concentration of treated ZnO NPs would be required to achieve the desired antibacterial functionality, which could potentially reduce the risk of developing cytotoxic effects due to overexposure.

Instead of silanization, Luo et al. [189] showed that PEGylation of ZnO (covalent attachment of PEG to ZnO) significantly reduced the cytotoxicity of ZnO, mainly by decreasing the cellular uptake of ZnO. The reduction in cellular uptake was linked to the hydrostatic repulsion between the hydrophilic PEG and the lipidic bilayer of the cell surface [189]. Moreover, PEGylation inhibited the protein binding ability of ZnO NPs, consequently preventing the proteins from binding on the surface of ZnO NPs to form protein-nanoparticle coronas, which may facilitate cellular uptake [189].

4.3. Surface engineering of PLA-ZnO composites

Other than modifying the surface of ZnO particles, another way for achieving strong antibacterial properties while minimizing the ZnO NPs concentration is to engineer the surface properties of PLA-ZnO composites. This is because surface properties such as surface topography, roughness, energy, charge, and wettability have a profound impact on antibacterial properties and tissue engineering functionality [195,196]. Hence, the surface properties of PLA-ZnO may be engineered to obtain the desired therapeutic properties while working at low nanofiller loadings. After a brief introduction to the effect of surface properties on antibacterial and tissue regenerative properties, this section presents and analytically discusses the literature specifically focused on surface engineering of PLA-ZnO composites for therapeutic purposes.

4.3.1. Effect of surface properties on antibacterial activity

SURFACE CHARGE: Surface charge affects the electrostatic interaction between the material surface and bacterial cells. Most bacteria possess a global negative charge. For gram positive bacteria, this stems from the teichoic acid, carboxyl, and amino groups found in the thick peptidoglycan layer [197]. As for gram-negative bacteria, this is due to the negatively charged LPS layer constituting the major glycolipid component of the bacterial outer membrane [198]. Since oppositely charged objects attract each other, positively charged surfaces typically possess stronger antibacterial activity [188], because the electrostatic attraction brings the bacterial cells close to the surface, and the stronger interaction promotes the antibacterial mechanisms. Interestingly, some studies revealed that negatively charged surfaces may also be able to interact with bacterial cells [199,200]. This arises from the ability of bacteria to subdue the electrostatic repulsion of like charges and bind tightly to negative surfaces through appendage-like fimbriae [195].

The effect of surface charge on biofilm inhibition remains disputable. Gottenbos et al. [201] observed that the initial adhesion of gram-positive and gram-negative bacteria was more rapid on positively charged (+12 mV) polymethacrylate surfaces than on the negatively charged (−12 mV, −18 mV) counterparts. The faster bacterial adhesion observed on the positively charged surfaces may promote quicker biofilm formation. However, in the long run, the strong electrostatic binding forces between positively charged surfaces and gram-negative bacteria impeded the subsequent growth of additional layers of gram-negative bacteria, which may aid in biofilm prevention. In contrast, both gram-negative and gram-positive bacteria continued to grow with time on negatively charged surfaces [201]. The study by Terada et al. [202] investigated the effect of surface charge on the initial adhesion of gram-negative bacteria and the subsequent biofilm formation. Positively and negatively charged polyethylene (PE) sheets were functionalized with diethylamine and sodium sulfite, respectively. The former, due to electrostatic attraction, strongly adhered to gram-negative bacteria and exhibited bactericidal properties [202]. Nonetheless, such bactericidal effects only lasted for a short period. Over time, the debris from damaged bacterial cells accumulated on the surface, providing ground for biofilm formation [202].

SURFACE TOPOGRAPHY: As illustrated in Fig. 12, surface topography, which is the arrangement of surface features such as valleys and peaks, may endow the material with antibacterial surface properties without altering its bulk properties. Generally, bacteria prefer rough surfaces to smooth ones, because surface protrusion protect bacteria from detachment when exposed to external shear forces [195,203,204].

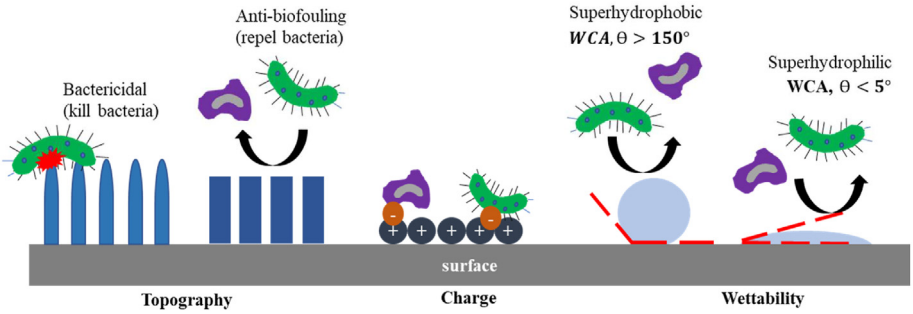

Fig. 12. Correlation between surface properties and antibacterial properties.

However, if topographical features are smaller than the cells themselves, bacteria will not be able to adhere [205].

Antibacterial surfaces can be classified into either anti-fouling (repel and prevent bacterial adhesion), bactericidal, or self-cleaning (the ability to self-clean so that bacteria do not remain attached) [206]. Such antibacterial surfaces can be commonly found in nature, thanks to the presence of micro/nano features in natural constructs. One of the best-known examples of a naturally occurring anti-fouling and self-cleaning surface is the lotus leaf. As seen in Fig. 13, the lotus leaf is covered with hierarchical nano- and micro-structures of papillae that are coated with a dense layer of wax tubules [207]. The small diameter and varying heights of the papillae minimize the contact area with water droplets and result in very low adhesion of water [207]. The additional epicuticular wax coating largely enhances the water repellency of the leaf [207]. The highly water repellent surface means that water droplets will roll off the surface of the leaf, carrying away contaminants so that any dirt particles cannot remain attached on the leaf, giving the surface its anti-fouling and self-cleaning properties. On the other hand, instead of repelling bacterial cells, insect wings such as cicada wings exhibit bactericidal capabilities [208,209]. This is due to the presence of sharp nano-scale pillars on the surface of the wings, which have been reported to penetrate the cells of *P. aeruginosa* and kill most of them within 5 min from first contact [209]. There are many other examples in the nature, i.e., butterfly and dragonfly wings, and gecko and shark skin, all of which are covered with micro/nanopatterns that present either anti-biofouling, bactericidal, or both antibacterial properties [208,210–213]. Inspired by nature, biomimetic strategies have been employed by numerous researchers to synthesize micro/nano-textured surfaces with effective antibacterial properties [214].

SURFACE WETTABILITY: Surface wettability can be measured through the water contact angle (WCA), which is the angle formed when a drop of liquid meets a solid surface. Angles above 90° indicate surface hydrophobicity, whereas angles below 90° indicate surface hydrophilicity [215]. Surfaces with extreme wettability, either super-low (superhydrophobic surfaces, WCA >150°) or super-high (superhydrophilic surfaces, WCA <5°), have excellent anti-biofouling and self-cleaning properties. For this reason, they have been extensively researched as surfaces to resist bacterial adhesion [216,217].

Lotus leaf is an example of superhydrophobic surface. As previously mentioned, water droplets cannot wet the surface of the lotus leaf and will simply roll off upon surface contact, picking up and carrying away dirt to enable the self-cleaning effect. In contrast, superhydrophilic surfaces make water droplets spread rapidly, forming a super-thin layer of water that diminishes the attraction of pollutants and establishes anti-fouling properties [218]. It is important to point out that surface wettability in air is deeply affected by the interplay between the surface energy of the solid, the surface tension of the liquid, and the solid-liquid interfacial tension. In the first instance, a high wetting surface has a strong surface energy that prevails on the surface tension of the liquid, and this causes the liquid droplets to spread out on the surface [219]. Nonetheless, surface energy by itself is usually insufficient to trigger extreme wetting behaviors and requires extra input from surface topography [220]. For example, the WCA measured on epitaxially grown *n*-perfluoroeicosane ($C_{20}F_{42}$) was around 119°, which is not yet indicative of superhydrophobicity, in spite of the presence of regularly aligned, closest hexagonally packed $–CF_3$ groups that are well-known for lowering the surface energy [221]. However, lotus leaves that do not have low surface energy groups like $–CF_3$ groups possess hierarchal microstructures that provide their surface with superhydrophobic characteristics. This clearly demonstrates the important effects of surface topography on the wettability of the material.

Ultimately, surface charge, topography, and wettability all act interdependently to define the antibacterial properties of the material.

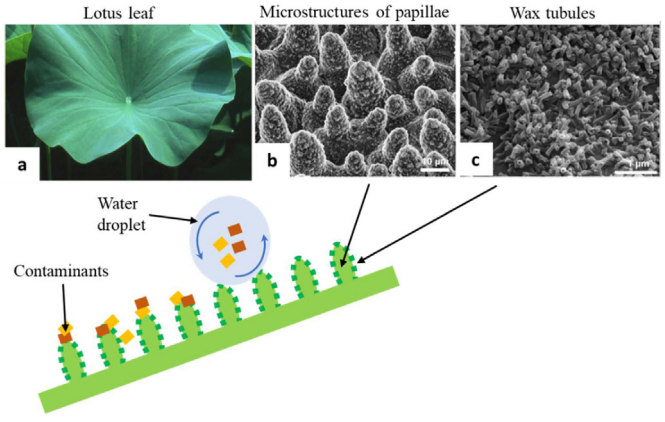

Fig. 13. (a) Lotus leaf. SEM images of the upper leaf side showing (b) the microstructures of papillae and (c) wax tubules on the microstructures. Adapted from Ref. [207] under the terms and conditions of Creative Common Attribution (CC BY) license.

4.3.2. *Effect of surface properties on tissue regenerative properties*

In terms of tissue regenerative properties, surface features such as surface charge [222], topography [223], and wettability [224] of tissue engineering scaffolds largely affect their interaction with cells. For example, surface charge affects the amount, type, and refolding degree of absorbed proteins, and therefore influences cell adhesion processes [222]. It is known that positively charged surfaces significantly improve cell proliferation and spreading especially at the initial stage of cell response [222].

Topographical features, including surface roughness, patterns, and porosities, are important physical properties of tissue-engineered scaffolds to provide contact guidance cues to improve cellular proliferation, migration, and differentiation. Much effort has been dedicated to design tissue engineering constructs with controlled topographical features such as the micro/nano patterns and porosities that mimic the ECM [225,226]. For example, microgrooves or grating patterns can assist in tissue regeneration processes in which the alignment and orientation of cells are essential [227].

The effect of surface wettability on the viability of tissue engineering scaffolds has also been widely explored in the literature [228]. Generally, cell adhesion is promoted by hydrophilic surfaces, as mammalian cells favorably attach to hydrophilic surfaces that enable the reorganization of fibronectin, which is crucial for the interaction between cells and adhered surfaces [229]. Moreover, hydrophilic surfaces have also been associated with enhanced cell spreading, proliferation, and differentiation [230].

4.3.3. *Effect of surface properties of PLA/ZnO on their therapeutic responses*

Currently, there are few contributions in the literature that specifically target the effect of surface properties on the antibacterial efficacy of PLA/ZnO composites, and even less is known regarding the consequences of surface properties on tissue engineering capabilities. To date, to the best of the Authors' knowledge, only two papers in the literature explicitly discuss the effect of surface properties of PLA/ZnO composites on their antibacterial properties [231,232].

The first study [231] employed a biomimetic approach to design ZnO nano-spicules on the surface of PLA in order to reproduce the protective function of sea urchin spicules (Fig. 14). The fabrication started with electrospinning and electrospraying to produce PLA microbeads and microfibers encapsulating ZnO nanoparticle seeds. Hydrothermal treatment was then used to stimulate the migration of ZnO NPs to the polymer surface, followed by their growth into ZnO nano-spicules in the form of hexagonal pillars.

ZnO nano-spicules converted PLA from its hydrophobic to a superhydrophillic surface having a WCA of less than 3°. This was because water molecules could easily penetrate through the nano-spicules and spread rapidly across the surface. Subsequently, the antibacterial efficacy of ZnO nano-spicules was compared against ZnO crystallites. The outcome proved that the former exhibited higher antibacterial properties, due to the dual antibacterial actions exerted by the nano-spicules, namely the production of ROS, and the physical stabbing of bacteria enabled by the sharp morphology of the nano-spicules. The superhydrophilic surface boosted the attraction of bacterial cells towards the sharp nano-spicules and promoted physical stabbing [231]. Interestingly, the structural differences between gram-positive and gram-negative bacteria led to different sensitivity towards the two antibacterial mechanisms. *S. aureus,* which has thicker peptidoglycan layers than *E. coli*, was better in resisting the physical rupturing induced by the nano-spicules [231]. On the other hand, *E. coli*, which has two distinct membranes, displayed higher resistance to ROS-driven cell membrane disruption compared to the single membrane system of *S. aureus* [231]. Noteworthy, cell viability tests were also conducted using human breast epithelial cells. Even though cell survival was confirmed, the cells did not spread lamellipodia. The spreading of lamellipodia contributes to cell movement, which is crucial for the migration and proliferation of cells during tissue regeneration [233]. Hence, the results obtained cannot confirm the tissue engineering viability of the PLA-ZnO spicules construct.

In the second study [232], composite films of PLA/Ag, PLA/ZnO, and PLA/TiO_2 were fabricated through melt-mixing of PLA with different loadings (0.5, 1.0, and 2.5 wt%) of NPs, followed by the extrusion of thin films (280–320 μm). The concentration, surface structure, morphology, size, and distribution of the NPs in the PLA matrix influenced the surface properties of the films. The general trend was that the nanofillers enhanced the roughness, surface energy, and wettability of the nanocomposites as compared to plain PLA films. The changes in surface properties had an impact on the final antibacterial properties of the films. For instance, the antibacterial activity against *E. coli* and *S. aureus* increased with the surface roughness of PLA/ZnO and PLA/TiO_2 films, due to the larger surface area and the increased hydrophilicity that provided higher bacterial inhibitory effects [232]. Notably, the enhancement in surface roughness and the subsequent improvement in antibacterial properties were not linear to the nanofiller loading. This was related to the preferential distribution of NPs into the inner layer of PLA that occurred with increasing filler loading (Fig. 15) [232]. As a result, higher surface roughness, and hence, higher antibacterial properties were obtained in films with relatively low nanofiller loadings. This demonstrated that the antibacterial efficacy of the material does not necessarily depend on the nominal concentration of the nanofiller. Rather, it can be affected by the nanofiller concentration close to the surface, which is responsible for the changes in surface physical, chemical, and morphological properties [232].

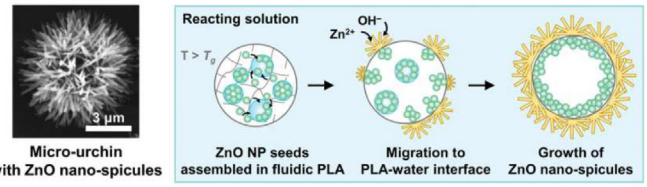

Fig. 14. ZnO nano-spicules mimicking sea urchin spicules and their fabrication process. Adapted from Ref. [231] with permission from Copyright Clearance Center on behalf of John Wiley and Sons.

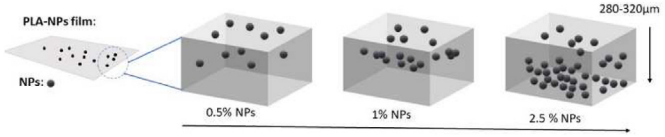

Fig. 15. Preferential distribution of NPs into the core of the polymer film with increasing filler loading. Reproduced from Ref. [232] under the terms and conditions of Creative Common Attribution (CC BY) license.

Evidently, the surface of PLA/ZnO composites play an important role in determining the antibacterial properties and possibly, the tissue engineering properties. Nonetheless, the effect of ZnO NPs on the surface properties and the subsequent influence on the therapeutic properties of PLA/ZnO composites have rarely been analyzed. More research is certainly needed to understand how the antibacterial and tissue engineering properties of PLA/ZnO composites can be improved through their surface properties.

5. Summary

The tailorable physicochemical properties and biodegradability of PLA, together with the multi-therapeutic potential of ZnO provide a versatile material platform for a variety of biomedical applications. Many researchers have explored the potential use of composites such as tissue engineering scaffolds, functional coatings, and wound dressings for antibacterial and tissue regenerative applications. Despite this, issues regarding the potential health hazards of ZnO NPs cannot be ignored. It is of utmost importance to maintain a balance between the medicinal properties and the cytotoxicity of ZnO NPs in the composite material. However, there are several challenges involved, with one fundamental issue being the antagonistic effects of ZnO concentration, since oftentimes increasing the filler loading simulateously enhances the therapeutic properties and worsen the cytotoxicity of the composite material. While the conventional approach to mitigating the cytotoxic effect of ZnO consists in modifying the surface properties of ZnO nanofillers, for example through silanization, new avenues to maximizing the therapeutic efficacy while reducing the filler loading are gradually being explored. A promising method is to exploit the biodegradability of PLA to control the release of ZnO for the delivery of safe and effective ZnO dosages required for specific treatments. Finally, the surface properties of the nanocomposites can be engineered to enhance their therapeutic effects and provide antibacterial properties without relying solely on increasing the concentration of ZnO.

Declaration of competing interest

Given Prof. Cuie Wen role as Editor-in-Chief of this journal, she was not involved in the editorial review or the decision to publish this article and had no access to information regarding its review.

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