Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [10] September 2021 : 01-22

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

REVIEW ARTICLE **OPEN ACCESS**

A Review on Diabetic Wound Infection and Its Treatment Using Zinc Oxide Nanoparticle Synthesized by Green Chemistry Techniques

M.Balalakshitha[1], K.Kolanjinathan[2*] ,T.Aishwarya[3] and B.Vinothini[4]
[1, 3&4]Ph.D., Research Scholar, Department of Microbiology, Faculty of science, Annamalai university
[2]Assistant professor, Department of Microbiology, Faculty of science, Annamalai University
*Corresponding Author E-mail: drkolanji@gmail.com

ABSTRACT
The review study emphasizes on delayed wound healing nature of diabetic wound infections and its treatment using novel metal oxide nanoparticles synthesized by green chemistry techniques. Diabetes is a physiological disorder of insulin deficiency leading to loss of uptake of glucose from blood leading to accumulation of glucose in blood. During this brief period of illness, any kind of external wound or an internal wound as a result of bacterial infection due to increased glucose in blood can cause serious complications such as diabetic foot ulcers including other diabetic wound infections with delayed wound healing process. The treatment options for this severe condition are very limited and usually leads to the amputation of the affected site especially vital organs including upper and lower limbs. Thus, there is a need for competent treatment for diabetic wound infection and delayed wound healing. Nanobiotechnology is one of the fields which shows promising therapy for diabetic wound infections. Various metals, polymers, etc. are all assessed for antimicrobial and accelerated wound healing ability and zinc is one of such options which shows promising results as it is normally associated with wound healing and is a cofactor for more than 300 enzymes. Nano crystals of zinc are oxidized and are regenerated as Anti-microbial zinc oxide nanoparticles. Novel techniques involving zinc oxide nanoparticle synthesis which are preferred from biological sources are known as green chemistry. Green synthesis methods are regarded as less hazardous, inexpensive and eco-friendly. Various variants of nanoparticles of zinc oxide along with their wound healing and antibacterial properties were analysed with a special reference towards zinc nanoparticles conjugated with naturally occurring polymers. With an in-depth analysis of the pathophysiological conditions of the disease along with its current golden standard market treatment including novel nanomaterial-based approaches, it was evidently proven that zinc oxide nanoparticle has a greater scope in diabetic wound infections and delayed wound healing.
Keywords: *Diabetic wound infections, diabetic foot ulcers, diabetic wound healing, zinc oxide nanoparticles, green synthesis, antibacterial nanoparticles.*

Received 29.08.2021 Revised 06.09.2021 Accepted 28.09.2021

INTRODUCTION

Wounds are known as the damage which affects the skin integrity, usually as a result of traumatic injury [1]. Skin wounds are usually regenerative and they anatomically and physiologically repair their own cells [2]. This natural healing process of the wounds is a result of various physiological chain and is influenced by various extrinsic and intrinsic factors and there are chances of disruption in these factors resulting in an inefficient healing process [3]. In cases of severe wound damage or limited regeneration of skin cells, skin grafting methods are a choice of treatment [4]. Diabetes is a different pathophysiological condition rather than an injury or a traumatic condition of the human body which can result in wounds or ulcerations, especially the foot region. It is a result of neuropathic condition arising due to hyperglycemia [5]. According to the World Health Organization, India had 31 million diabetic cases as of 2000 which is projected to be around 79 million by 2030 [6]. The Diabetes Country Profiling of India by WHO as on 2016 shows more than 1 lakh deaths as a result of diabetes between the age group of 30 to 69 and more than 90 thousand deaths of age group above 70. The data also reveals that 2% of deaths in the country is due to diabetes [7]. Thus, a necessity of developing a viable and cost-effective treatment for diabetic wounds has emerged. In the recent decade, there has been rising interest in the field of nanobiotechnology especially due to their biomedical application. As a result, various nanoparticles have been developed and assessed for antimicrobial properties and wound healing processes [8,9]. Of all the nanoparticles which have been developed, zinc oxide nanoparticle has been determined to be a promising

nanotherapeutic agent for diabetic wound infections and its associated wound healing. This is made possible as the zinc element is associated with normal wound healing and its function in more than 300 enzymes [10]. Similarly, nanoparticle synthesis by means of green sources or biological sources have been developed and is considered safer, less-toxic and cheap when compared to that of conventional nanoparticle synthesis methods [11]. Thus, the present study reviews on diabetic wound infection and its associated complications including delayed wound healing along with the role of zinc oxide nanoparticles (ZnO NPs) in diabetic wound infections and wound healing along with the significance of green synthesis of ZnO NPs.

DIABETES: DEFINITION, CLASSIFICATION AND PATHOGENESIS

Diabetes mellitus or shortly known as DM is described by an increased in the levels of blood glucose known as hyperglycaemia. This is due to an idiopathic autoimmune attack resulting in the destruction of βcells of the pancreas and thereby resulting in insulin deficiency [12, 13]. These β cells of pancreas controls the levels of blood glucose in the body and whose destruction is termed as diabetes mellitus [14]. Carbohydrate metabolism and the resulting insulin effect are the basis of pathophysiology of DM. Consumption of carbohydrate compounds by the human body are broken down as smaller or lower molecules such as glucose. The resulting glucose molecule is responsible for energy production inside the cells; thus, it enters the bloodstream to increase glucose level in the blood known as glycaemia. Glycaemia results in secretion of pancreatic βcells and which binds to cellular receptors and to aid in the entry of glucose molecules into the cells for energy production and also result in lowering the glucose levels in the blood. This physiological function of glucose-cell interaction for energy production is disturbed or even disrupted completely if there is no secretion of pancreatic βcells and the glucose levels in the blood does not lower but also increases resulting in hyperglycaemia. Another case of hyperglycaemia is also seen as a result of target cells fails in utilizing the pancreatic βcell or the insulin. Diabetes mellitus is classified into two types., Type 1 DM and Type 2 DM. The Type 1 diabetes mellitus is an insulin-dependent variant where the autoimmune cells destroy the cells of the insulin and thus there is a dependency on insulin for survival. Type 2 DM is the most commonly seen diabetes which is caused by resistance towards insulin in the peripheral tissues, increased glucose production in the liver and defect in the secretion of β cells of the pancreas or insulin. It is also known as insulin independent where the glucose molecules fail to enter the target cells and results in accumulated glucose in the blood. This can lead to increased insulin production known as hyperinsulinemia.

DIABETIC WOUNDS: AN INTRODUCTION

Patients diagnosed with diabetes mellitus are also characterized by other co-morbid conditions such as renal failure, cardio-vascular disease and skin wounds [15]. Skin wounds including ulcerations as a result of diabetic wounds are a common problem and is usually present in the lower extremities of the body such as the foot region. One such prevalent diabetic wound is the ulceration of the foot region known as diabetic foot ulcer (DFU). DFU is an important case in diabetic patients as most patients reach hospitals for DFU [16]. DFU can either be developed by direct or indirect trauma. Once the development of DFU, the wound will progress to a damaged condition along with the loss of sensation and result in peripheral arterial disease and can onset cell necrosis and finally result in amputation of the infected foot [17]. Nearly 80% of all lower limb amputations are a result of diabetic foot ulcer [18]. However, studies by the Action to Control Cardiovascular Risk in Diabetes also known as ACCORD revealed that the amputations in the lower limb as a result of DFU can be reduced if the patients maintain normal blood glucose level or when receive antidiabetic therapy [19]. In case of diabetic wounds, there are various factors including peripheral arterial disease (PAD), peripheral neuropathy, immunosuppression and loss of glycaemic control are responsible for wound development. However, neuropathy is the most important factor leading to diabetic wounds [20]. Loss of glycaemic control is the next factor which can cause vascular disease in DM patients as a result of dysfunction of the endothelial cells and increased thromboxane A2 [20]. Another important cause of diabetic wounds leading to limp amputation is the disruption in the wound healing process. DM patients develops imbalanced angiogenesis as a result of imbalance between angiogenic factors such as TGG-β, FGF2, etc and angiostatic factors such as endostatins, thrombospondins, etc. This can result in the delay of wound healing process [21]. In case of wound healing, diabetic wounds neither enter into proliferation nor remodelling phases but stays in inflammatory phase. This can affect the synthesis of growth factors and immune cells such as microcirculatory cells and causes lack of availability of energy at the wound site [22]. Thus, delayed wound healing, decreased growth factors and disrupted levels of microcirculatory cells can cause chronic ulcer [23]. All these lack of growth factors, essential peptides and microcirculatory cells can cause further infection of the foot leading to amputation of the affected limb [24].

PATHOPHYSIOLOGY OF DIABETIC FOOT ULCER (DFU)

As stated earlier, several criteria such as PAD, neuropathy, ischemia and diabetic wound infections of microbial origin are responsible for the pathophysiology of DFU [25]. Trauma to the foot in the presence of sensory neuropathy is an important component cause of ulceration [26].

a. Neuropathy

Increased blood glucose levels or hyperglycaemia results in neuropathy causing diabetic foot ulcers [27]. During the hyperglycaemic conditions, enzymes such as aldose reductase and sorbitol dehydrogenase are increasingly stimulated which converts intracellular glucose into sorbitol and fructose. These by-products of glucose accumulate in the blood and results in decreased nerve cell myoinositol synthesis [28]. This accumulation also results in the reduction of nicotinamide adenine dinucleotide phosphate (NADP). NADPs plays an important role in detoxification of reactive oxygen species (ROS) which is essential for nitric oxide (NO) synthesis, a vasodilator. Thus, there is reduced synthesis of the NO vasodilator causing vasoconstriction which leads to ischemia and the nerve cell oxidative stress is also increased [29, 30]. All these physiological changes affect the sensory, autonomic and motor nervous systems. The drying of foot is stimulated as a result of autonomic neuropathy leading to lack of moisturization of the foot surface which encourages spreading of infections.

b. Peripheral Arterial Disease (PAD)

Diabetic foot ulceration can be caused by PAD, but various factors other than diabetes mellitus such as smoking, renal dysfunction, age, hyperlipidaemia, hypertension and inflammatory markers are all associated with peripheral arterial disease [31]. PAD caused by diabetes is of two types., macrovascular disease and microvascular disease. As a result of reduction in vasodilators, dysfunction and abnormalities of the endothelial cells in smooth muscles are developed. This leads to the constriction of the blood arteries present in the foot [32]. Thickening of blood capillaries and arteriolar wall thickening aided by atherosclerosis will cause major constrictions in several major arteries of the foot which results in ischemia [33].

DIABETIC WOUND INFECTIONS

Infections as a result of microbial sources are an important element to consider in diabetic wounds and is crucial and challenging to manage and treat [34]. The normal microflora of the skin consists of various bacterial species; however, 105 bacterial species were identified to be clinically infectious [35]. Damages caused by either direct or indirect trauma in the skin encourages surface bacteria to infest into underlying tissue. As a result of this microbial penetration, the inflammatory cells cause inflammation by releasing protease enzymes and reactive oxygen species to the infection site [36, 37]. The infested bacterial secretes endotoxins and an increased level of these endotoxins are release at the site which increases the proinflammatory cytokines as an immune response. This immune response to the bacterial toxin reduces collagen deposition along with decrease in growth factor production which plays a crucial role in wound healing. Thus, wound healing is delayed [38]. Later, the bacterial contamination of the damaged skin progresses to bacterial colonization the production of biofilms [39]. A bacterial biofilm is the encapsulation of a consortium or a group of bacteria in a polymeric substance. This polymeric substance known as extracellular polymeric substance (EPS) is synthesized by the same bacteria using protein, polysaccharide and DNA [40]. 60% of chronic wound biopsy specimens are identified with biofilms, whereas 6% of acute wound biopsy specimens identified biofilms [36]. The bacterial biofilms represent greater clinical infectious stated sue to its resistance towards antimicrobial therapies and acts as a barrier for wound healing [41]. Certain bacterial biofilms show multiple tolerance to antimicrobials including genotypic and phenotypic antimicrobial resistance [42]. Some bacteria such as *Pseudomonas aeruginosa* shows multiple antimicrobial resistance mechanisms such as efflux system expression, decreased permeability, target modification and antibiotic inactivation enzyme production [43]. Other bacteria including vancomycin-resistant enterococcal bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae* [44]. The drug resistance by these bacteria are developed as a result of inappropriate use of antibiotics and antimicrobial therapy [44]. Bacterial biofilm-based infections are not susceptible to antimicrobial and antibiotic therapies; thus, a development of more sophisticated and well-developed anti-biofilm therapy is crucial [45]. Diabetic chronic wounds characterized by bacterial biofilms delays wound healing and also causes high inflammation as a result of increased free radicals and prolonged stimulation of inflammatory cytokines and nitric oxides [39].

The bacteriological profiling of diabetic wounds and foot ulcers identified that the most prominent infectious agent to be Gram-positive *Staphylococcus aureus*. Other Gram-positive bacteria include β-haemolytic *Streptococci*. The Gram-negative bacterial species include *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella* [46]. A normal human skin microflora Gram-positive Staphylococcus epidermidis will convert itself to an infectious agent of diabetic wound when exposed to the systemic

circulation of the wound site [47]. All these bacteria are treated by conventional antibiotic and antimicrobial therapy, however as stated earlier, the treatment of bacterial infections using antibiotics can cause antibiotic resistance or they encapsulate themselves using extracellular polymeric substances (EPS) to form biofilms which provides optimum environment for bacterial cell proliferation and survival [48]. Studies on biofilms shows that they are surface associated and the consortium of bacteria communicates within the biofilm via quorum sensing (QS). These quorum sensing signals regulates protease production, gene expression and other necessary signals necessary for the bacteria present in the biofilm to survive [49]. Due to the bacterial biofilm and their sophisticated mechanism, high levels of antibiotic and antimicrobial tolerance can be observed.

WOUND HEALING MECHANISM

Whenever there is an injury at the skin, a complex mechanism is initiated for the forced wound healing. This healing results in restoration of the damaged tissue at the wound site. This healing is a cascade of events which occurs in a sequential order [50, 51]. The steps involved in wound healing are as follows., haemostasis, inflammation, proliferation and remodelling. During haemostasis, vasoconstriction happens by thrombin which activates platelet aggregation. This results in production of various growth factors which helps in fibroblast migration and proliferation. Thus, formation of thrombus is seen at the wound site [52]. The mast cells release histamine and serotonin which increase vascular permeability at wound site. This opens up the endothelial junction and results in neutrophil and monocyte migration towards the wound site and promotes inflammation [23]. Removal of dead cell and infecting microorganisms at the injured site is aided by macrophage and lymphocyte migration [53]. Tissue proliferation is promoted by tissue reactions as a result of release of various cytokines and growth factors which are induced by tissue debris [54, 55]. After 48 hours of tissue damage, newly synthesized epithelial tissue is formed at the damage site as a result of wound healing process which is initiated by macrophages. A rapid growth phase was proceeded by both collagen and the endothelial cells. As a result of this, angiogenesis is initiated. Then, maturation of the tissue at the wound site is seen which undergoes remodelling and results in scar formation. Oxygen supply is very important throughout the healing process [23, 35]. At normal conditions, acute wounds in healthy individuals heals within 2 – 3 weeks. But, any change or external internal interaction in the healing process can delay the process and the wound can progress into a chronic wound [23]. Diabetes is one such factor which affects the healing process leading to delayed healing.

Interaction of Diabetes in Wound Healing Mechanism

When compared to that of normal wound healing, the macrophages responsible for the inflammation phase of the wound healing stays for a longer duration at the site of the wound and produces increased ratio of pro-inflammatory cytokines including tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) to reactive oxygen species (ROS). This results in continued inflammation at the site of wound. In case of wound healing, the general cytokine cascade is disturbed during diabetes due to poor phagocytosis of apoptotic cells or efferocytosis by the macrophages due to increased apoptotic cells. An increase in the ratio of proinflammatory cytokines such as TNF-α and interleukin 1 beta (IL-1β) with that of matrix metalloproteinase 9 (MMP-9) when assisted with a decreased in anti-inflammatory signals such as TGF-β, CD206, IGF-1 and IL-10 results in decreased angiogenesis and abnormal apoptosis of keratinocytes and fibroblasts [56, 58]. Diabetes also interrupts the differentiation fibroblasts into myofibroblasts during wound healing process. This causes the mechanical tension of extracellular matrix (ECM) to be significantly reduced and resulting in improper wound closure as alpha smooth muscle actin (α-SMA) is absent [58, 60]. The matrix metalloproteinase (MMP) enzymes present during the normal wound healing degrades disorganized or disarranged collagen. The impaired wound healing due to diabetes will cause an imbalance between tissue inhibitor of metalloproteinases (TIMPs) and MMPs and leads to improper ECM degradation as well as deposition. The imbalance between TIMPs and MMPs are seen as reduced TIMPs expression and increased MMPs expression resulting in increased levels of pro-inflammatory cytokines and pro-fibrotic cytokines. Comparative to acute wounds, chronic wounds has 60 times higher levels of MMPs [60]. Persistent inflammation due to diabetic wounds causes increased protease activity helps in ECM and growth factor degradation and also results in collagen deposition [61, 62]. Thus, all the above-mentioned factors along with an incompatible microenvironment for the cellular and molecular healing of wounds causes impaired wound healing in diabetic wounds [63].

DIABETIC WOUND HEALING AND ITS ASSOCIATED COMPLICATIONS

Diabetes mellitus patients are present with various factors which affects the wound healing process. Wounds characterized by either diabetes or any other sources are affected by DM. This shows us that

both wounds as a result of any external injury or any surgical operations and diabetic wounds or diabetic foot ulcers are affected by the presence of DM [64].

a. High Blood Sugar Levels

As the mechanism is stated earlier, increased blood glucose levels result in decreased wound healing process as the elevated glucose levels affects proper functioning of immune system and prevents energizing cells with oxygen and other nutrients and causes prolonged inflammation [64].

b. Neuropathy

Nervous systems are drastically affected by diabetes. Sensory nervous system involving the ability to sense or feel any sensation over the skin as well as internal organs of the lower extremities such as limbs, foot skins, etc are lost and complicates treatment as there is no sensation of pain. Any kind of cuts, blister, burns etc are heavily infected [64].

c. Poor Blood Circulation

Poor blood circulation as a result of accumulated glucose in diabetic patients causes peripheral vascular diseases. As a result, the vascular system is constricted and results in much more lower blood circulation. The constriction of blood flow makes the blood difficult to circulate at the lower extremities and results in the inhibition of angiogenesis and causes thickening of blood and results in delayed wound healing [65].

d. Immune System Deficiency

The immune system is very well affected by diabetes as the immune cells sent for the healing process at the site of the wound reduced gradually and promotes delayed wound healing and increases the risk of further infections. This also prevents or obstructs the immune cells to fight off any future microbial infections which accumulated at the wound site due to the increased amount of glucose present in the blood which promotes the growth of bacteria. Any type of infection in a DM patient if left untreated can cause severe infection spreading locally and can cause sepsis or gangrene [64].

CURRENT DIABETIC WOUND THERAPY

As conventional wound therapy, wound dressings are applied as immediate wound therapy to provide a protective wound barrier at the wound site. However, to aid in diabetic wound, an ideal dressing must possess characteristics such as semi-permeability to oxygen and water, biocompatible, hypoallergenic, must promote tissue renewal process and also have to be affordable by the patients [37]. Dressing such as semi-permeable polyurethane, hydrocolloid and calcium alginate are to provide moist environment and promote tissue renewal [66]. But to manage ulcerated or heavily infected wounds, specialized dressing with antibacterial or antibiotic embeddings are used [37]. Tetracyclines, quinolines, cephalosporins and aminoglycosides are usually used as wound dressing embeddings. Due to the development of antibiotic or antibacterial resistance, topical and oral treatments are not recommended. Several non-antibiotic antimicrobial topical medications are sometimes preferred due to its ease of application [67-69]. Traditional non-antibiotic antimicrobial agents such as honey and essential oils are used along with wound dressings preferably [70]. Treatment of multidrug resistance bacteria is done using essential oils as bacteria cannot develop antimicrobial resistance towards these essential oils [71]. Wound dressings based on hydrogels are also used as they allow diffusion of gases and maintains moist conditions. Hydrogel-based dressings also helps in avoiding any dressing-based infections as they absorb wound exudates. Other dressing options are met with the usage of synthetic polymers including polyvinyl alcohol and also natural polymers including as alginate, chitin and chitosan where chitosan already possess antimicrobial properties [72]. Another class of dressings used in diabetic wound therapy is silver products including silver nitrate and silver sulfadiazine as they release silver ions which possess potent antibacterial property. They are also effective against bacterial biofilms and multidrug resistant bacteria. Studies reveals that a growth factor derived from platelet which is a recombinant human homodimer PDGF-BB of 100 g/g of 0.01% becaplermin gel as a topical treatment for diabetic foot ulcers as they induce granulation with zero to minor side-effects [66]. Vacuum-assisted closure (VAC) therapy is also a type of wound therapy used for diabetic wounds by establishing suction by negative pressure. This therapy also reduces edema of the tissue and micromechanical stretching force is applied to individual cells so as to promote angiogenesis and proliferation. This therapy can also aid in the granulation of tissues after surgical removal of severe wound debris [66]. Another therapy in diabetic wound is the treatment using bioengineered substitutes of skin to restore dermal architecture of skin. Some of the bioengineered skin substitutes present in the market are Integra © and Alloderm©. Integra© consists of double layered bovine collage which is rich in chondroitin sulphate with a silicon sheet and replaces skin graft. Alloderm© is a skin graft of cadaveric human skin which is allogenic. Another wound therapy option is the use of transplanted cells which are embedded into the dermal matrices which can result in accelerated healing. Apligraf© and Dermagraft-TC© are some transplanted cell-based wound therapy

found in the market. Apligraf© uses neonatal keratinocytes along with bovine type 1 collagen as dermal matrix. Dermagraft-TC© employs the use of a nylon mesh which is integrated with fibroblast cells.

DIABETIC WOUND THERAPY COMPLICATIONS

Bacterial biofilms are the most challenging factor in wound healing therapy and has greater significance in treatment evaluation of wound therapy [73]. Research study on bacteria pathogens of the infected wounds and their drug resistance revealed that samples from 213 patients comprising of diverse wounds showed 28 distinct species of bacteria [74]. Infections on surgical wounds, chronic wounds, burns and ulcerations causes delayed healing and are known to fail at times [75].

First, the inefficiency of various traditional antibiotics to treat biofilms are attributed to different factors. Poor penetration of antibiotics into biofilms, metabolic inactivation due to lack of nutrients and gases and adaptive stress response are some major criteria providing resistance towards antibiotics [76]. Biofilm surfaces are identified with charged pockets [77]. Positively charged antibiotics fail to penetrate negatively charges biofilms [78]. If at all an antibiotic is to enter a biofilm, then the biofilm should diffuse through the matrix of the biofilm to arrive at the bacterial cells to proceed with the antimicrobial processes. Antibiotic compounds such as beta-lactams and aminoglycosides will be inactivated by being dissolved by the solutes of the matrix which makes it harder for the penetration of the antibiotic to the depth of the biofilm and the process is known as mass transporter limitation [79, 80]. The same process takes place when gases and nutrients are transferred to the biofilm, thus denoting that the nutrients and gases fails to reach the bottom of the biofilms and fails to grow and divide exponentially unlike the bacteria present at the top surface. However, growing bacterial cells and cells in stationary phase are untouched by the antibiotics as they do not work on metabolically inactive bacteria [81]. These bacteria present at the bottom stimulates their own stress response mechanisms and switch metabolic pathways and remains as persistent bacteria rather than growing bacteria [76]. Phenotypic changes are also seen in some bacteria to survive longer period of time and also by resisting the antibiotics. Thus, these persistent bacterial cells are known as persisters [82]. Comparative to free growing bacteria, Staphylococcus aureus biofilms have increased number of persisters [83]. The limitations of gases and nutrients in the biofilms, promotes the transformation of regular bacterial cells into persister cells. These cells also sense the environmental changes from regular bacterial cells through quorum sensing (QS). This QS signalling helps cells to coordinate expression if genes and nucleotide signalling in order to collectively survive in the biofilms [84]. QS signalling also expresses virulence factors only upon reaching high cell density and as a result this ensures that the host immune cells do not supress the virulence property of the bacteria. Polymicrobial biofilms are known to change phenotypic characters with the help QS signals which makes biofilms more difficult to treat [85]. All these complexities of the wound infections in diabetes mellitus patients made it challenging to be treated. Still, the aforementioned treatments are still in use today for diabetic wound therapy. However, there is still a search for a proper treatment methodology to overcome every aspect of limitations posed by these infections which is also cost-effective.

Scope of Nanotechnology

Recent times have witnessed some progress in developing an anti-biofilm agent but there are several limitations including its complexity and required capital. This resulted in the search of novel bioresources [37]. A promising approach is provided by nanotechnology in development of treatment of drug resistant biofilms as medicals devices with this technology have been previously developed [86, 87]. Nanoparticles are characterized by their bactericidal and fungicidal properties and is used in various therapeutic approaches in wound care which denotes the importance of nanomedicine for developing novel antimicrobial agents for drug resistant bacteria [86, 88].

Nanotechnology has revolutionized conventional therapies of various pathogenic infections. Nanoparticles derived from polysaccharides or polymers from plant source, synthetic sources, non-metals and metals have been used in treating pathogenic infections when used with biologically active components [89-92].

Nanotechnology advancements have helped in developing nanomaterials which are biocompatible and using as an innovative treatment for diabetic patients suffering from complications of wound healing. The synthesized nanomaterials have been useful in speeding the process of wound healing and acts as diabetic wound healing therapy which includes both wounds and ulcerations especially diabetic foot ulcers. The treatment by nanoparticles can be achieved by various aspects such as using the pure form of synthesized nanoparticles, endogenous bearing molecules, conjugated bioactive compounds, drug delivery systems, etc [8, 9]. Nanoparticles based on metals for instance copper, silver, gold and their oxides along with cerium dioxide, titanium dioxide, yttrium trioxide and zinc oxide have been very well characterized with their antimicrobial properties and can be used in diabetic wound healing [93, 94]. Zeta

potential estimates the particle surface charge which influences receptor binding capacity as well as the penetrability of cellular barriers [95].

Nanoparticle-based Drug Delivery for Diabetic Wound Healing

Therapeutically active components including nitric oxide, antioxidants, antimicrobials, growth factors and nucleic acids and its delivery to any damaged site or tissue for the stimulation of cell proliferation, cell migration, collagen secretion and angiogenesis and also antimicrobial compounds have been a good treatment option at the current stage and also paves way for wound infection therapy and wound healing [96]. Nanofibers have also received much attention in biomedical sciences due to its similarity in propagating extracellular matrix (ECM) environment [97, 98]. The characters of nanofibers such as increased porosity, increased ratio of surface area to volume, mechanical properties which can be re-engineered and encapsulation of nanoparticles and biologically active compounds to regulate the release of drugs in the cell matrix [99, 100]. The fields of tissue engineering and drug delivery have seen the rise of 3D polymer networks which are hydrophilic known as hydrogels which is of greater significance in these fields. High water content, biocompatibility and tuneable viscoelasticity are the characters of a network of polymer known as the hydrogels [101, 102]. Incorporation of hydrogels with bioactive nanoparticles have paved way towards topical treatment in wounds and burns due to its drug delivery characteristics. These hydrogels have been useful in the application over localized tissues with controlled release of drugs and minimized burst but also maintaining the nanoparticle structural integrity [103]. Nanoparticles which are not of polymeric origin like silver, gold, etc., are very much used as they exhibit good anti-inflammatory and antimicrobial properties [104]. Still, there was a need for anti-biofilm technology to be developed for effective wound healing especially in chronic diabetic wounds. These criteria have been met by silver NPs [105]. Silver nanoparticles (Ag NPs) are widely used in treating chronic ulcers and wounds especially those that have been presented with antimicrobial-resistance bacteria. Silver NPs were also used for its anti-inflammatory properties and resulting in accelerated healing of wounds [106, 107]. Gold nanoparticles (Au NPs) are also a very good nanotherapeutic and have been used in tissue engineering, wound healing and targeted drug delivery, however, they cannot exhibit antimicrobial property as a stand-alone nanomaterial and have to be incorporated with other biologically active compounds [108, 109]. Usage of zinc (Zn) in treating both type 1 and 2 of diabetes mellitus is prevalent as the mineral itself is enrolled in the function of more than 300 enzymes and promotes absorption of glucose by the adipose tissue and skeletal muscles and reduces blood sugar accumulation [110]. Biocompatibility of ZnO NPs have been very well explored along with their therapeutic usage in disease such as diabetes, infections and inflammation and melanoma are studied and concludes ZnO NPs to be potent therapeutic agents in diabetic wound healing [111, 112]. Other therapeutically important nanoparticles are ceramic NPs incorporated with inorganic compounds [113]. NPs based on lipids are considered safe and efficient in delivery both hydrophobic and hydrophilic drugs [114]. Polymeric NPs such as chitosan are natural polymers and widely used due to its better biocompatibility and antibacterial activity. These NPs can be encapsulated with other natural components like curcumin, aloe vera, vitamin E which can further increase wound healing [115, 116]. Synthetic polymer-based NPs are developed using poly('-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG). These polymers are Food and Drug Administration (FDA) approved. PLGA is the most preferred synthetic NPs for therapeutic use as they help in releasing lactate, stimulates proliferation of cells and reduces time taken for wound healing in diabetic rat models [117, 118].

Metal Oxides (ZnO) NPs for Diabetic Wounds

As stated earlier, metal-based nanoparticles especially metal oxides have found its way in biomedical applications as these metals are the minerals which are actively present in the human body and even low quantities showed better bioactivity. Zinc oxide (ZnO) nanoparticles (NPs) have been very well established due its applications in topical creams as antiseptics and anti-inflammatories. They are also studied well for their resistance towards bacteria especially those in diabetic wounds and thus results in an accelerated wound healing process [119, 120]. Studies involving the making of dressings of calcium alginate embedded with ZnO NPs and used in chronic diabetic wounds and were assessed as effective treatment [121]. ZnO NPs have been very well studied due to applications in skin infections including wounds. The mechanism of ZnO NPs have been summarized as i. keratinocytes migration which is promoted by cofactor in enzyme complexed and ii. development of perforations of cell membranes of bacteria. Thus, Zinc oxide NPs are considered as a promising metal oxide NP in the treatment of skin infections and speeding up wound healing process. One other metal oxide which has been promising in the healing of excision type wounds is titanium dioxide (TiO_2) nanoparticle [122].

Role of Zinc in wound healing

Zinc is a crucial element which is necessary for growth in humans which has been characterized for various biological functions [10]. An impaired uptake of zinc in the small intestine can cause

acrodermatitis enteropathica, a severe heredity deficiency of zinc. The impairment in the zinc update is due to the ZIP-4 gene mutation, this gene is responsible for encoding zinc membranous transporter protein. The symptoms of this zinc deficiency known as acrodermatitis enteropathica will gradually develop to a condition with wound healing impairment and reduced resistance towards infections. Distribution of zinc is widespread in the environment and is found in air, water and almost all food items [123]. Carbonic anhydrase was the first identified metalloenzyme which requires zinc as its cofactor [124]. As time progressed, more than 300 enzymes have been identified with zinc as an essential component. Metzincin superfamily of the metallo-endopeptidases are the enzymes which are critical for wound healing [125].

The zinc ions are involved in neurotransmission and intercellular signalling like calcium ions [126]. Metallothioneins (MTs) along with zinc transporter proteins mediates these signalling mechanisms. Almost 20% of intracellular zinc comprises the MTs complex and thus play a unique role in the metabolism of zinc in both healthy and diseased states. The MTs are cysteine-rich, ubiquitous low molecular proteins which regulates intracellular zinc supply to the enzymes, zinc depots and gene regulatory molecules. They also protect the cells from the toxic effects of elevated zinc exposure. One molecule of metallothionein can bind seven ions of zinc. Several physiological roles of zinc in wound healing are listed below.

- Inducing metallothioneins
- Cofactor in enzymes such as matrix metalloproteins
- Cofactor in transcription factors such as zinc-finger proteins
- Epithelialization enhancement
- Boosting innate immune response
- Antiseptic effects

The antimicrobial and wound healing properties of Zinc NPs have been investigated extensively for their role in ulcer therapy. They can promote wound healing in case of wounds and foot ulcers present in diabetic patients or any other wound or ulcerations which are associated with allergic dermatitis.

Zinc oxide Nanoparticles

Zinc oxide (ZnO) is a metal oxide possessing n-type semiconducting property and its nanoparticle ZnO NP have been established for its several applications in the fields of biomedicine, electronics and optics for the past 5 to 6 years [127-132]. Though several other metal oxide NPs have been developed in various domains of science, ZnO NPs have been of utmost interest due to its inexpensiveness and safer production methods [133]. The United States FDA have enlisted Zinc metal oxide as generally recognized as safe (GRAS) [134]. ZnO NPs have exhibited various properties such as high catalytic activity, semi conductivity, optical activity, UV filtration, anti-inflammatory, antimicrobial and wound healing properties [11, 135-140]. Cosmetic use of ZnO NPs as lotions is also carried out due to their UV filtration property and various other properties such in the field of biomedicals such as anti-cancer, antibacterial, anti-diabetic, etc have also been studied [141-146]. Even when ZnO NPs are used for drug delivery, their cytotoxicity is to be evaluated briefly [147]. ZnO NPs synthesized biologically showed efficient antibacterial effect even at low concentrations rather than chemically synthesized ZnO NP [148-150]. Other properties and uses of ZnO NPs include removal of arsenic and sulphur in water, disposal of aquatic weed, protein adsorption, paint and manufacturing industries, dental applications along with pyroelectric and piezoelectric properties [151-153]. Distinct morphologies such as nanorod, nanoflower, nanoflake, nanowire and nanobelt are all reported [154, 155]. As zinc element have been studied extensively for its role in normal wound healing processes and zinc oxide nanoparticles have showed promising results in terms of its antibacterial and antidiabetic properties, ZnO NPs are used as therapeutic agents in diabetic wound infections and diabetic wound healing.

Different Methods for Synthesizing Nanoparticles

Both chemical and physical methods are used for NP synthesis, however, there have been significant rise in green synthesis or green chemistry of NPs in recent years. The green synthesis involves the use of biological sources for NP synthesis [156-158]. The physical methods of nanoparticle synthesis use physical forces which attracts nanoscale particles together to form large and stable nanostructures as a result of colloidal dispersion and also involved expensive equipment and should be provided with large working area, high temperatures and pressure. Techniques such as vapor condensation, amorphous crystallization, physical fragmentation, etc. are used in the physical methods of NP synthesis [159-165]. The chemical basis of NP synthesis is highly toxic and hazardous and these toxic chemicals are be retained in NPs and affects the environmental safety aspect of the usage of NPs [166, 167]. Both physical and chemical means of NP synthesis requires stabilizing agent and capping agents [168-171]. This clearly indicates that ZnO NP synthesis by both chemical and physical methods are either expensive or

environmentally hazardous, thus the biological or green NP synthesis of ZnO has a widespread interest as the method is inexpensive, environmentally safe and efficient. The green synthesis of the biological means of NP synthesis are explained in brief along with its types of sources used in later part of the review.

Conventional Synthesis of ZnO NPs

ZnO NPs and their bioactivity is affected by several parameters suc h as distribution of size, particle reactivity, morphology of nanoparticles and surface chemistry. Thus, it is necessary to develop the NPs in uniform size and morphology so as to provide maximum bioactivity [172].

a. Chemical Precipitation

Chemical precipitation is the oldest and popular approach of ZnO NP synthesis. The process is carried out by a purified zinc precursor such as zinc nitrate, zinc acetate and zinc sulphate treated with a precipitator such as ammonium hydroxide or sodium hydroxide [173]. The process involved increasing the pH to 10 using the alkali precipitators to obtain zinc hydroxide which is converted to zinc oxide using high temperatures. The zinc oxide precipitate is then dried at 100°C for 6 hours to obtain the nanostructures of zinc oxide with a size distribution of 100nm. A complication agglomerate formation by the nano-oxide precursors used in the chemical precipitation process is seen [174-177].

b. Sol- Gel Method

The sol-gel method of ZnO NP synthesis involved three steps [178],

1. Preparation of Zinc Precursor

Zinc acetate which acts as a precursor for ZnO NP is dissolved in ethanol and refluxed at atmospheric pressure and boiled at 80°C. This is then stirred to collect the condensate along with a hygroscopic reaction mixture [172].

2. Preparation of ZnO Clusters

Obtained hygroscopic mixture is added with lithium hydroxide and is diluted to ethanolic solution so as to make transparent suspension using an ultrasonic bath [172].

3. Crystal Growth

Crystal growth is an automated process which occurs at room temperature; however, the amount of lithium hydroxide should be controlled as they can influence the shape and size of the formed NPs. Sodium hydroxide also replace lithium hydroxide as the alkali [179].

c. Solid state Pyrolytic Method

This method was an easy operation with low cost. The procedure involves the mixture of zinc acetate with that of sodium carbonate at room temperature and is subjected to pyrolysis and results in the formation of sodium acetate which is cleaned using deionized water and the zinc oxide nanoparticles were obtained by thermal decomposition where the resulting nanoparticle size were altered using changing the temperature of pyrolysis [180].

d. Solution-free Mechanochemical Method

This process of ZnO NP synthesis is of two steps. The first step is grinding oxalic acid and zinc acetate powder mixtures to obtain zinc oxalate nanoparticles [181]. The next step involves the thermal decomposition of zinc oxalate NPs at high temperatures to obtain zinc oxide NPs. Though the process is considered simple and inexpensive, the time duration of grinding highly affects the size of the resulting ZnO NPs [182].

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES

Green synthesis or biological methods are developed and preferred as it is eco-friendly [11]. Plant extracts, bacteria, fungus, algae are some of the biological sources for zinc oxide nanoparticle synthesis. Some examples of plant extract-based ZnO NP synthesis are leaves of *Cochlospermum religiosum* (L.), *Azadirachta indica* (L.), *Aloe barbadensis*, *Andrographis paniculate* and *Plectranthus amboinicus* and seeds of *Physalis alkekengi* (L.) and peels of *Nephelium lappaceum* (L.) and flower extract of *Jacaranda mimosifolia*, *Trifolium pratense* and rhizome extracts of *Zingiber officinale* and root extracts of *Polygala tenuifolia* [11, 183, 192, 193, 184-191].

a. Plant extract-based Zinc Oxide Nanoparticle Synthesis

ZnO NP synthesis have been performed in various plant part extracts such as stem, leaf, seed, fruit and root which comprises of several phytochemicals and is easy to obtain, less time-consuming, inexpensive and free of impurities to proceed with the synthesis [194]. Plants are specifically selected for NP synthesis in large-scale to produce sustainable NPs of different shape and size according to our needs [192]. The process involved in NP synthesis is metal oxide bio-reduction to lower valence metal nanoparticles aided by phytochemicals in plant like amino acids, polysaccharides, phenol, terpenoids, vitamins, alkaloids etc [192, 194]. The procedure consists of sterilizing the plant part, drying and crushing them, hydrating them at the desired concentration and finally boiling them under a magnetic stirrer under specified time period [192, 194-197]. Then the solution is filtered and is mixed with 0.5mM zinc precursor which is hydrated

such as zinc sulphate, zinc oxide, or zinc nitrate and is boiled and mixed appropriately at specific temperatures for a time period [196, 197]. During the incubation period the colour change of the mixture to yellow visually confirms zinc NP synthesis. Various conditions such as temperature, extract concentration, pH, incubation time are all optimized [196, 197]. Crystal NPs are obtained by centrifugation of the mixture and air drying the pellets using hot air oven after being confirmed using UV-Vis Spectrophotometry [198]. Characterization of Zinc NPs is done by bioanalytical instruments such as Energy Dispersion Analysis of X-ray (EDAX), X-ray Diffractometer (XRD), Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR) Spectroscopy, Field Emission Scanning Electron Microscopy (FE-SEM), Transmission Electron Microscopy (TEM), Thermal-gravimetric Differential Thermal Analysis (TG-DTA), Raman Spectroscopy, Atomic Force Spectroscopy (AFM), X-ray Photoelectron Microscopy (XPS), Photoluminescence Analysis (PL), Dynamic Light Scattering (DLS), UV-Visible Diffuse Reflectance Spectroscopy (UV-DRS) and Attenuated Total Reflectance (ATR) [152, 168, 184, 186, 187, 199-202].

b. Bacteria-based Zinc Oxide Nanoparticle Synthesis

Bacterial synthesis of Zinc oxide NPs is one of the preferred green synthesis of NPs after plant derivative-based synthesis. However, the sampling, screening and handling of microorganisms are time-consuming and the media used for bacterial growth must be monitored well as it can interfere with the nanoparticle shape and size. The nanoparticle synthesis is same as the plant source NP synthesis however, zinc precursors are added to bacterial cultures in the culture media and the obtained NPs were characterized accordingly. *Bacillus licheniformis* produced zinc oxide nanoflower structure which were eco-friendly and showed photocatalysis property [203]. *Rhodococcus sp.* derived nanoparticles showed biodegradation properties and metabolic activity towards hydrophobic compounds [204]. *Rhodococcus pyridinivorans* produced spherical nanoparticle structure when zinc sulphate was used as a precursor [205]. *Aeromonas hydrophila* produced both oval and spherical nanostructure when zinc oxide was used as precursor [206]. Antioxidant activity of NPs synthesized using the bacterial *Pseudomonas aeruginosa* rhamnolipids showed better results when compared with that of bare zinc oxide NPs [207-208].

c. Macroalgae and Microalgae-based Zinc Oxide Nanoparticle Synthesis

Zinc oxide NP synthesis are done only in a limited scale but have been extensively used for gold and silver NPs [209]. Microalgae were useful in ZnO NP synthesis as they were able to degrade toxic metals into lesser toxic form [210]. Hexagonal wurtzite nanostructures along with sulphated polysaccharides and hydroxyl groups were seen *Sargassum muticum* derived ZnO NPs. However, *Sargassum myriocystum* which belongs to the same Sargassaceae family showed varied sizes of nanostructure along with carbonyl and hydroxyl groups [211].

d. Fungi-based Zinc Oxide Nanoparticle Synthesis

Fungal-based NPs of zinc oxide have been very much useful especially for large-scale production and also economically viable [212]. Fungal strains are preferred to that of bacteria due to its bioaccumulation of metals and better tolerance to metals [213]. The mycelium of *Aspergillus fumigatus* is used for ZnO NP synthesis and produced average sized NPs but formation of agglomerates was seen after 90 days [214]. Primary or secondary amine, primary alcohol, amides and aromatic nitro compounds were seen in NPs synthesized using *Aspergilllus terreus* [215]. *Aspergillus sp.* were the widely studied fungal source of ZnO NP synthesis. *Candida albicans* derived NPs also showed similar results to that of the previous one [216].

e. Other Biological Sources for Zinc Oxide Nanoparticle Synthesis

Other green sources of ZnO NPs were any biocompatible chemicals. These chemicals are used due to their fast and economic process and also to avoid any side products or intermediates during the nucleation and synthesis of NPs and helps in size and shape-controlled synthesis of nanoparticles [217–219]. Synthesis of NPs using wet chemical method and coating the NPs on a cotton fabric enhances efficiency of antibacterial effect up to 99.99% [218].

DIABETIC WOUND HEALING AND ANTIBACTERIAL CHARACTERIZATION OF ZNO NPS AND ITS VARIANTS

Topical applications of zinc helped on improving re-epithelialization of wounds and reduce inflammation along with inhibiting bacterial growth. Zinc acts as a cofactor in metalloproteinases and promotes ECM regeneration. Embedding ZnO NPs with hydrogels of chitosan showed strong and efficient antibacterial activity and makes the combination for the development of an efficient dressing material for wounds [220-222]. Regulation of keratinocyte migration and auto-phagocytosis is met by zinc and is understood as a critical factor in wound repair. When biological fluids are infused or incorporated with ZnO NPs, it hydrated rapidly to form hydrated Zinc oxide which is also a bactericidal agent [233].

Zinc oxide nanoparticles which are green synthesized from aloe vera leaf extract were assessed for their antibacterial properties towards various drug resistant bacterial strains. The bacterial strains were *E. coli* and *P. aeruginosa* which are positive for extended spectrum beta lactamases (ESBL) along with

methicillin-resistant *S. aureus* (MRSA). The antibacterial study showed bacterial inhibition activity against ESBL *E. coli* and *P. aeruginosa* at 2200µg and MRSA at 2400µg, the bactericidal activity of *Zinc oxide nanoparticles* against ESBL *E. coli* and *P. aeruginosa* was observed at 2300µg and MRSA strain at 2700µg. The yielded result reveals the potential of ZnO NPs in antibacterial activity against drug-resistant bacterial strains and also anti-biofilm potential [187].

Another study was done on the anti-biofilm and anti-adherence property of the zinc oxide NPs against methicillin resistant *S. aureus* with vancomycin as control. The study results showed that almost 50% of pre-formed bacterial biofilm was broken or penetrated by ZnO NPs at a concentration of 13.5µg. Similarly, antibacterial activity against MRSA was also recorded at a concentration of 65.4µg of ZnO NPs. Thus, ZnO NPs are a promising anti-biofilm therapeutic with anti-adherence properties [224].

Though zinc and its application have its own potential over increasing wound healing, there is a necessity to develop new materials made of zinc oxide to deliver wound healing properties along with antibacterial effects which led to the development of zinc oxide quantum dots (QDs) which is incorporated into hydrogels. Hydrogels are biocompatible polymers usually derived from natural sources and is biodegradable. Hydrogels made from microporous chitosan is used to develop zinc oxide composite bandages. Thus, encapsulation of zinc oxide NPs with various biomaterials are used to deliver zinc oxide nanotherapeutics [10].

Encapsulated ZnO NPs

As stated earlier, though the zinc oxide NPs have been very much useful and showed promising results for reduced wound infections and increased wound healing processes, their biocompatibility and biodegradability are considered as a major treatment problem. This problem is addressed using encapsulation and incorporation of zinc oxide nanoparticles into hydrogels. Various hydrogels from differing sources are present, however for biocompatibility reasons, hydrogels derived from biological sources such as chitosan, alginate and cellulose are preferred for NP encapsulation. These polymers when incorporated with ZnO NPs can acts as better treatment method for diabetic wound infections and delayed wound healing [225-228].

a.ZnO and Cellulose

Though cellulose is obtained from various natural sources, bacterial cellulose (BC) is preferred as they have better tensile strength and crystallinity. Thus, bacterial celluloses incorporated with ZnO NPs and their antibacterial, antifungal, cytotoxicity properties were assessed using BCs without ZnO NPs and ZnO NPs without being encapsulated in BCs were used as control. When BCs are loaded with ZnO NPs, a slight reduction in the proliferation of human dermal fibroblast cells after a period of 72 hours is seen when compared to both the controls. The surface adherence of *E. coli*, *B. subtilis* and *C. albicans* were predominantly prevented using the hydrogel biofilm [229]. When the same BC-ZnO hydrogel biofilm was prepared by solution plasma method and assessed for inhibition zone against *E.coli* and *S. aureus* showed 3.33±0.29 mm and 5.33±0.29 mm in diameter [230]. When BC-ZnO NPs were used as topical cream for wound treatment for 15 days, the wound area was assessed to be 98.3±7.6 mm2, whereas negative control and BC showed 234.6±5.7mm2 and 143.±7.5 mm2, denoting that the ZnO NP encapsulated by bacterial cellulose showed reduction in the wound area [231]. When electrospun nanofibers of ZnO is incorporated in 5% of poly(3-hydroxybutyrate-co-3- hydroxy valerate (PHBV), a bacterial biodegradable polymer, 2.9mm and 3.5mm zone of inhibition against *E.coli* and *S. aureus* were observed [232]. Studies involving incorporation on ZnO NPs with TEMP-oxidized nanofibrilated cellulose (NFC) with either 5% or 10% of polyethylene glycine (PEG) showed reduction in the bleeding time of wounds especially in 5% PEG. Antibacterial assay of ZnO-NFC-PEG was assessed and the results showed that 5% PEG variant showed antimicrobial activity against *S. epidermidis*, *S. aureus* and *E.coli* where *S. aureus* were seen to be higher [233]. As the extracellular matrix (ECM) components can aid the process of wound healing, one such component of ECM is heparin sulphated glycosaminoglycan which binds to growth factors like platelet derived growth factor (PDGF-BB) and vascular endothelial-cell growth factor (VEGF-A165) which enhances wound healing [234]. Thus, ZnO NPs which are heparinized and incorporated in poly vinyl alcohol(PVA) and carboxymethyl cellulose (CMC) hydrogels revealed 83% cell viability in human dermal fibroblast cells [235]. To increase oxygen permeability which is an important factor in wound healing, mesoporous silica-based hydrogels are used. Mobil composition of matter (MCM-41) is a silicate based mesoporous material which is used along with CMC hydrogels for encapsulation of ZnO NPs. The results of gas permeability of the MCM-41/CMC/ZnO NP hydrogel was 500% and with 100% of swelling and it revealed effective antimicrobial activity against *S. aureus* and *E. coli* [236].

b. ZnO and Chitosan

The antibacterial assay of ZnO NP encapsulated on chitosan hydrogels showed efficient activity against *S. aureus* and *E. coli* [237]. The inhibition zone diameters (IZD) of the ZnO-Chitosan nanocomposite against *S. aureus*, *B. subtilis*, *K. pneumoniae* and *E. coli* were 22.5mm, 21mm, 24.5mm and 25.5mm; thus, showing

efficient antimicrobial activity especially against gram-negative bacteria [238]. The antibacterial activity can be further enhanced when ZnO NPs are combined with other metal-based NPs such as silver nanoparticles (Ag NPs). When Ag/ZnO nanoparticles were incorporated on chitosan, the antibacterial activity increased further even against drug resistant strains such as drug-resistant P. aeruginosa, drug-resistance *E. coli* and methicillin-resistant *S. aureus* (MRSA). Also, the Ag/ZnO NP on chitosan also showed closure of wound in 7 days and the cytotoxic effect of Ag/ZnO when assessed against human normal hepatocytes showed 100% cell viability [239]. Biofilms of ZnO-Chitosan can be prepared using PVA which showed significant increase in wound healing in 7 days and was comparatively higher to phosphate-buffered saline (PBS), chitosan/PVA and chitosan alone. The biofilm also showed efficient antibacterial activity against *E. coli* and *S. aureus* with 19mm and 20mm IZDs [240]. Heparinized variant of ZnO NPs-chitosan-PVA showed cell viability at 89% with 48 hours incubation and showed more than 70% efficient activity against *E. coli* and *S. aureus*. ZnO NP-chitosan hydrogels when added with keratin can increase wound healing up to 92% after 14 days[241].

The chitosan-based hydrogels are also incorporated with zinc oxide QDs and their results yielded good results in terms of antibacterial and increased wound healing effects by enhancing swelling capacity and blood clotting [242]. Studies on cell attachment and infiltration showed clear penetration and attachment on cells. Scaffold-based nanocomposites are developed using chitosan-silk sericin (CHT/SS) which is incorporated with ZnO NPs and showed antibacterial activity against *E. coli* and *S. aureus* [243]. This nanocomposite also showed improved attachment, proliferation and growth of immortalized human keratinocyte cell line (HaCaT cell line) without any secondary effects [244]. Another nanocomposite was developed using ZnO NPs-gelatin-chondroitin 4-sulphate which showed significant decrease in wounds up to 14% to 35% [245].

c. ZnO Np encapsulated with alginate

Sodium alginate-based encapsulation of ZnO NPs is accompanied by natural gums such as acacia due to its higher viscosity and are associated with formation of hydrogels [246]. At a concentration of 1000µg, the ZnO NP-sodium alginate – gum acacia hydrogel showed increased antibacterial effects on B. cereus and P. aeruginosa [247]. Alginate-based wound dressings are known preserve moisture and absorb wound exudates [248]. Thus, alginate-based nanocomposites can be useful in treating diabetic foot ulcers, infected pressure ulcers and wound infections [249]. Use of cellulose fibres can improve mechanical properties of the hydrogel such as the Young's modulus. Thus, ZnO NPs- cellulose fibre along with 1.5% sodium alginate showed increased Young's modulus of 379 MPa which indicates the increased strength of the hydrogel [250]. As the biodegradability of chitosan is higher than that of alginate, they both are combined to form a hydrogel with ZnO NPs which provided reduced wound exposure along with significant biocompatibility, biodegradability and antibacterial effect [251]. Another combination of hydrogel sources with ZnO NPs were sodium alginate with carboxymethyl chitosan [252]. The hydrogel when compared with Kaltostat® alginate dressing, showed better biocompatibility, haemostatic ability and antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* [253].

FUTURE PERSPECTIVE

The review suggests that there is a need for development of nanoparticle-based products for diabetic wound infection and delayed wound healing in clinical perspective. Though various research has been done on nanobiotechnology, development of commercially available nanotherapeutic products is considered as the need of the hour. Biofilm production by bacterial strains owing to its multi-drug resistance is an important criterion to be evaluated for the developing product and much more research on anti-biofilm properties of these nanoparticles have to be conducted. On the contrary, the development of these nanotherapeutics such as zinc oxide nanoparticles and its nanocomposite variants have to be developed via natural sources including the green synthesis of nanoparticles and use of natural polymers. Similarly, green synthesis of zinc oxide nanoparticles has to be studied much more extensively to make the synthesis techniques, a more efficient and eco-friendlier and the products to be cheap and commercially viable.

CONCLUSION

Diabetic wound infection and its resulting delayed wound healing is a widespread problem affecting millions of people worldwide. Though many studies were done on the pathophysiological condition, it was in the recent decades that the use of nanoparticles have been employed in the treatment of this condition. There are various ongoing research studies in all the aspects of developing this clinically critical nanotherapeutic agent. Initially, the development of a competent, less toxic, element-based nanoparticle has been employed and then its synthesis methods are optimized to obtain safe and cost-effective nanoparticles and then the combination of the synthesized nanoparticle with various

therapeutic agents and its delivery system is studied. The present study analysed zinc nanoparticles as a nanotherapeutic agent for diabetic wound infections and delayed wound healing due to diabetes, and thereby suggests that green synthesis of zinc oxide nanoparticles to be efficient and safe way for nanoparticle synthesis and its delivery mechanism and mechanism is more efficient when combined with natural polymers and addition of other therapeutic agents are also advised if necessary for the efficient antibacterial activity towards diabetic wound infections and also to accelerate wound healing process in diabetic wounds.

List of Abbreviations

DFU – diabetic foot ulcer; PAD – peripheral arterial disease; DM – diabetes mellitus; NADP – nicotinamide adenine dinucleotide phosphate; NO – nitric oxide; QS – quorum sensing; ROS – reactive oxygen species; EPS – extracellular polymeric substance; TNF – tumour necrosis factor; MRSA – methicillin resistant *Staphylococcus aureus*; IL – interleukin; ECM – extracellular matrix; SMA – smooth muscle actin; MMP – matrix metalloprotein; VAC – vacuum-assisted closure; TIMP – tissue inhibitor metalloproteinase; Ag – silver; NP – nanoparticles; Au – gold; Zn – zinc; ZnO – zinc oxide; PLGA – poly lactic co-glycolic acid; PCL – polycaprolactone; PLA – poly(lactic acid); PEG – polyethylene glycol; FDA – Food and drug administration; TiO_2 – Titanium dioxide; MT – metallothioneins; GRAS – generally recognized as safe; L. – Linnaeus; AFM – atomic force spectroscopy; XRD – X-ray diffractometer; EDAX – energy dispersion analysis of X-ray; FTIR – Fourier transform infrared spectroscopy; TG-DTA – thermal-gravimetric differential thermal analysis; FE-SEM – field emission scanning electron microscopy; SEM – scanning electron microscopy; TEM – transmission electron microscopy; PL – photoluminescence analysis; XPS – X-ray photoelectron spectroscopy; DLS – dynamic light scattering; UV-DRS – ultraviolet diffuse reflectance spectroscopy; ATR – attenuated total reflectance; ESBL – extended spectrum beta lactamases; QD – quantum dots; BC – bacterial cellulose; NFC – nanofibrilated cellulose; PDGF – platelet-derived growth factor; PHBV - poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PVA – polyvinyl alcohol; VEGF – vascular endothelial-cell growth factor; CMC – carboxymethyl cellulose; MCM – mobil composition of matter; IZD – inhibition zone diameter; PBS – phosphate-buffered saline; CHT/SS – chitosan/silk sericin; HaCaT – immortalized human keratinocyte cell line.

REFERENCES

1. Parani, M., Lokhande, G., Singh, A., & Gaharwar, A. K. (2016). Engineered nanomaterials for infection control and healing acute and chronic wounds. *ACS applied materials & interfaces*, *8*(16), 10049-10069.
2. Gurtner, G. C., Werner, S., Barrandon, Y., & Longaker, M. T. (2008). Wound repair and regeneration. *Nature*, *453*(7193), 314-321.
3. Guo, S. A., & DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of dental research*, *89*(3), 219-229.
4. Dreifke, M. B., Jayasuriya, A. A., & Jayasuriya, A. C. (2015). Current wound healing procedures and potential care. *Materials Science and Engineering: C*, *48*, 651-662.
5. Buch, P. J., Chai, Y., & Goluch, E. D. (2019). Treating polymicrobial infections in chronic diabetic wounds. *Clinical microbiology reviews*, *32*(2), e00091-18.
6. Diabetes Facts.
7. Country profile: India. Journal of the Indian Medical Association. 97 (9); 1999: 377-378, 383.
8. Kalashnikova, I., Das, S., & Seal, S. (2015). Nanomaterials for wound healing: scope and advancement. *Nanomedicine*, *10*(16), 2593-2612.
9. Vellayappan, M. V., Jaganathan, S. K., & Manikandan, A. (2016). Nanomaterials as a game changer in the management and treatment of diabetic foot ulcers. *RSC advances*, *6*(115), 114859-114878.
10. Lansdown, A. B., Mirastschijski, U., Stubbs, N., Scanlon, E., & Ågren, M. S. (2007). Zinc in wound healing: theoretical, experimental, and clinical aspects. *Wound repair and regeneration*, *15*(1), 2-16.
11. Elumalai, K., & Velmurugan, S. (2015). Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of Azadirachta indica (L.). *Applied Surface Science*, *345*, 329-336.
12. Gadelkarim, M., Abushouk, A. I., Ghanem, E., Hamaad, A. M., Saad, A. M., & Abdel-Daim, M. M. (2018). Adipose-derived stem cells: effectiveness and advances in delivery in diabetic wound healing. *Biomedicine & Pharmacotherapy*, *107*, 625-633.
13. Xu, A., Wang, Y., Lam, K. S., & Vanhoutte, P. M. (2010). Vascular actions of adipokines: molecular mechanisms and therapeutic implications. *Advances in pharmacology*, *60*, 229-255.
14. Hossen, M. S., Ali, M. Y., Jahurul, M. H. A., Abdel-Daim, M. M., Gan, S. H., & Khalil, M. I. (2017). Beneficial roles of honey polyphenols against some human degenerative diseases: a review. *Pharmacological Reports*, *69*(6), 1194-1205.
15. Okonkwo, U. A., & DiPietro, L. A. (2017). Diabetes and wound angiogenesis. *International journal of molecular sciences*, *18*(7), 1419.
16. Hicks, C. W., Selvarajah, S., Mathioudakis, N., Sherman, R. L., Hines, K. F., Black III, J. H., & Abularrage, C. J. (2016). Burden of infected diabetic foot ulcers on hospital admissions and costs. *Annals of vascular surgery*, *33*, 149-158.

17. Monteiro-Soares, M., Ribeiro-Vaz, I., & Boyko, E. J. (2019). Canagliflozin should be prescribed with caution to individuals with type 2 diabetes and high risk of amputation. *Diabetologia*, *62*(6), 900-904.
18. Pecoraro, R. E., Reiber, G. E., & Burgess, E. M. (1990). Pathways to diabetic limb amputation: basis for prevention. *Diabetes care*, *13*(5), 513-521.
19. Goldman, M. P., Clark, C. J., Craven, T. E., Davis, R. P., Williams, T. K., Velazquez-Ramirez, G., ... & Edwards, M. S. (2018). Effect of intensive glycemic control on risk of lower extremity amputation. *Journal of the American College of Surgeons*, *227*(6), 596-604.
20. Aumiller, W. D., & Dollahite, H. A. (2015). Pathogenesis and management of diabetic foot ulcers. *Journal of the American Academy of PAs*, *28*(5), 28-34.
21. Tahergorabi, Z., & Khazaei, M. (2012). Imbalance of angiogenesis in diabetic complications: the mechanisms. *International journal of preventive medicine*, *3*(12), 827.
22. Wu, J., Zheng, Y., Song, W., Luan, J., Wen, X., Wu, Z., ... & Guo, S. (2014). In situ synthesis of silver-nanoparticles/bacterial cellulose composites for slow-released antimicrobial wound dressing. *Carbohydrate polymers*, *102*, 762-771.
23. Rajendran, N. K., Kumar, S. S. D., Houreld, N. N., & Abrahamse, H. (2018). A review on nanoparticle based treatment for wound healing. *Journal of Drug Delivery Science and Technology*, *44*, 421-430.
24. Tsourdi, E., Barthel, A., Rietzsch, H., Reichel, A., & Bornstein, S. R. (2013). Current aspects in the pathophysiology and treatment of chronic wounds in diabetes mellitus. *BioMed research international*, *2013*.
25. Frykberg, R. G., Zgonis, T., Armstrong, D. G., Driver, V. R., Giurini, J. M., Kravitz, S. R., ... & Vanore, J. V. (2006). Diabetic foot disorders: a clinical practice guideline (2006 revision). *The journal of foot and ankle surgery*, *45*(5), S1-S66.
26. Reiber, G. E., Vileikyte, L. O. R. E. T. T. A., Boyko, E. D., Del Aguila, M., Smith, D. G., Lavery, L. A., & Boulton, A. J. (1999). Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes care*, *22*(1), 157-162.
27. Bowering, C. K. (2001). Diabetic foot ulcers. Pathophysiology, assessment, and therapy. *Canadian Family Physician*, *47*(5), 1007-1016.
28. Tang, W., Martin, K. A., & Hwa, J. (2012). Aldose reductase, oxidative stress, and diabetic mellitus. *Frontiers in pharmacology*, *3*, 87.
29. Clayton, W., & Elasy, T. A. (2009). A review of the pathophysiology, classification, and treatment of foot ulcers in diabetic patients. *Clinical Diabetology*, *10*(5), 209-216.
30. Simmons, Z., & Feldman, E. L. (2002). Update on diabetic neuropathy. *Current opinion in neurology*, *15*(5), 595-603.
31. Juster-Switlyk K, Smith AG. Updates in diabetic peripheral neuropathy. F1000Research. 5 2016:
32. Avogaro, A., Albiero, M., Menegazzo, L., de Kreutzenberg, S., & Fadini, G. P. (2011). Endothelial dysfunction in diabetes: the role of reparatory mechanisms. *Diabetes care*, *34*(Supplement 2), S285-S290.
33. Noor, S., Zubair, M., & Ahmad, J. (2015). Diabetic foot ulcer—a review on pathophysiology, classification and microbial etiology. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *9*(3), 192-199.
34. Bumpus, K., & Maier, M. A. (2013). The ABC's of wound care. *Current cardiology reports*, *15*(4), 1-6.
35. Han, G., & Ceilley, R. (2017). Chronic wound healing: a review of current management and treatments. *Advances in therapy*, *34*(3), 599-610.
36. Morton, L. M., & Phillips, T. J. (2016). Wound healing and treating wounds: Differential diagnosis and evaluation of chronic wounds. *Journal of the American Academy of Dermatology*, *74*(4), 589-605.
37. Negut, I., Grumezescu, V., & Grumezescu, A. M. (2018). Treatment strategies for infected wounds. *Molecules*, *23*(9), 2392.
38. Lin, Y. H., Hsu, W. S., Chung, W. Y., Ko, T. H., & Lin, J. H. (2016). Silver-based wound dressings reduce bacterial burden and promote wound healing. *International wound journal*, *13*(4), 505-511.
39. Leaper, D., Assadian, O., & Edmiston, C. E. (2015). Approach to chronic wound infections. *British Journal of Dermatology*, *173*(2), 351-358.
40. Kirketerp-Møller, K., Zulkowski, K., & James, G. (2011). Chronic wound colonization, infection, and biofilms. In *Biofilm infections* (pp. 11-24). Springer, New York, NY.
41. Percival, S. L., & McCarty, S. M. (2015). Silver and alginates: role in wound healing and biofilm control. *Advances in wound care*, *4*(7), 407-414.
42. Ciofu, O., Rojo-Molinero, E., Macià, M. D., & Oliver, A. (2017). Antibiotic treatment of biofilm infections. *Apmis*, *125*(4), 304-319.
43. Bassetti, M., Vena, A., Croxatto, A., Righi, E., & Guery, B. (2018). How to manage Pseudomonas aeruginosa infections. *Drugs in context*, *7*.
44. Khan, H. A., Ahmad, A., & Mehboob, R. (2015). Nosocomial infections and their control strategies. *Asian pacific journal of tropical biomedicine*, *5*(7), 509-514.
45. Wu, H., Moser, C., Wang, H. Z., Høiby, N., & Song, Z. J. (2015). Strategies for combating bacterial biofilm infections. *International journal of oral science*, *7*(1), 1-7.
46. Gardner, S. E., & Frantz, R. A. (2008). Wound bioburden and infection-related complications in diabetic foot ulcers. *Biological research for nursing*, *10*(1), 44-53.
47. Christensen, G. J. M., & Brüggemann, H. (2014). Bacterial skin commensals and their role as host guardians. *Beneficial microbes*, *5*(2), 201-215.

48. Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M., & Marrie, T. J. (1987). Bacterial biofilms in nature and disease. *Annual Reviews in Microbiology*, *41*(1), 435-464.
49. Whiteley, M., Diggle, S. P., & Greenberg, E. P. (2017). Progress in and promise of bacterial quorum sensing research. *Nature*, *551*(7680), 313-320.
50. Atala, A., Irvine, D. J., Moses, M., & Shaunak, S. (2010). Wound healing versus regeneration: role of the tissue environment in regenerative medicine. *MRS bulletin*, *35*(8), 597-606.
51. Eming, S. A., Martin, P., & Tomic-Canic, M. (2014). Wound repair and regeneration: mechanisms, signaling, and translation. *Science translational medicine*, *6*(265), 265sr6-265sr6.
52. Evans, N. D., Oreffo, R. O., Healy, E., Thurner, P. J., & Man, Y. H. (2013). Epithelial mechanobiology, skin wound healing, and the stem cell niche. *Journal of the mechanical behavior of biomedical materials*, *28*, 397-409.
53. Koh, T. J., & DiPietro, L. A. (2011). Inflammation and wound healing: the role of the macrophage. *Expert reviews in molecular medicine*, *13*.
54. Ho, J. K., & Hantash, B. M. (2013). The principles of wound healing. *Expert Review of Dermatology*, *8*(6), 639-658.
55. Pakyari, M., Farrokhi, A., Maharlooei, M. K., & Ghahary, A. (2013). Critical role of transforming growth factor beta in different phases of wound healing. *Advances in wound care*, *2*(5), 215-224.
56. Patel, S., Srivastava, S., Singh, M. R., & Singh, D. (2019). Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. *Biomedicine & Pharmacotherapy*, *112*, 108615.
57. Cho, H., Blatchley, M. R., Duh, E. J., & Gerecht, S. (2019). Acellular and cellular approaches to improve diabetic wound healing. *Advanced drug delivery reviews*, *146*, 267-288.
58. Xu, F., Zhang, C., & Graves, D. T. (2013). Abnormal cell responses and role of TNF-in impaired diabetic wound healing. *BioMed research international*, *2013*.
59. Chitturi, R. T., Balasubramaniam, A. M., Parameswar, R. A., Kesavan, G., Haris, K. M., & Mohideen, K. (2015). The role of myofibroblasts in wound healing, contraction and its clinical implications in cleft palate repair. *Journal of international oral health: JIOH*, *7*(3), 75.
60. Nguyen, T. T., Mobashery, S., & Chang, M. (2016). Roles of matrix metalloproteinases in cutaneous wound healing. *Wound Healing-New insights into Ancient Challenges*, 37-71.
61. McCarty, S. M., & Percival, S. L. (2013). Proteases and delayed wound healing. *Advances in wound care*, *2*(8), 438-447.
62. Ayuk, S. M., Abrahamse, H., & Houreld, N. N. (2016). The role of matrix metalloproteinases in diabetic wound healing in relation to photobiomodulation. *Journal of diabetes research*, *2016*.
63. Kasiewicz, L. N., & Whitehead, K. A. (2017). Recent advances in biomaterials for the treatment of diabetic foot ulcers. *Biomaterials science*, *5*(10), 1962-1975.
64. Vijayakumar, V., Samal, S. K., Mohanty, S., & Nayak, S. K. (2019). Recent advancements in biopolymer and metal nanoparticle-based materials in diabetic wound healing management. *International journal of biological macromolecules*, *122*, 137-148.
65. Chen, H., Jia, P., Kang, H., Zhang, H., Liu, Y., Yang, P., ... & Deng, L. (2016). Upregulating Hif-1α by hydrogel nanofibrous scaffolds for rapidly recruiting angiogenesis relative cells in diabetic wound. *Advanced healthcare materials*, *5*(8), 907-918.
66. Miller, M. C., & Nanchahal, J. (2005). Advances in the modulation of cutaneous wound healing and scarring. *BioDrugs*, *19*(6), 363-381.
67. Høiby, N., Bjarnsholt, T., Moser, C., Bassi, G. L., Coenye, T., Donelli, G., ... & Zimmerli, C. E. E. W. (2015). ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. *Clinical microbiology and infection*, *21*, S1-S25.
68. Mihai, M. M., Giurcãneanu, C., Popa, L. G., Nitipir, C., & Popa, M. I. (2015). Controversies and challenges of chronic wound infection diagnosis and treatment. *Mod. Med*, *22*, 375-381.
69. Gao, Y., Han, Y., Cui, M., Tey, H. L., Wang, L., & Xu, C. (2017). ZnO nanoparticles as an antimicrobial tissue adhesive for skin wound closure. *Journal of Materials Chemistry B*, *5*(23), 4535-4541.
70. Mori, H. M., Kawanami, H., Kawahata, H., & Aoki, M. (2016). Wound healing potential of lavender oil by acceleration of granulation and wound contraction through induction of TGF-β in a rat model. *BMC complementary and alternative medicine*, *16*(1), 1-11.
71. Orchard, A., & van Vuuren, S. (2017). Commercial essential oils as potential antimicrobials to treat skin diseases. *Evidence-Based Complementary and Alternative Medicine*, *2017*.
72. Kumar, S., Lakshmanan, V. K., Raj, M., Biswas, R., Hiroshi, T., Nair, S. V., & Jayakumar, R. (2013). Evaluation of wound healing potential of β-chitin hydrogel/nano zinc oxide composite bandage. *Pharmaceutical research*, *30*(2), 523-537.
73. Metcalf, D. G., & Bowler, P. G. (2016). Clinician perceptions of wound biofilm. *International wound journal*, *13*(5), 717-725.
74. Bessa, L. J., Fazii, P., Di Giulio, M., & Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. *International wound journal*, *12*(1), 47-52.
75. Kininogen, M. W., & Weight, L. M. (2016). Journal of Diabetes & Metabolism.
76. Stewart, P. S. (2002). Mechanisms of antibiotic resistance in bacterial biofilms. *International journal of medical microbiology*, *292*(2), 107-113.
77. Kurniawan, A., & Fukuda, Y. (2016). Electric charge characteristics of biofilms formed on various surfaces. *The Journal of Pure and Applied Chemistry Research*, *5*(2), 95.

78. Tseng, B. S., Zhang, W., Harrison, J. J., Quach, T. P., Song, J. L., Penterman, J., ... & Parsek, M. R. (2013). The extracellular matrix protects P seudomonas aeruginosa biofilms by limiting the penetration of tobramycin. *Environmental microbiology*, *15*(10), 2865-2878.
79. Gordon, C. A., Hodges, N. A., & Marriott, C. (1988). Antibiotic interaction and diffusion through alginate and exopolysaccharide of cystic fibrosis-derived Pseudomonas aeruginosa. *Journal of Antimicrobial Chemotherapy*, *22*(5), 667-674.
80. Nichols, W. W., Dorrington, S. M., Slack, M. P., & Walmsley, H. L. (1988). Inhibition of tobramycin diffusion by binding to alginate. *Antimicrobial agents and chemotherapy*, *32*(4), 518-523.
81. Brown, M. R. W. (2018). *Microbiological quality assurance: a guide towards relevance and reproducibility of inocula*. CRC Press.
82. Lewis, K. (2001). Riddle of biofilm resistance. *Antimicrobial agents and chemotherapy*, *45*(4), 999-1007.
83. Conlon, B. P., Nakayasu, E. S., Fleck, L. E., LaFleur, M. D., Isabella, V. M., Coleman, K., ... & Lewis, K. (2013). Activated ClpP kills persisters and eradicates a chronic biofilm infection. *Nature*, *503*(7476), 365-370.
84. Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. *Annual Reviews in Microbiology*, *55*(1), 165-199.
85. Proctor, R. A., Von Eiff, C., Kahl, B. C., Becker, K., McNamara, P., Herrmann, M., & Peters, G. (2006). Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nature Reviews Microbiology*, *4*(4), 295-305.
86. Ramasamy, M., & Lee, J. (2016). Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices. *BioMed Research International*, *2016*.
87. Franci, G., Falanga, A., Galdiero, S., Palomba, L., Rai, M., Morelli, G., & Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. *Molecules*, *20*(5), 8856-8874.
88. Simões, D., Miguel, S. P., Ribeiro, M. P., Coutinho, P., Mendonça, A. G., & Correia, I. J. (2018). Recent advances on antimicrobial wound dressing: A review. *European Journal of Pharmaceutics and Biopharmaceutics*, *127*, 130-141.
89. Kesharwani, P., Gorain, B., Low, S. Y., Tan, S. A., Ling, E. C. S., Lim, Y. K., ... & Pandey, M. (2018). Nanotechnology based approaches for anti-diabetic drugs delivery. *Diabetes research and clinical practice*, *136*, 52-77.
90. Gorain, B., Tekade, M., Kesharwani, P., Iyer, A. K., Kalia, K., & Tekade, R. K. (2017). The use of nanoscaffolds and dendrimers in tissue engineering. *Drug discovery today*, *22*(4), 652-664.
91. Parayath, N. N., Parikh, A., & Amiji, M. M. (2018). Repolarization of tumor-associated macrophages in a genetically engineered nonsmall cell lung cancer model by intraperitoneal administration of hyaluronic acid-based nanoparticles encapsulating microRNA-125b. *Nano letters*, *18*(6), 3571-3579.
92. Choudhury, H., Gorain, B., Pandey, M., Khurana, R. K., & Kesharwani, P. (2019). Strategizing biodegradable polymeric nanoparticles to cross the biological barriers for cancer targeting. *International journal of pharmaceutics*, *565*, 509-522.
93. Colon, G., Ward, B. C., & Webster, T. J. (2006). Increased osteoblast and decreased Staphylococcus epidermidis functions on nanophase ZnO and TiO2. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*, *78*(3), 595-604.
94. Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. (2008). Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. *FEMS microbiology letters*, *279*(1), 71-76.
95. Ferrari, M. Nano geometry: Beyond drug delivery. Nat. Nano technol. 2008, 3, 131–132.
96. Goh, E. T., Kirby, G., Jayakumar, R., Liang, X. J., & Tan, A. (2016). Accelerated wound healing using nanoparticles. In *Nanoscience in Dermatology* (pp. 287-306). Academic Press.
97. Ezhilarasu, H., Ramalingam, R., Dhand, C., Lakshminarayanan, R., Sadiq, A., Gandhimathi, C., ... & Srinivasan, D. K. (2019). Biocompatible aloe vera and tetracycline hydrochloride loaded hybrid nanofibrous scaffolds for skin tissue engineering. *International journal of molecular sciences*, *20*(20), 5174.
98. Ramalingam, R., Dhand, C., Leung, C. M., Ezhilarasu, H., Prasannan, P., Ong, S. T., ... & Arunachalam, K. D. (2019). Poly-ε-caprolactone/gelatin hybrid electrospun composite nanofibrous mats containing ultrasound assisted herbal extract: Antimicrobial and cell proliferation study. *Nanomaterials*, *9*(3), 462.
99. Shan, X., Liu, C., Li, F., Ouyang, C., Gao, Q., & Zheng, K. (2015). Nanoparticles vs. nanofibers: a comparison of two drug delivery systems on assessing drug release performance in vitro. *Designed Monomers and Polymers*, *18*(7), 678-689.
100. Xavier, M. V., Macedo, M. F., Benatti, A. C. B., Jardini, A. L., Rodrigues, A. A., Lopes, M. S., ... & Kharmandayan, P. (2016). PLLA synthesis and nanofibers production: viability by human mesenchymal stem cell from adipose tissue. *Procedia CIRP*, *49*, 213-221.
101. Slaughter, B. V., Khurshid, S. S., Fisher, O. Z., Khademhosseini, A., & Peppas, N. A. (2009). Hydrogels in regenerative medicine. *Advanced materials*, *21*(32-33), 3307-3329.
102. Wang, F., Gao, W., Thamphiwatana, S., Luk, B. T., Angsantikul, P., Zhang, Q., ... & Zhang, L. (2015). Hydrogel retaining toxin-absorbing nanosponges for local treatment of Methicillin-resistant Staphylococcus aureus infection. *Advanced Materials*, *27*(22), 3437-3443.
103. Chai, Q., Jiao, Y., & Yu, X. (2017). Hydrogels for biomedical applications: their characteristics and the mechanisms behind them. *Gels*, *3*(1), 6.
104. Mauricio, M. D., Guerra-Ojeda, S., Marchio, P., Valles, S. L., Aldasoro, M., Escribano-Lopez, I., ... & Victor, V. M. (2018). Nanoparticles in medicine: a focus on vascular oxidative stress. *Oxidative Medicine and Cellular Longevity*, *2018*.

105. Kalantari, K., Mostafavi, E., Afifi, A. M., Izadiyan, Z., Jahangirian, H., Rafiee-Moghaddam, R., & Webster, T. J. (2020). Wound dressings functionalized with silver nanoparticles: promises and pitfalls. *Nanoscale*, *12*(4), 2268-2291.
106. Chaloupka, K., Malam, Y., & Seifalian, A. M. (2010). Nanosilver as a new generation of nanoproduct in biomedical applications. *Trends in biotechnology*, *28*(11), 580-588.
107. Alarcon, E. I., Griffith, M., & Udekwu, K. I. (2015). Silver nanoparticle applications. *Springer International Publishing. doi*, *10*, 978-3.
108. Volkova, N., Yukhta, M., Pavlovich, O., & Goltsev, A. (2016). Application of cryopreserved fibroblast culture with Au nanoparticles to treat burns. *Nanoscale research letters*, *11*(1), 1-6.
109. Ding, Y., Jiang, Z., Saha, K., Kim, C. S., Kim, S. T., Landis, R. F., & Rotello, V. M. (2014). Gold nanoparticles for nucleic acid delivery. *Molecular therapy*, *22*(6), 1075-1083.
110. El-Gharbawy, R. M., Emara, A. M., & Abu-Risha, S. E. S. (2016). Zinc oxide nanoparticles and a standard antidiabetic drug restore the function and structure of beta cells in Type-2 diabetes. *Biomedicine & Pharmacotherapy*, *84*, 810-820.
111. Huang, X., Zheng, X., Xu, Z., & Yi, C. (2017). ZnO-based nanocarriers for drug delivery application: From passive to smart strategies. *International journal of pharmaceutics*, *534*(1-2), 190-194.
112. Jin, S. E., & Jin, H. E. (2019). Synthesis, characterization, and three-dimensional structure generation of zinc oxide-based nanomedicine for biomedical applications. *Pharmaceutics*, *11*(11), 575.
113. Yang, L., Sheldon, B. W., & Webster, T. J. (2010). Nanophase ceramics for improved drug delivery. *Am Ceram Soc Bull*, *89*(2), 24-32.
114. Kraft, J. C., Freeling, J. P., Wang, Z., & Ho, R. J. (2014). Emerging research and clinical development trends of liposome and lipid nanoparticle drug delivery systems. *Journal of pharmaceutical sciences*, *103*(1), 29-52.
115. Lin, Y. H., Lin, J. H., & Hong, Y. S. (2017). Development of chitosan/poly-γ-glutamic acid/pluronic/curcumin nanoparticles in chitosan dressings for wound regeneration. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *105*(1), 81-90.
116. Blažević, F., Milekić, T., Romić, M. D., Juretić, M., Pepić, I., Filipović-Grčić, J., ... & Hafner, A. (2016). Nanoparticle-mediated interplay of chitosan and melatonin for improved wound epithelialisation. *Carbohydrate polymers*, *146*, 445-454.
117. Chereddy, K. K., Vandermeulen, G., & Préat, V. (2016). PLGA based drug delivery systems: Promising carriers for wound healing activity. *Wound Repair and Regeneration*, *24*(2), 223-236.
118. Sharma, S., Parmar, A., Kori, S., & Sandhir, R. (2016). PLGA-based nanoparticles: A new paradigm in biomedical applications. *TrAC trends in analytical chemistry*, *80*, 30-40.
119. Jaiswal, M., Gupta, A., Dinda, A. K., & Koul, V. (2015). An investigation study of gelatin release from semi-interpenetrating polymeric network hydrogel patch for excision wound healing on W istar rat model. *Journal of Applied Polymer Science*, *132*(25).
120. Deepachitra, R., Lakshmi, R. P., Sivaranjani, K., Chandra, J. H., & Sastry, T. P. (2015). Nanoparticles embedded biomaterials in wound treatment: a review. *J. Chem. Pharm. Sci*, *8*, 324-328.
121. Tulip G, Hamburg H. Biopolymers & Bioplastics,
122. Archana, D., Singh, B. K., Dutta, J., & Dutta, P. K. (2015). Chitosan-PVP-nano silver oxide wound dressing: in vitro and in vivo evaluation. *International journal of biological macromolecules*, *73*, 49-57.
123. Prasad, A. S. (1995). Zinc: an overview. *Nutrition (Burbank, Los Angeles County, Calif.)*, *11*(1 Suppl), 93-99.
124. Keilin, D., & Mann, T. (1941). Activity of carbonic anhydrase within red blood corpuscles. *Nature*, *148*(3756), 493-496.
125. Toriseva, M., & Kähäri, V. M. (2009). Proteinases in cutaneous wound healing. *Cellular and Molecular Life Sciences*, *66*(2), 203-224.
126. Fukada, T., Yamasaki, S., Nishida, K., Murakami, M., & Hirano, T. (2011). Zinc homeostasis and signaling in health and diseases. *JBIC Journal of Biological Inorganic Chemistry*, *16*(7), 1123-1134.
127. Sundrarajan, M., Ambika, S., & Bharathi, K. (2015). Plant-extract mediated synthesis of ZnO nanoparticles using Pongamia pinnata and their activity against pathogenic bacteria. *Advanced powder technology*, *26*(5), 1294-1299.
128. Vanathi, P., Rajiv, P., Narendhran, S., Rajeshwari, S., Rahman, P. K., & Venckatesh, R. (2014). Biosynthesis and characterization of phyto mediated zinc oxide nanoparticles: a green chemistry approach. *Materials Letters*, *134*, 13-15.
129. Jamdagni, P., Khatri, P., & Rana, J. S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity. *Journal of King Saud University-Science*, *30*(2), 168-175.
130. Prasad, K., & Jha, A. K. (2009). ZnO nanoparticles: synthesis and adsorption study. *Natural Science*, *1*(02), 129.
131. Patil, B. N., & Taranath, T. C. (2016). Limonia acidissima L. leaf mediated synthesis of zinc oxide nanoparticles: a potent tool against Mycobacterium tuberculosis. *International journal of mycobacteriology*, *5*(2), 197-204.
132. Gunalan, S., Sivaraj, R., & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International*, *22*(6), 693-700.
133. Selvarajan, E., & Mohanasrinivasan, V. (2013). Biosynthesis and characterization of ZnO nanoparticles using Lactobacillus plantarum VITES07. *Materials Letters*, *112*, 180-182.
134. Pulit-Prociak, J., Chwastowski, J., Kucharski, A., & Banach, M. (2016). Functionalization of textiles with silver and zinc oxide nanoparticles. *Applied Surface Science*, *385*, 543-553.
135. Mirzaei, H., & Darroudi, M. (2017). Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *Ceramics International*, *43*(1), 907-914.

136. Patel, V., Berthold, D., Puranik, P., & Gantar, M. (2015). Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. *Biotechnology Reports*, *5*, 112-119.
137. Stan, M., Popa, A., Toloman, D., Dehelean, A., Lung, I., & Katona, G. (2015). Enhanced photocatalytic degradation properties of zinc oxide nanoparticles synthesized by using plant extracts. *Materials Science in Semiconductor Processing*, *39*, 23-29.
138. Sherly, E. D., Vijaya, J. J., Selvam, N. C. S., & Kennedy, L. J. (2014). Microwave assisted combustion synthesis of coupled ZnO–ZrO2 nanoparticles and their role in the photocatalytic degradation of 2, 4-dichlorophenol. *Ceramics International*, *40*(4), 5681-5691.
139. Sangeetha, G., Rajeshwari, S., & Venckatesh, R. (2011). Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. *Materials Research Bulletin*, *46*(12), 2560-2566.
140. Sheikhloo, Z., Salouti, M., & Katiraee, F. (2011). Biological synthesis of gold nanoparticles by fungus Epicoccum nigrum. *Journal of Cluster Science*, *22*(4), 661-665.
141. Wodka, D., Bielanska, E., Socha, R. P., Elzbieciak-Wodka, M., Gurgul, J., Nowak, P., ... & Kumakiri, I. (2010). Photocatalytic activity of titanium dioxide modified by silver nanoparticles. *ACS applied materials & interfaces*, *2*(7), 1945-1953.
142. Hassani Sangani, M., Nakhaei Moghaddam, M., & Forghanifard, M. M. (2015). Inhibitory effect of zinc oxide nanoparticles on pseudomonas aeruginosa biofilm formation. *Nanomedicine Journal*, *2*(2), 121-128.
143. Hameed, A. S. H., Karthikeyan, C., Ahamed, A. P., Thajuddin, N., Alharbi, N. S., Alharbi, S. A., & Ravi, G. (2016). In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing Escherichia coli and Klebsiella pneumoniae. *Scientific reports*, *6*(1), 1-11.
144. Movahedi, F., Masrouri, H., & Kassaee, M. Z. (2014). Immobilized silver on surface-modified ZnO nanoparticles: As an efficient catalyst for synthesis of propargylamines in water. *Journal of Molecular Catalysis A: Chemical*, *395*, 52-57.
145. Martínková, L., Uhnáková, B., Pátek, M., Nešvera, J., & Křen, V. (2009). Biodegradation potential of the genus Rhodococcus. *Environment international*, *35*(1), 162-177.
146. Jain, N., Bhargava, A., & Panwar, J. (2014). Enhanced photocatalytic degradation of methylene blue using biologically synthesized "protein-capped" ZnO nanoparticles. *Chemical Engineering Journal*, *243*, 549-555.
147. Ma, H., Williams, P. L., & Diamond, S. A. (2013). Ecotoxicity of manufactured ZnO nanoparticles–a review. *Environmental Pollution*, *172*, 76-85.
148. Vimala, K., Sundarraj, S., Paulpandi, M., Vengatesan, S., & Kannan, S. (2014). Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and Bcl-2 expression in breast and colon carcinoma. *Process biochemistry*, *49*(1), 160-172.
149. Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N., Rene, E. R., Sharma, N. C., & Sahi, S. V. (2017). Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in Leucaena leucocephala seedlings: a physiochemical analysis. *Plant Physiology and Biochemistry*, *110*, 59-69.
150. Narayanan, J., Ramji, R., Sahu, H., & Gautam, P. (2010). Synthesis, stabilisation and characterisation of rhamnolipid-capped ZnS nanoparticles in aqueous medium. *IET nanobiotechnology*, *4*(2), 29-34.
151. Jha, A. K., Prasad, K., & Kulkarni, A. R. (2007). Microbe-mediated nanotransformation: cadmium. *Nano*, *2*(04), 239-242.
152. Rajeshkumar, S. (2016). Synthesis of silver nanoparticles using fresh bark of Pongamia pinnata and characterization of its antibacterial activity against gram positive and gram negative pathogens. *Resource-Efficient Technologies*, *2*(1), 30-35.
153. Nagajyothi, P. C., An, T. M., Sreekanth, T. V. M., Lee, J. I., Lee, D. J., & Lee, K. D. (2013). Green route biosynthesis: Characterization and catalytic activity of ZnO nanoparticles. *Materials Letters*, *108*, 160-163.
154. Rajeshkumar, S., Malarkodi, C., Paulkumar, K., Vanaja, M., Gnanajobitha, G., & Annadurai, G. (2014). Algae mediated green fabrication of silver nanoparticles and examination of its antifungal activity against clinical pathogens. *International journal of Metals*, *2014*.
155. Zarrinkhameh, M., Zendehnam, A., & Hosseini, S. M. (2015). Fabrication of polyvinylchloride based nanocomposite thin film filled with zinc oxide nanoparticles: morphological, thermal and optical characteristics. *Journal of Industrial and Engineering Chemistry*, *30*, 295-301.
156. Afifi, M., Almaghrabi, O. A., & Kadasa, N. M. (2015). Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin-induced diabetic rat testes. *BioMed Research International*, *2015*.
157. Chen, J., Liu, X., Wang, C., Yin, S. S., Li, X. L., Hu, W. J., ... & Zheng, H. L. (2015). Nitric oxide ameliorates zinc oxide nanoparticles-induced phytotoxicity in rice seedlings. *Journal of hazardous materials*, *297*, 173-182.
158. Vitosh, ML, Warncke, DD and Lucas,RE, 1994. Secondary and Micronutrients for vegetables and field crops
159. Vidya, C., Hiremath, S., Chandraprabha, M. N., Antonyraj, M. L., Gopal, I. V., Jain, A., & Bansal, K. (2013). Green synthesis of ZnO nanoparticles by Calotropis gigantea. *Int J Curr Eng Technol*, *1*(1), 118-120.
160. Aladpoosh, R., & Montazer, M. (2015). The role of cellulosic chains of cotton in biosynthesis of ZnO nanorods producing multifunctional properties: mechanism, characterizations and features. *Carbohydrate polymers*, *126*, 122-129.
161. Krupa, A. N. D., & Vimala, R. (2016). Evaluation of tetraethoxysilane (TEOS) sol–gel coatings, modified with green synthesized zinc oxide nanoparticles for combating microfouling. *Materials Science and Engineering: C*, *61*, 728-735.
162. Elumalai, K., Velmurugan, S., Ravi, S., Kathiravan, V., & Ashokkumar, S. (2015). RETRACTED: green synthesis of zinc oxide nanoparticles using Moringa oleifera leaf extract and evaluation of its antimicrobial activity.

163. Mitra, S., Patra, P., Pradhan, S., Debnath, N., Dey, K. K., Sarkar, S., ... & Goswami, A. (2015). Microwave synthesis of ZnO@ mSiO2 for detailed antifungal mode of action study: understanding the insights into oxidative stress. *Journal of colloid and interface science*, *444*, 97-108.
164. Aladpoosh, R., Montazer, M., & Samadi, N. (2014). In situ green synthesis of silver nanoparticles on cotton fabric using Seidlitzia rosmarinus ashes. *Cellulose*, *21*(5), 3755-3766.
165. Rajkuberan, C., Prabukumar, S., Sathishkumar, G., Wilson, A., Ravindran, K., & Sivaramakrishnan, S. (2017). Facile synthesis of silver nanoparticles using Euphorbia antiquorum L. latex extract and evaluation of their biomedical perspectives as anticancer agents. *Journal of Saudi Chemical Society*, *21*(8), 911-919.
166. Yuvakkumar, R., Suresh, J., Saravanakumar, B., Nathanael, A. J., Hong, S. I., & Rajendran, V. (2015). Rambutan peels promoted biomimetic synthesis of bioinspired zinc oxide nanochains for biomedical applications. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *137*, 250-258.
167. Sabir, S., Arshad, M., & Chaudhari, S. K. (2014). Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. *The Scientific World Journal*, *2014*.
168. Ambika, S., & Sundrarajan, M. (2015). Green biosynthesis of ZnO nanoparticles using Vitex negundo L. extract: spectroscopic investigation of interaction between ZnO nanoparticles and human serum albumin. *Journal of Photochemistry and Photobiology B: Biology*, *149*, 143-148.
169. Kavithaa, K., Paulpandi, M., Ponraj, T., Murugan, K., & Sumathi, S. (2016). Induction of intrinsic apoptotic pathway in human breast cancer (MCF-7) cells through facile biosynthesized zinc oxide nanorods. *Karbala International Journal of Modern Science*, *2*(1), 46-55.
170. Ravikumar, S., Gokulakrishnan, R., & Boomi, P. (2012). In vitro antibacterial activity of the metal oxide nanoparticles against urinary tract infectious bacterial pathogens. *Asian Pacific Journal of Tropical Disease*, *2*(2), 85-89.
171. Kumari, B., Sharma, S., Singh, N., Satsangi, V. R., Dass, S., & Shrivastav, R. (2015). Chemically etched ZnO thin films, with surface-evolved nano-ridges, for efficient photoelectrochemical splitting of water. *Journal of Solid State Electrochemistry*, *19*(5), 1311-1320.
172. Jiang, J., Pi, J., & Cai, J. (2018). The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorganic chemistry and applications*, *2018*.
173. Khan, M. M., Khan, M. W., Alhoshan, M., AlSalhi, M. S., & Aldwayyan, A. S. (2010). Influences of Co doping on the structural and optical properties of ZnO nanostructured. *Applied Physics A*, *100*(1), 45-51.
174. Bettini, S., Pagano, R., Bonfrate, V., Maglie, E., Manno, D., Serra, A., ... & Giancane, G. (2015). Promising piezoelectric properties of new ZnO@ octadecylamine adduct. *The Journal of Physical Chemistry C*, *119*(34), 20143-20149.
175. Pagano, R., Quarta, A., Pal, S., Licciulli, A., Valli, L., & Bettini, S. (2017). Enhanced solar-driven applications of ZnO@ Ag patchy nanoparticles. *The Journal of Physical Chemistry C*, *121*(48), 27199-27206.
176. Bettini, S., Pagano, R., Valli, L., & Giancane, G. (2016). Enhancement of Open Circuit Voltage of a ZnO-Based Dye-Sensitized Solar Cell by Means of Piezotronic Effect. *Chemistry–An Asian Journal*, *11*(8), 1240-1245.
177. Fracasso, G., Ghigna, P., Nodari, L., Agnoli, S., Badocco, D., Pastore, P., ... & Amendola, V. (2018). Nanoaggregates of iron poly-oxo-clusters obtained by laser ablation in aqueous solution of phosphonates. *Journal of colloid and interface science*, *522*, 208-216.
178. Spanhel, L., & Anderson, M. A. (1991). Semiconductor clusters in the sol-gel process: quantized aggregation, gelation, and crystal growth in concentrated zinc oxide colloids. *Journal of the American Chemical Society*, *113*(8), 2826-2833.
179. Rani, S., Suri, P., Shishodia, P. K., & Mehra, R. M. (2008). Synthesis of nanocrystalline ZnO powder via sol–gel route for dye-sensitized solar cells. *Solar Energy Materials and Solar Cells*, *92*(12), 1639-1645.
180. Wang, Z., Zhang, H., Zhang, L., Yuan, J., Yan, S., & Wang, C. (2002). Low-temperature synthesis of ZnO nanoparticles by solid-state pyrolytic reaction. *Nanotechnology*, *14*(1), 11.
181. Shen, L., Bao, N., Yanagisawa, K., Domen, K., Gupta, A., & Grimes, C. A. (2006). Direct synthesis of ZnO nanoparticles by a solution-free mechanochemical reaction. *Nanotechnology*, *17*(20), 5117.
182. Pardeshi, S. K., & Patil, A. B. (2009). Effect of morphology and crystallite size on solar photocatalytic activity of zinc oxide synthesized by solution free mechanochemical method. *Journal of Molecular Catalysis A: Chemical*, *308*(1-2), 32-40.
183. Mahendra, C., Murali, M., Manasa, G., Ponnamma, P., Abhilash, M. R., Lakshmeesha, T. R., ... & Sudarshana, M. S. (2017). Antibacterial and antimitotic potential of bio-fabricated zinc oxide nanoparticles of Cochlospermum religiosum (L.). *Microbial pathogenesis*, *110*, 620-629.
184. Fu, L., & Fu, Z. (2015). Plectranthus amboinicus leaf extract–assisted biosynthesis of ZnO nanoparticles and their photocatalytic activity. *Ceramics International*, *41*(2), 2492-2496.
185. Rajakumar, G., Thiruvengadam, M., Mydhili, G., Gomathi, T., & Chung, I. M. (2018). Green approach for synthesis of zinc oxide nanoparticles from Andrographis paniculata leaf extract and evaluation of their antioxidant, anti-diabetic, and anti-inflammatory activities. *Bioprocess and biosystems engineering*, *41*(1), 21-30.
186. Qian, Y., Yao, J., Russel, M., Chen, K., & Wang, X. (2015). Characterization of green synthesized nano-formulation (ZnO–A. vera) and their antibacterial activity against pathogens. *Environmental Toxicology and Pharmacology*, *39*(2), 736-746.
187. Ali, K., Dwivedi, S., Azam, A., Saquib, Q., Al-Said, M. S., Alkhedhairy, A. A., & Musarrat, J. (2016). Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. *Journal of colloid and interface science*, *472*, 145-156.

188. Yuvakkumar, R., Suresh, J., Nathanael, A. J., Sundrarajan, M., & Hong, S. I. (2014). Novel green synthetic strategy to prepare ZnO nanocrystals using rambutan (Nephelium lappaceum L.) peel extract and its antibacterial applications. *Materials Science and Engineering: C*, *41*, 17-27.
189. Nagajyothi, P. C., Cha, S. J., Yang, I. J., Sreekanth, T. V. M., Kim, K. J., & Shin, H. M. (2015). Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using Polygala tenuifolia root extract. *Journal of Photochemistry and Photobiology B: Biology*, *146*, 10-17.
190. Janaki, A. C., Sailatha, E., & Gunasekaran, S. (2015). Synthesis, characteristics and antimicrobial activity of ZnO nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *144*, 17-22.
191. Dobrucka, R., & Długaszewska, J. (2016). Biosynthesis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract. *Saudi journal of biological sciences*, *23*(4), 517-523.
192. Qu, J., Yuan, X., Wang, X., & Shao, P. (2011). Zinc accumulation and synthesis of ZnO nanoparticles using Physalis alkekengi L. *Environmental pollution*, *159*(7), 1783-1788.
193. Sharma, D., Sabela, M. I., Kanchi, S., Mdluli, P. S., Singh, G., Stenström, T. A., & Bisetty, K. (2016). Biosynthesis of ZnO nanoparticles using Jacaranda mimosifolia flowers extract: synergistic antibacterial activity and molecular simulated facet specific adsorption studies. *Journal of Photochemistry and Photobiology B: Biology*, *162*, 199-207.
194. Aruoja, V., Dubourguier, H. C., Kasemets, K., & Kahru, A. (2009). Toxicity of nanoparticles of CuO, ZnO and TiO2 to microalgae Pseudokirchneriella subcapitata. *Science of the total environment*, *407*(4), 1461-1468.
195. Qu, J., Luo, C., & Hou, J. (2011). Synthesis of ZnO nanoparticles from Zn-hyperaccumulator (Sedum alfredii Hance) plants. *Micro & Nano Letters*, *6*(3), 174-176.
196. Ochieng, P. E., Iwuoha, E., Michira, I., Masikini, M., Ondiek, J., Githira, P., & Kamau, G. N. (2015). Green route synthesis and characterization of ZnO nanoparticles using Spathodea campanulata. *Int. J. Biochem. Phys*, *23*, 53-61.
197. Rajeshkumar, S., Malarkodi, C., Vanaja, M., & Annadurai, G. (2016). Anticancer and enhanced antimicrobial activity of biosynthesizd silver nanoparticles against clinical pathogens. *Journal of molecular structure*, *1116*, 165-173.
198. Yasmin, A., Ramesh, K., & Rajeshkumar, S. (2014). Optimization and stabilization of gold nanoparticles by using herbal plant extract with microwave heating. *Nano convergence*, *1*(1), 1-7.
199. Arfat, Y. A., Benjakul, S., Prodpran, T., Sumpavapol, P., & Songtipya, P. (2014). Properties and antimicrobial activity of fish protein isolate/fish skin gelatin film containing basil leaf essential oil and zinc oxide nanoparticles. *Food Hydrocolloids*, *41*, 265-273.
200. Bhuyan, T., Mishra, K., Khanuja, M., Prasad, R., & Varma, A. (2015). Biosynthesis of zinc oxide nanoparticles from Azadirachta indica for antibacterial and photocatalytic applications. *Materials Science in Semiconductor Processing*, *32*, 55-61.
201. Mari, A., Mookkaiah, R., & Elayaperumal, M. (2019). Emblica officinalis leaf extract mediated synthesis of zinc oxide nanoparticles for antibacterial and photocatalytic activities. *Asian Journal of Green Chemistry*, *3*(4), 418-431.
202. Udayabhanu, G. N., Nagabhushana, H., Basavaraj, R. B., Raghu, G. K., Suresh, D., Rajanaika, H., & Sharma, S. C. (2016). Green, non-chemical route for the synthesis of ZnO superstructures, evaluation of its applications towards photocatalysis, photoluminescence and bio-sensing. *Cryst. Growth Des*, *16*(12), 6828-6840.
203. Raliya, R., & Tarafdar, J. C. (2013). ZnO nanoparticle biosynthesis and its effect on phosphorous-mobilizing enzyme secretion and gum contents in Clusterbean (Cyamopsis tetragonoloba L.). *Agricultural Research*, *2*(1), 48-57.
204. Otari, S. V., Patil, R. M., Nadaf, N. H., Ghosh, S. J., & Pawar, S. H. (2012). Green biosynthesis of silver nanoparticles from an actinobacteria Rhodococcus sp. *Materials Letters*, *72*, 92-94.
205. Tripathi, R. M., Bhadwal, A. S., Gupta, R. K., Singh, P., Shrivastav, A., & Shrivastav, B. R. (2014). ZnO nanoflowers: novel biogenic synthesis and enhanced photocatalytic activity. *Journal of Photochemistry and Photobiology B: Biology*, *141*, 288-295.
206. Mehta, S. K., Kumar, S., Chaudhary, S., Bhasin, K. K., & Gradzielski, M. (2009). Evolution of ZnS nanoparticles via facile CTAB aqueous micellar solution route: a study on controlling parameters. *Nanoscale research letters*, *4*(1), 17-28.
207. Kundu, D., Hazra, C., Chatterjee, A., Chaudhari, A., & Mishra, S. (2014). Extracellular biosynthesis of zinc oxide nanoparticles using Rhodococcus pyridinivorans NT2: multifunctional textile finishing, biosafety evaluation and in vitro drug delivery in colon carcinoma. *Journal of photochemistry and photobiology B: Biology*, *140*, 194-204.
208. Singh, B. N., Rawat, A. K. S., Khan, W., Naqvi, A. H., & Singh, B. R. (2014). Biosynthesis of stable antioxidant ZnO nanoparticles by Pseudomonas aeruginosa rhamnolipids. *PLoS One*, *9*(9), e106937.
209. Thema, F. T., Manikandan, E., Dhlamini, M. S., & Maaza, M. (2015). Green synthesis of ZnO nanoparticles via Agathosma betulina natural extract. *Materials Letters*, *161*, 124-127.
210. Bird, S. M., El-Zubir, O., Rawlings, A. E., Leggett, G. J., & Staniland, S. S. (2016). A novel design strategy for nanoparticles on nanopatterns: interferometric lithographic patterning of Mms6 biotemplated magnetic nanoparticles. *Journal of Materials Chemistry C*, *4*(18), 3948-3955.
211. Rajiv, P., Rajeshwari, S., & Venckatesh, R. (2013). Bio-Fabrication of zinc oxide nanoparticles using leaf extract of Parthenium hysterophorus L. and its size-dependent antifungal activity against plant fungal pathogens. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *112*, 384-387.

212. Azizi, S., Ahmad, M. B., Namvar, F., & Mohamad, R. (2014). Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalga Sargassum muticum aqueous extract. *Materials Letters*, *116*, 275-277.
213. Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M., & Thamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *Journal of hazardous materials*, *146*(1-2), 270-277.
214. Pavani, K. V., Kumar, N. S., & Sangameswaran, B. B. (2012). Synthesis of lead nanoparticles by Aspergillus species. *Polish Journal of Microbiology*, *61*(1), 61-63.
215. Hoffmann, M. R., Martin, S. T., Choi, W., & Bahnemann, D. W. (1995). Environmental applications of semiconductor photocatalysis. *Chemical reviews*, *95*(1), 69-96.
216. Mashrai, A., Khanam, H., & Aljawfi, R. N. (2017). Biological synthesis of ZnO nanoparticles using C. albicans and studying their catalytic performance in the synthesis of steroidal pyrazolines. *Arabian Journal of Chemistry*, *10*, S1530-S1536.
217. Nagarajan, S., & Kuppusamy, K. A. (2013). Extracellular synthesis of zinc oxide nanoparticle using seaweeds of gulf of Mannar, India. *Journal of nanobiotechnology*, *11*(1), 1-11.
218. Asokan, A., Ramachandran, T., Ramaswamy, R., Koushik, C. V., & Muthusamy, M. (2010). Preparation and characterization of zinc oxide nanoparticles and a study of the anti-microbial property of cotton fabric treated with the particles. *Journal of Textile and Apparel, Technology and Management*, *6*(4).
219. Gharagozlou, M., Baradaran, Z., & Bayati, R. (2015). A green chemical method for synthesis of ZnO nanoparticles from solid-state decomposition of Schiff-bases derived from amino acid alanine complexes. *Ceramics International*, *41*(7), 8382-8387.
220. Păunica-Panea, G., Ficai, A., Marin, M. M., Marin, Ş., Albu, M. G., Constantin, V. D., ... & Ghica, M. V. (2016). New collagen-dextran-zinc oxide composites for wound dressing. *Journal of Nanomaterials*, *2016*.
221. McClain, P. E., Wiley, E. R., Beecher, G. R., Anthony, W. L., & Hsu, J. M. (1973). Influence of zinc deficiency on synthesis and cross-linking of rat skin collagen. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *304*(2), 457-465.
222. Prockop, D. J., Sieron, A. L., & Li, S. W. (1998). Procollagen N-proteinase and procollagen C-proteinase. Two unusual metalloproteinases that are essential for procollagen processing probably have important roles in development and cell signaling. *Matrix Biology*, *16*(7), 399-408.
223. Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., ... & Mohamad, D. (2015). Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nano-micro letters*, *7*(3), 219-242.
224. Kemung, H. M., Tan, L. T. H., Khaw, K. Y., Ong, Y. S., Chan, C. K., Low, D. Y. S., ... & Goh, B. H. (2020). An optimized anti-adherence and anti-biofilm assay: Case study of zinc oxide nanoparticles versus MRSA biofilm. *Progress In Microbes & Molecular Biology*, *3*(1).
225. Rasool, A., Ata, S., & Islam, A. (2019). Stimuli responsive biopolymer (chitosan) based blend hydrogels for wound healing application. *Carbohydrate polymers*, *203*, 423-429.
226. Sajjad, W., Khan, T., Ul-Islam, M., Khan, R., Hussain, Z., Khalid, A., & Wahid, F. (2019). Development of modified montmorillonite-bacterial cellulose nanocomposites as a novel substitute for burn skin and tissue regeneration. *Carbohydrate polymers*, *206*, 548-556.
227. Shahzad, A., Khan, A., Afzal, Z., Umer, M. F., Khan, J., & Khan, G. M. (2019). Formulation development and characterization of cefazolin nanoparticles-loaded cross-linked films of sodium alginate and pectin as wound dressings. *International journal of biological macromolecules*, *124*, 255-269.
228. Prasanna, A. P. S., Niranjan, R., Kaushik, M., Devasena, T., Kumar, J., Chelliah, R., ... & Swaminathan, S. (2018). Metal oxide curcumin incorporated polymer patches for wound healing. *Applied Surface Science*, *449*, 603-609.
229. Dincă, V., Mocanu, A., Isopencu, G., Busuioc, C., Brajnicov, S., Vlad, A., ... & Suchea, M. (2020). Biocompatible pure ZnO nanoparticles-3D bacterial cellulose biointerfaces with antibacterial properties. *Arabian Journal of Chemistry*, *13*(1), 3521-3533.
230. Janpetch, N., Saito, N., & Rujiravanit, R. (2016). Fabrication of bacterial cellulose-ZnO composite via solution plasma process for antibacterial applications. *Carbohydrate polymers*, *148*, 335-344.
231. Khalid, A., Khan, R., Ul-Islam, M., Khan, T., & Wahid, F. (2017). Bacterial cellulose-zinc oxide nanocomposites as a novel dressing system for burn wounds. *Carbohydrate polymers*, *164*, 214-221.
232. Abdalkarim, S. Y. H., Yu, H. Y., Song, M. L., Zhou, Y., Yao, J., & Ni, Q. Q. (2017). In vitro degradation and possible hydrolytic mechanism of PHBV nanocomposites by incorporating cellulose nanocrystal-ZnO nanohybrids. *Carbohydrate polymers*, *176*, 38-49.
233. Shefa, A. A., Taz, M., Hossain, M., Kim, Y. S., Lee, S. Y., & Lee, B. T. (2019). Investigation of efficiency of a novel, zinc oxide loaded TEMPO-oxidized cellulose nanofiber based hemostat for topical bleeding. *International journal of biological macromolecules*, *126*, 786-795.
234. Ishihara, J., Ishihara, A., Fukunaga, K., Sasaki, K., White, M. J., Briquez, P. S., & Hubbell, J. A. (2018). Laminin heparin-binding peptides bind to several growth factors and enhance diabetic wound healing. *Nature communications*, *9*(1), 1-14.
235. Joorabloo, A., Khorasani, M. T., Adeli, H., Mansoori-Moghadam, Z., & Moghaddam, A. (2019). Fabrication of heparinized nano ZnO/poly (vinylalcohol)/carboxymethyl cellulose bionanocomposite hydrogels using artificial neural network for wound dressing application. *Journal of Industrial and Engineering Chemistry*, *70*, 253-263.
236. Rakhshaei, R., & Namazi, H. (2017). A potential bioactive wound dressing based on carboxymethyl cellulose/ZnO impregnated MCM-41 nanocomposite hydrogel. *Materials Science and Engineering: C*, *73*, 456-464.

237. Yusof, N. A. A., Zain, N. M., & Pauzi, N. (2019). Synthesis of ZnO nanoparticles with chitosan as stabilizing agent and their antibacterial properties against Gram-positive and Gram-negative bacteria. *International journal of biological macromolecules*, *124*, 1132-1136.
238. Bharathi, D., Ranjithkumar, R., Chandarshekar, B., & Bhuvaneshwari, V. (2019). Preparation of chitosan coated zinc oxide nanocomposite for enhanced antibacterial and photocatalytic activity: as a bionanocomposite. *International journal of biological macromolecules*, *129*, 989-996.
239. Lu, Z., Gao, J., He, Q., Wu, J., Liang, D., Yang, H., & Chen, R. (2017). Enhanced antibacterial and wound healing activities of microporous chitosan-Ag/ZnO composite dressing. *Carbohydrate polymers*, *156*, 460-469.
240. Gutha, Y., Pathak, J. L., Zhang, W., Zhang, Y., & Jiao, X. (2017). Antibacterial and wound healing properties of chitosan/poly (vinyl alcohol)/zinc oxide beads (CS/PVA/ZnO). *International journal of biological macromolecules*, *103*, 234-241.
241. Zhai, M., Xu, Y., Zhou, B., & Jing, W. (2018). Keratin-chitosan/n-ZnO nanocomposite hydrogel for antimicrobial treatment of burn wound healing: Characterization and biomedical application. *Journal of Photochemistry and Photobiology B: Biology*, *180*, 253-258.
242. Jayakumar, R., Prabaharan, M., Nair, S. V., Tokura, S., Tamura, H., & Selvamurugan, N. (2010). Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. *Progress in Materials Science*, *55*(7), 675-709.
243. Karahaliloglu, Z., Kilicay, E., & Denkbas, E. B. (2017). Antibacterial chitosan/silk sericin 3D porous scaffolds as a wound dressing material. *Artificial cells, nanomedicine, and biotechnology*, *45*(6), 1172-1185.
244. Tarnow, P., Ågren, M., Steenfos, H., & Jansson, J. O. (1994). Topical zinc oxide treatment increases endogenous gene expression of insulin-like growth factor-1 in granulation tissue from porcine wounds. *Scandinavian journal of plastic and reconstructive surgery and hand surgery*, *28*(4), 255-259.
245. Ågren, M. S. (1990). Studies on zinc in wound healing.
246. Parwani, L., Bhatnagar, M., Bhatnagar, A., Sharma, V., & Sharma, V. (2019). Gum acacia-PVA hydrogel blends for wound healing. *Vegetos*, *32*(1), 78-91.
247. Raguvaran, R., Manuja, B. K., Chopra, M., Thakur, R., Anand, T., Kalia, A., & Manuja, A. (2017). Sodium alginate and gum acacia hydrogels of ZnO nanoparticles show wound healing effect on fibroblast cells. *International journal of biological macromolecules*, *96*, 185-191.
248. Shi, M., Zhang, H., Song, T., Liu, X., Gao, Y., Zhou, J., & Li, Y. (2019). Sustainable dual release of antibiotic and growth factor from pH-responsive uniform alginate composite microparticles to enhance wound healing. *ACS applied materials & interfaces*, *11*(25), 22730-22744.
249. Satish, A., Aswathi, R., Maria, J. C., & Korrapati, P. S. (2019). Triiodothyronine impregnated alginate/gelatin/polyvinyl alcohol composite scaffold designed for exudate-intensive wound therapy. *European Polymer Journal*, *110*, 252-264.
250. Varaprasad, K., Raghavendra, G. M., Jayaramudu, T., & Seo, J. (2016). Nano zinc oxide–sodium alginate antibacterial cellulose fibres. *Carbohydrate polymers*, *135*, 349-355.
251. Gong, C. P., Luo, Y., & Pan, Y. Y. (2019). Novel synthesized zinc oxide nanoparticles loaded alginate-chitosan biofilm to enhanced wound site activity and anti-septic abilities for the management of complicated abdominal wound dehiscence. *Journal of Photochemistry and Photobiology B: Biology*, *192*, 124-130.
252. Wang, Y. T., Shi, T. Q., Fu, J., & Zhu, H. L. (2019). Discovery of novel bacterial FabH inhibitors (Pyrazol-Benzimidazole amide derivatives): Design, synthesis, bioassay, molecular docking and crystal structure determination. *European journal of medicinal chemistry*, *171*, 209-220.
253. Mohandas, A., Sudheesh Kumar, P. T., Raja, B., Lakshmanan, V. K., & Jayakumar, R. (2015). Exploration of alginate hydrogel/nano zinc oxide composite bandages for infected wounds. *International journal of nanomedicine*, *10*(Suppl 1), 53.

CITATION OF THIS ARTICLE

M.Balalakshitha, K.Kolanjinathan ,T.Aishwarya and B.Vinothini. A Review on Diabetic Wound Infection and Its Treatment Using Zinc Oxide Nanoparticle Synthesized by Green Chemistry Techniques. Bull. Env. Pharmacol. Life Sci., Vol 10[10] September 2021 : 01-22.