PERSPECTIVE ARTICLE

PLGA based drug delivery systems: Promising carriers for wound healing activity

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ABSTRACT

Wound treatment remains one of the most prevalent and economically burdensome healthcare issues in the world. Current treatment options are limited and require repeated administrations which led to the development of new therapeutics to satisfy the unmet clinical needs. Many potent wound healing agents were discovered but most of them are fragile and/or sensitive to in vivo conditions. Poly(lactic-co-glycolic acid) (PLGA) is a widely used biodegradable polymer approved by food and drug administration and European medicines agency as an excipient for parenteral administrations. It is a well-established drug delivery system in various medical applications. The aim of the current review is to elaborate the applications of PLGA based drug delivery systems carrying different wound healing agents and also present PLGA itself as a wound healing promoter. PLGA carriers encapsulating drugs such as antibiotics, anti-inflammatory drugs, proteins/peptides, and nucleic acids targeting various phases/signaling cycles of wound healing, are discussed with examples. The combined therapeutic effects of PLGA and a loaded drug on wound healing are also mentioned.

Wound care is one of the major economic burdensome problems in the healthcare system.[1] Pathological conditions such as diabetes, arterial, and venous insufficiency and lymphedema may lead to serious chronic wounds and associated complications.[2] Consequently, wound management appears as one of the serious problems that the clinical sector is facing today.

WOUND HEALING

According to Wound Healing Society, a wound is due to the disruption of normal anatomical structure and function and healing is a dynamic complex process involving restoration of anatomic continuity and function.[3] Wounds have been classified based on their appearance, number of layers disrupted, cause of injury and repair process. In addition, based on the repair process, wounds can be acute (complete healing and minimal scarring) or chronic (slow healing and repetitive tissue insult). Acute wounds are caused by mechanical and chemical injuries and different types of burns like radiation, thermal sources, and so forth.[4] whereas chronic wounds include diabetic ulcers like bedsores or pressure sores and leg ulcers because of ischemia or venous reasons.[5,6] The healing of wound is a complex biological process, which involves an interdependent, and overlapping sequence of physiological actions. Concisely, the important phases are as follows: inflammation, proliferation and maturation[7] which have been displayed in the Figure 1. The wound healing is initiated by the bleeding which flushes the microbes and antigens resulting in clotting and hemostasis.[9] Clotting factors released start the clotting cascade. Clotting process is not merely a visible event but also serves as a dynamic infiltration of incoming inflammatory cells, fibroblasts and growth factors.[10] Fibrin monomers are produced by the proteolytic breaking of

CC	Curcumin
ECM	Extra cellular matrix
EGF	Epidermal growth factor
EMA	European medicines agency
FDA	Food and drug administration
FGF	Fibroblast growth factor
GMP	Good manufacturing practices
GPx	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
HGF	Hepatocyte growth factor
IGF	Insulin-like growth factor
IL	Interleukin
NO	Nitric oxide
NP	Nanoparticles
PDGF	Platelet-derived growth factor
PEG	Polyethylene glycol
PHD	Prolylhydroxylase
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PMN	Polymorphonuclear cells
PMS-CM PLGA	Microparticulate scaffolds coupled with cytomodulin
PPAR	Peroxisome proliferator-activated receptor
rhEGF	Recombinant human epidermal growth factor
S1P	Sphingosine-1-phosphate
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VEGF	Vascular endothelial growth factor

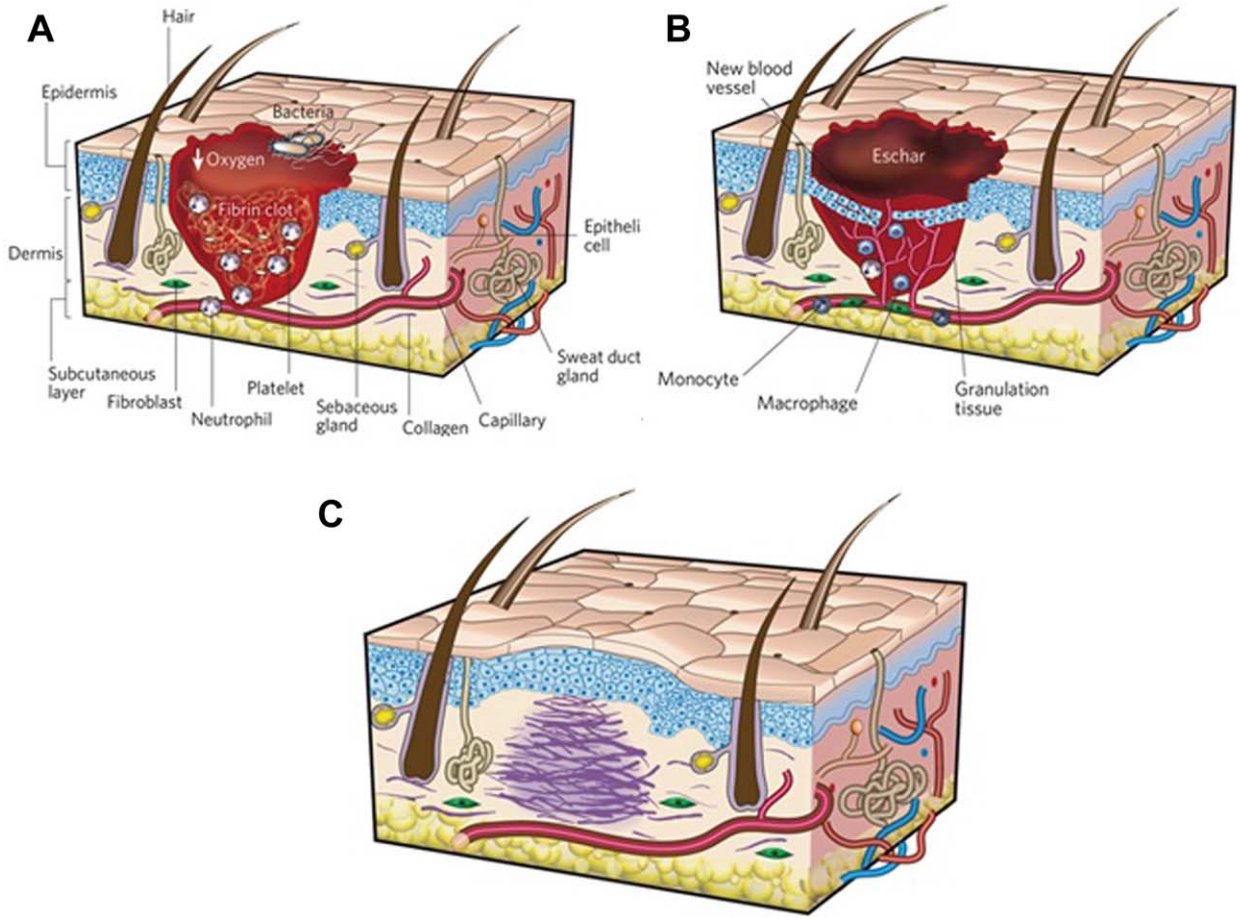


Figure 1. Different phases of wound healing. There are three classic stages of wound repair: (A) Hemostasis and inflammation, (B) Proliferation, and (C) Maturation. (A) Inflammation. This stage lasts until about 24–48 h after injury. The wound is characterized by clot formation and an internal hypoxic (ischemic) environment in which a fibrin clot has formed. Bacteria, neutrophils and platelets infiltrate into the wound. Normal skin appendages (such as hair follicles and sweat duct glands) are still present in the skin outside the wound. (B) Proliferation. This stage occurs about 2–10 days after injury. The picture illustrates a wound at 5-10 days and an eschar (scab) has formed on the surface of the wound. Most cells from the previous stage of repair have migrated from the wound, and new blood vessels now populate the area. Epithelial cells migration can be clearly observed under the eschar. (C) Maturation. This stage lasts for a days to year or longer. Disorganized collagen has been laid down by fibroblasts that have migrated into the wound. The wound has contracted near its surface and the reepithelialized wound is slightly higher than the surrounding surface. The healed region does not contain normal skin appendages.[8]

fibrinogen by thrombin, cross link with each other and bind to platelets to form the clot. Also fibrin serves as a repository for growth factors and cytokines. Fibroblast growth factor (FGF)-2 and vascular endothelial growth factor (VEGF) bind fibrin and stimulate angiogenesis, whereas insulin-like growth factor (IGF)-1 binds fibrin and stimulates stromal cell function and proliferation. The infiltrated platelets function as reservoirs for the growth factors like platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, FGF-2, VEGF, hepatocyte growth factor (HGF), IGF, epidermal growth factor (EGF), and sphingosine-1-phosphate (S1P) which influence many cells- including fibroblasts, keratinocytes, and endothelial cells and participate in all phases of wound healing.[11]

Inflammation proceeds simultaneously involving a release of protein rich exudates leading to vasodilation. This stage lasts for about 24–48 hours after injury. Different cells are involved in various functions during inflammation phase:(1) Mast cells promote vasodilation by producing histamine and leukotrines C_4 and D_4; (2) Polymorphonuclear cells (PMNs) serve as early cleaners of cellular debris, bacteria and are major source of several proinflammatory cytokines, such as interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor (TNF)-α; (3) Fibrocytes are involved in collagen and cytokine production; and (4) Eosinophils induce the production of TGF-α leading to reepithelialization of keratinocytes.

Migration and proliferation of fibroblasts and epithelial cells follow the inflammation phase. This stage occurs

about 2–10 days after injury. Fibroblasts produce collagen based extra cellular matrix (ECM) that replaces the provisional fibrin based matrix and also aid in wound contraction. Coupled with angiogenesis, ingrowth of capillaries and lymphatic vessels into the wound forms the granulation tissue and the fibroblasts synthesize collagen giving strength and form to the skin.[12] Local environmental changes in the wound such as increased lactate, decreased pH, and low oxygen tension stimulate angiogenesis. Several growth factors particularly VEGF, FGF, angiopoietin, TGF-β and ECM, and cytokines, produced during the inflammatory phase of wound healing also stimulate and regulate angiogenesis.[13]

The reepithelialization process progresses with the migration and proliferation of keratinocytes from the close proximity of wound edges and hair bulges. Factors such as low calcium, high magnesium, produced peptides and proteins, pH changes and hypoxia contribute mostly to the keratinocyte migration, proliferation and differentiation. Growth factors FGF-2, -7, -10 and TGF-β have positive influence on reepithelialization.[14]

Maturation or tissue remodeling involves formation of cellular connective tissue and strengthening of newly formed epithelium.[15] Macrophages and fibroblasts play a key role in remodeling. The clinical manifestations involved in maturation include wound contraction, decreased redness and thickness and increased strength. Cellular mechanisms involved in remodeling are extracellular matrix reshaping by cross-linking collagens, maturation of cells and apoptosis.[16,17]

Wound healing processes occur in a systematic way aiming to heal the injury. Healing process needs the active participation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets and production of growth factors and cytokines released by these cell types.[18,19] The severe disturbances in the aforesaid cellular functions could potentially impair the wound healing in diabetic conditions. There are many known physiological factors that can contribute to wound healing deficiencies in diabetic patients. These include decreased or impaired growth factor production, angiogenic response, macrophage function, collagen accumulation, epidermal barrier function, quantity of granulation tissue, keratinocyte and fibroblast migration and proliferation, number of epidermal nerves, bone healing, and balance between the accumulation of ECM components and their remodeling by MMPs.[20,21] The distinctive feature of diabetic patient is neuropathy and angiopathy, which could be causative for the impairment of healing. As the patients are unable to mount an adequate inflammatory response, they also lack ability to fight infections. This may lead to sepsis and require limb amputation.[22]

WOUND MANAGEMENT

A successful treatment of wounds requires assessment of the patient condition and the wound in particular because systemic problems often impair wound healing; conversely, nonhealing wounds may herald systemic pathology. For health care practitioners, the first step would be educating the patient. In parallel, depending on the cause, type, age and severity of wound, presence of infection and other disorders/diseases, wound care and management are adopted. The current treatment options need repetitive doses, changing bandages, attention of nurse, hospitalization making them more and more expensive and limited. A major drawback is the frequent change of dressing, with an increased risk of infections. Particularly in case of chronic wounds where there is insufficient blood flow and local edema, healing of wounds is very difficult without any active treatment.[23] Different devices, dressings, drugs and delivery systems have been extensively investigated aiming to accelerate wound healing and expanding their applicability to several kinds of wounds.

Both the scientific and industrial facts underline the urgent need of research and development in wound care domain. Many potent wound healing agents were discovered but most of them are fragile and/or sensitive to in vivo conditions emphasizing that there should be an efficient delivery these agents to improve the existing wound care treatments. Indeed, this reduces the cost and increases applicability to wide range of wounds/patients and higher efficacy. Poly(lactic-co-glycolic acid) (PLGA) is one of the best drug delivery systems available for these purposes.

POLY(LACTIC-CO-GLYCOLIC ACID)

PLGA

PLGA, the copolymer of poly lactic acid and poly glycolic acid, is one of the widely used biodegradable polymers in medical applications. PLGA is approved by FDA and EMA for parenteral administration because of its high biocompatibility.[24] The commercial availability of GMP PLGA, favourable degradation in physiological conditions, possibility of tuning surface and physio-chemical properties, sustained drug release, presented PLGA as the most interesting polymeric drug carrier in many clinical applications.[25] PLGA undergoes hydrolysis, breaking its ester linkages to form lactic acid and glycolic acid monomers that can be easily metabolized by Krebs cycle[26] (Figure 2A). The increase in carboxylic end groups facilitates the further autocatalysis. Any change in PLGA molecular weight, chemical composition and surface modification may lead to change in its rate of degradation.[28]

Effects of lactate in wound healing

Lactate plays an important role in wound healing processes. One of the physiological consequences during wound healing process is the hypoxia which arises due to the microcirculatory damage and increased oxygen consumption by inflammatory cells.[29] The hypoxia atmosphere promotes the anaerobic respiration involving glycolysis and produces large amount of lactate as one of the end products.[30] The rapidly multiplying newly recruited and already existing cells and activated leukocytes and macrophages also release lactate due to oxidative burst producing reactive oxygen species which is meant to kill the bacteria and other invasive microbes.[31]

Lactate also produced as a byproduct of glycolysis pathway. Thomas K. Hunt and colleagues revealed interesting findings about how lactate involve in the wound healing. Hunt stated that increase in lactate levels in wounds was a major signal for collagen synthesis and wound repair.[32] The proposed mechanism was the activation of collagen prolylhydroxylase (PHD) in fibroblasts through a lactate-dependent decrease in PHD mono-ADPribosylation. Collagen prolylhydroxylase is an enzyme that controls procollagen hydroxylation and collagen maturation processes.[32] Lactate dehydrogenase 1 (enzyme

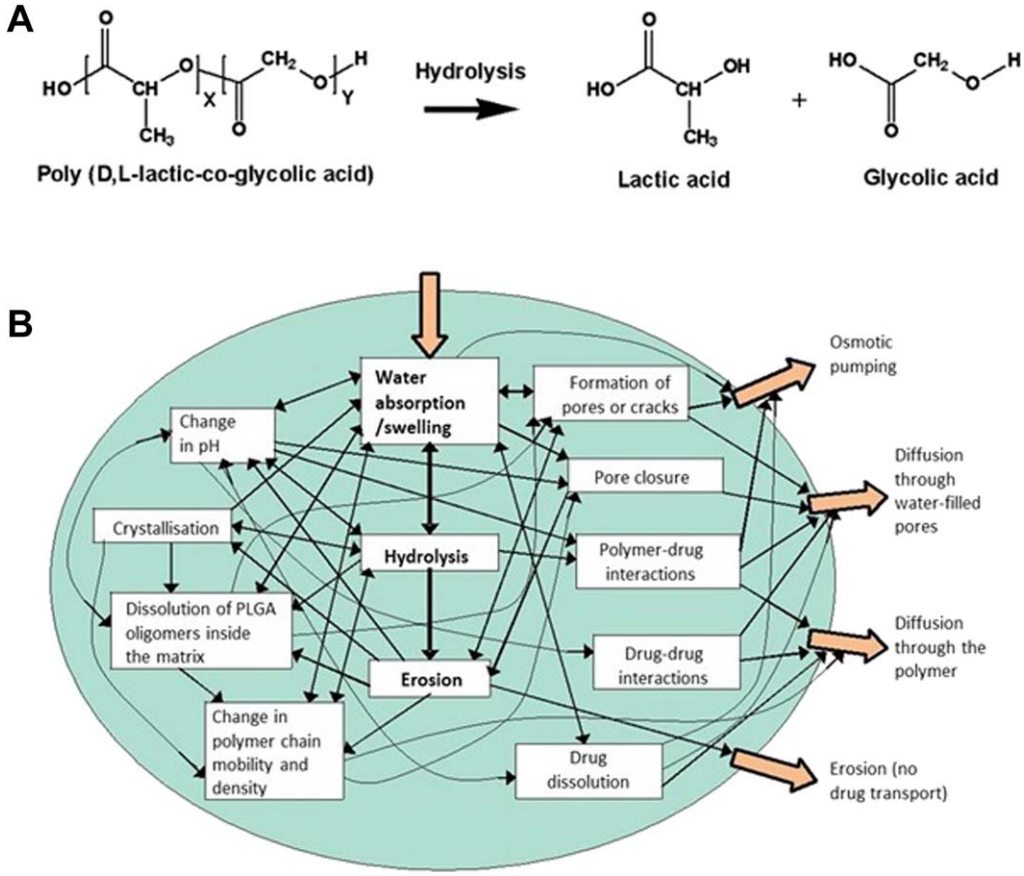


Figure 2. (A) Hydrolysis of PLGA. X and Y represent the number of units of lactic acid and glycolic acid respectively. (B) Complex web picture showing different processes involved in drug release from PLGA.[27]

catalyzes lactate to pyruvate) and cytoplasmic ADP-ribosyl transferase competes with each other resulting in limited availability of NAD^+ for PHD mono-ADP-ribosylation/inactivation. In macrophages and endothelial cells, similar competition occurs that lead to decreased mono-ADP-ribosylation/inactivation of VEGF. This is a major signaling agent for proangiogenesis.[33,34] Increase procollagen and VEGF transcription in fibroblasts were found to be influenced by substrate competition between LDH1 and nuclear poly-ADP-ribose polymerases.[32,34] In vitro data suggested that lactate can stimulate proliferation, migration and capillary formation of endothelial cells.[35,36] Evidences also showed that lactate induced angiogenesis and recruitment of endothelial progenitor cells and vasculogenesis in vivo.[35,37]

PLGA—itself a wound healing agent

Porporato et al. revealed that the application of exogenous lactate could accelerate angiogenesis and wound healing processes, suggesting PLGA as the most suitable polymers to supply lactate to accelerate reparative angiogenesis and wound healing.[38] Thorough understanding of the physical, chemical and biological properties of the PLGA and poly (lactic acid) (PLA) helps in choosing PLGA. As lactic acid is hydrophobic and hence lactide-rich PLA polymers are less hydrophilic, absorb less water and subsequently degrade more slowly.[39] When PLA and PLGA tested for lactate release studies in vivo, PLGA showed significant 4-fold increase in the plasma level of lactate compared to untreated mice and PLA did not release significant amounts of lactate indicating that PLGA can be exploited for sustained supply of lactate for the treatment of wounds. As oral administration of sodium lactate solution showed weaker activity than PLGA, Porporato et al. reported the use of PLGA as one of the strategies to supply lactate to achieve the goal of angiogenesis and wound healing. It was observed that nearly 60% decrease in wounded area in comparison to control group after 10 days of injury. PLGA appeared therefore as a realistic way to supply lactate therapeutically.[38]

PLGA as a drug carrier

Different drugs could be administered targeting different phases or signaling cycles of wound healing where PLGA based carriers could act as active drug carriers. Many innovative works have been reported in the literature, which highlighted the use of PLGA as a potential drug delivery

system to carry highly potent and fragile drugs. Because of its ability to deliver varied types of cargo efficiently and compatibility with other polymers, PLGA can be a first choice carrier. Moreover, if the wound remains superficial, it is sufficient that the formulated PLGA carrier could stay on the wound surface and release the encapsulated drug promoting the healing process.

Drug release profile plays an important role in determining the dose and dosage. PLGA carriers have a typical biphasic curve for drug release. The encapsulated drug type, concentration of drug and polymer hydrophobicity determines the rate of initial burst phase. During the second phase, the water hydrolyses the matrix and the drug is released progressively from the thicker layer of PLGA matrix.[40] Fredenberg et al. described the complex picture of physicochemical process occurring within the PLGA carrier polymer matrix leading to the release of drugs or biomolecules (Figure 2B).[27] The release profiles of drug molecules from PLGA carriers play an important role in wound treatment.

PLGA delivery systems in wound management

PLGA nanofibers/membranes

PLGA nanofibers have been developed for many biomedical applications because they are versatile, inexpensive, scalable and reliable.[41] Particularly in wound care domain they can also serve as a dressing material.[42] PLGA nanofibers have been produced with biodegradable polymers blended with active wound healing agents. The developed drug blended nanofibers enhance thermal stability and minimize in vivo degradability of loaded drug. In a view of a translational approach, PLGA nanofibers would be a good choice. Electrospinning has been widely used to produce nanofibers of different morphology.[43] Major challenges associated with this technique are choosing a good organic solvent to prepare the drug-PLGA blend, optimizing the condition of temperature, distance and voltage between spinneret and the fiber collector and other processing parameters which influence fiber diameter, porosity, strength, thickness of scaffold, drug loading.[44]

Chen et al. reported PLGA based novel biodegradable sandwich-structured nanofibrous membranes, prepared with a mixture of PLGA, collagen and antimicrobials (vancomycin, gentamicin, and lidocaine). Electrospinning method was used to produce these membranes with PLGA/collagen as the surface layers and PLGA/drugs as the core. In vivo release studies showed that the PLGA nanofibrous membranes exhibited a biphasic pattern with an abrupt initial burst phase followed by a sustained release. In a rat infected (*Staphylococcus aureus* and *Escherichia coli*) wound model, the nanofibrous membranes were functionally active in treating wound infections and significantly accelerated wound healing in in the early stages of healing. Histological examination of the wound tissues showed that the treated group exhibited greater healing and reepithelialized epidermis with newly synthesized fibrous tissue and sparse inflammatory cells in the dermis and subcutis.[45] Similar in vivo studies were performed to understand the interactions of fusidic acid-loaded PLGA ultrafine fibers with wound bacteria (*S. aureus*). Results showed that the incisions treated with these ultrafine fibers remained clean throughout the study indicating the early and sustained eradication of bacteria during healing process. This suggests that the PLGA ultrafine fibers were able to deliver fusidic acid sustainably to the wound, protecting tissues from reinfection.[46]

PLGA membranes containing (1,3)-(1,6)-β-D-glucan (natural immune stimulatory molecule) membranes accelerated wound healing by improving the interaction, proliferation of cells, and angiogenesis.[47] Wounds covered by Ginsenoside-Rg3 loaded hydrophilic PLGA electrospun fibrous membranes coated with chitosan showed faster healing and higher reepithelialization. These fibrous membranes also inhibited hypertrophic scars of the skin.[48] Silk fibroin and PLGA electrospun fibrous scaffolds showed significant decrease in the wound area in excision wound model in diabetic rats than that of either pure silk fibroin or pure PLGA scaffolds.[49] PLGA electrospun membranes containing Epigallocatechin-3-O-gallate exhibited faster wound healing in full-thickness wounds created in nude mice. The underlying mechanisms include enhanced cell infiltration, reepithelialization, and angiogenesis[50] PLGA-metformin electrospun nanofibrous membranes improved wound healing and reepithelialization in diabetic rats relative to the respective controls.[51] The same group also reported that electrospun nanofibrous metformin-loaded collagen/PLGA scaffold membranes were more hydrophilic than collagen/PLGA membranes and exhibited a greater water-containing capacity. When tested in vivo, metformin-loaded collagen/PLGA membranes markedly promoted the healing of diabetic wounds by increasing collagen deposition and reepithelialization.[52]

PLGA microspheres

PLGA microspheres and their associated scaffolds are one of the most promising carriers for the sustained release of drugs to wounds. Mittal et al. reported that porous PLGA microparticulate scaffolds coupled with cytomodulin (PMS-CM) showed increase in hydrophilicity compared to the controls. In vitro studies on human dermal fibroblasts revealed that cell distribution, cell spreading and actin production were significantly higher on PMS-CM than the control groups. In a full-thickness wound mouse model, PMS-CM showed great wound healing effect by reducing inflammatory responses, enhancing fibroblast proliferation and early formation of the scar tissue. Thus, PLGA microparticulate scaffolds ensured efficient delivery of cytomodulin to wounded area.[53] Gainza et al. reported the use of recombinant human epidermal growth factor (rhEGF) loaded PLGA-Alginate microspheres for active healing diabetic wounds. rhEGF-PLGA microspheres treated Wistar rats showed a statistically significant decrease of the wound area, a complete reepithelialization by day 11 and an earlier resolution of the inflammatory process.[54]

Co-delivery of different types of drugs both in terms of activity and chemical nature can be delivered with PLGA microspheres. PLGA microspheres simultaneously delivered chlorhexidine and PDGF to wounds and stimulated healing of infected wounds in a rat model. The dual delivery system decreased infection and induced higher levels of mature vasculature.[55] Thus, PLGA based drug delivery system can simultaneously deliver hydrophobic and/or hydrophilic molecules to wounds.

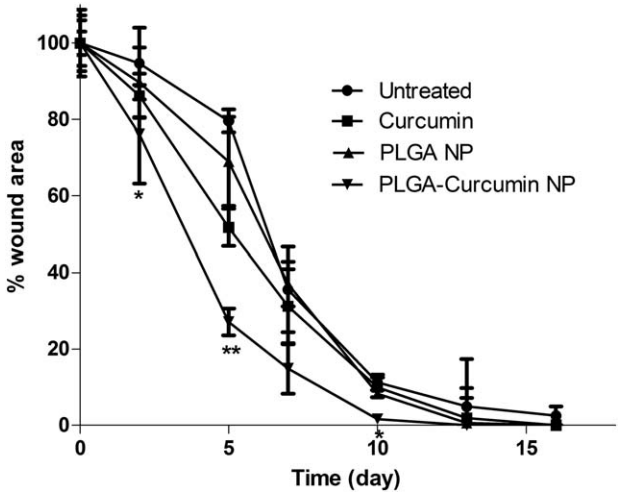


Figure 3. PLGA-CC NP accelerated wound healing in non-diabetic mice. Wounds closure over time was measured as percent of original area (day 5 $n = 10$; day 10 $n = 7$; data as mean ± SD).[88]

Due to its versatile properties, PLGA can be combined with other polymers to formulate different microparticle scaffolds to promote wound healing. A well-supported dermal substitute made of PLGA knitted mesh integrated with collagen-chitosan scaffold to obtain a PLGA knitted mesh-reinforced collagen-chitosan scaffold showed enhanced angiogenesis and dermal regeneration in a full-thickness skin wound rat model. The combination use of PLGA mesh and collagen-chitosan scaffold greatly improved the mechanical strength and physicochemical properties of the delivery system.[56] Similarly, a topical application of double-layer PLA-PLGA microparticles encapsulating peroxisome proliferator-activated receptor β/δ (PPARβ/δ) agonist GW501516 provided an earlier and sustained dose of GW501516 to the diabetic wound and reduced the oxidative wound microenvironment to accelerate healing. The reported mechanism was an early GW501516-mediated activation of PPARβ/δ stimulated glutathione peroxidase1 (GPx1) and catalase expression in fibroblasts. GPx1 and catalase scavenged excessive hydrogen peroxide (H_2O_2) accumulation in diabetic wound beds, thereby prevented H_2O_2-induced ECM modification and facilitated keratinocyte migration. These activities altogether accelerated the wound healing process.[57]

PLGA hydrogels

Maintaining hydration of wound is one of the idea characteristics of wound treatment. A biocompatible moist PLGA based drug delivery system encapsulating antibiotics was developed to promote dermal wound healing. PLGA microspheres encapsulating gentamicin and serratiopeptidase were incorporated into polyvinyl alcohol-gelatin slurry and casted into films to prepare multiphase hydrogel. The prepared hydrogel showed biphasic release pattern. During in vivo studies, the hydrogel formulation showed greater wound contraction, tensile strength and reepithelialization.[58] A thermosensitive gel loaded with KSL-W (cationic antimicrobial peptide) encapsulated PLGA microspheres showed antimicrobial activity against the targeted microorganism *S. epidermidis* effective wound healing in combat-related injuries.[59]

PLGA has been described for gene delivery in a variety of diseases and several PLGA-based DNA delivery systems have been developed. The choice of the formulation methodology had a direct impact on the particle size and the encapsulation efficiency of DNA.[60] Particularly in the context of DNA delivery in wounds, biodegradable thermosensitive hydrogels made of triblock copolymers of PEG-PLGA-PEG were successfully used for delivery of pDNA. These hydrogels were described as nontoxic and able to locally deliver pDNA in skin wounds.[61] By themselves, the PEG-PLGA-PEG hydrogels promoted wound healing by moisture retention to maintain homeostatic environment. Moreover, an accelerated reepithelialization was induced in a diabetic mouse model when PEG-PLGA-PEG hydrogels were loaded with a TGF-β1 encoding plasmid.[62]

PLGA nanoparticles

PLGA nanoparticles (NP) have been successfully proved as efficient carriers of drugs and biomolecules for the treatment of various ailments.[63–65] PLGA NP are one of the approaches to encapsulate the poorly soluble drugs or hydrophobic drugs.[28] PLGA NP may present extensive opportunities for exploration of new ways for sustained and controlled local release of loaded drugs for wound healing.

Direct delivery of VEGF and bFGF at the wound site in a sustained and controllable way without loss of bioactivity promotes wound healing. Poly(ether)urethane-polydimethylsiloxane/fibrin-based scaffold containing PLGA nanoparticles loaded with VEGF and bFGF (scaffold/GF-loaded NPs) were evaluated in diabetic mice (db/db) full-thickness dorsal skin wound model. Treatment with scaffolds containing growth factors induced complete reepithelialization, with enhanced granulation tissue formation/maturity and collagen deposition compared to the other groups.[66]

Different studies have found evidence of demonstrated using PLGA NP for the controlled release of antibiotics for wound repair using PLGA NP. *S. aureus* causes several types of infections ranging from minor to post-surgery skin infections.[67] Local administration of antibiotics at wound sites can provide limited dose and dosage, low occurrence of bacterial resistance, systemic toxicity and patient adherence, particularly for children.[68] Due to its low cost and potent activity against Gram-negative bacteria like *E. coli* and *Pseudomonas aeruginosa*, gentamicin is still a preferred drug for treatment of wounds, initial suspected bacterial infection and septicemia.[69,70] However, the activity of gentamicin is limited by its poor intracellular penetration and accumulation in lysozymes resulting in administration of high dose regimen in order to achieve therapeutic concentration levels. Gentamicin loaded PLGA NP have showed high tissue compatibility, reduced systemic toxicity and promising results by overcoming the foresaid drawbacks of gentamicin when applied locally in the form of cream or ointment.[68,71] Nitric oxide (NO) releasing PLGA-polyethylenimine NP composed of PLGA and polyethylenimine/diazeniumdiolate exhibited prolonged NO release profile over 6 days without any burst release and a concentration dependent potent bactericidal efficacy against methicillin-resistant *S. aureus* and *P. aeruginosa*. Furthermore, these nanoparticles accelerated wound healing and epithelialization in a mouse model of a MRSA-infected wound.[72]

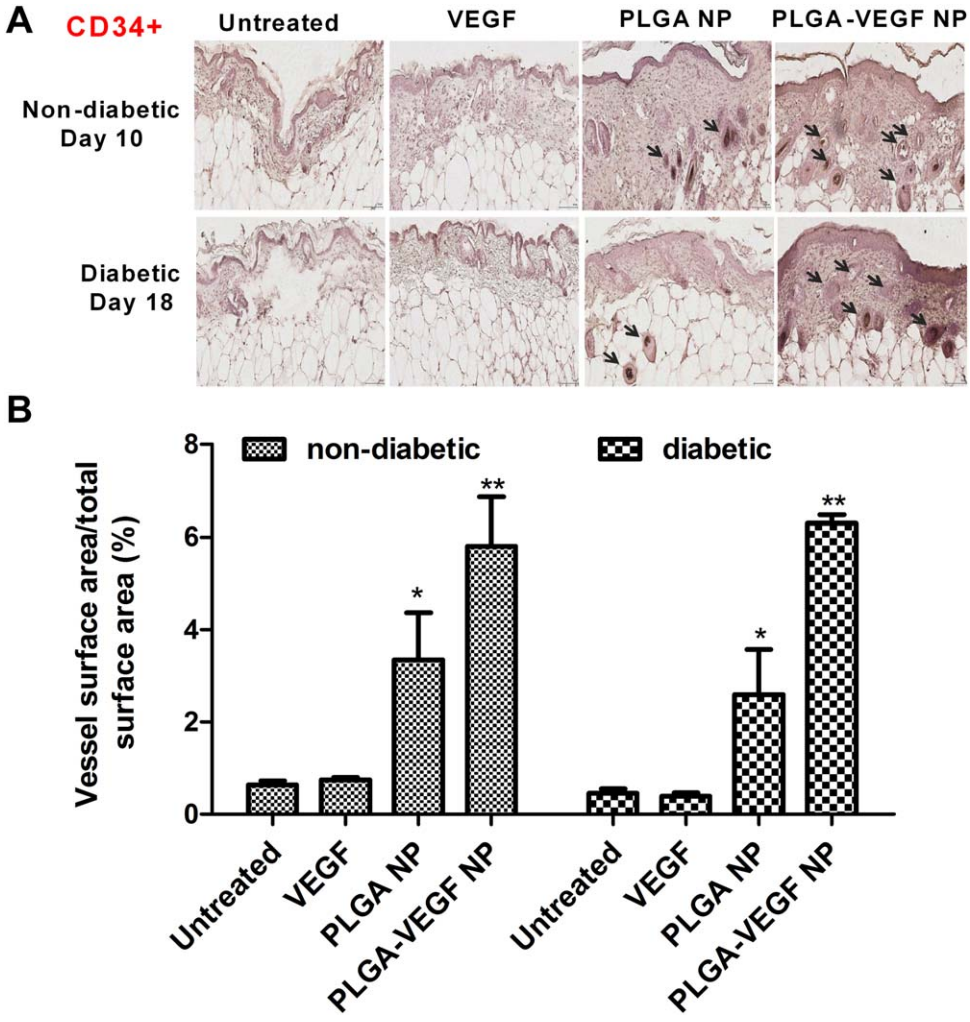


Figure 4. PLGA-VEGF NP enhanced the angiogenesis. Sections of nondiabetic and diabetic wounds were stained with CD34 immunohistochemistry staining. (A) Representative pictures of IHC staining of CD34+ marking (Scale bar = 100 μm, arrows indicate infiltrating blood capillaries), (B) Quantification of vessel area representing the extent of CD34+ marking (mean ± SD, $n = 3$). Statistical significance compared with untreated group.[95]

Until now, only a few studies were focused on PLGA-aided delivery of nucleic acids for wound healing. Gene silencing is another attractive therapy for wounds. siRNA delivery using PLGA carriers to achieve gene silencing is under serious debate. For instance, local p53 silencing using an agarose gel containing siRNA resulted in faster wound healing with wound closure in a diabetic mouse model.[73] siRNA is extremely vulnerable and RNase as well as harsh encapsulation methods can compromise its integrity. Also, a sustained release is needed because silencing effect of siRNA is very short. Several strategies have been engaged such as the use of cationic excipients for improving the loading efficiency, cellular uptake and the endosomal escape of siRNA-containing PLGA nanoparticles.[74]

COMBINED THERAPEUTIC EFFECTS OF PLGA AND LOADED DRUG ON WOUND HEALING

In the aforementioned literature, drug delivery using PLGA based carriers showed significant higher activity than the controls. But the major limitation of these works was none evaluated the therapeutic activity of PLGA (lactate release) in wound healing. Hence, further sections of this review solely focus on combined therapeutic effects of PLGA and the loaded drug on wound healing activity.

Curcumin loaded PLGA nanoparticles

Curcumin (CC) is one of the well-known wound healing agents in Indian Ayurveda and Chinese medicine.[75,76] Both in normal and diabetic-impaired wounds when used topically, CC has been reported as a promising wound healing and antimicrobial agent.[77,78] The promotion of wound healing includes increasing granulation tissue and enhancing the biosynthesis of TGF-β1 and proteins in ECM.[79,80] Also, it is reported that CC inhibits H_2O_2 induced oxidative damage in human keratinocytes and fibroblasts by inducing the down regulation of PI3K/AKT/NFκB pathway.[81,82] The major drawbacks associated with CC administration are its poor water solubility, photosensitivity and low stability.[83]

CC when encapsulated in PLGA, has showed good dispersion in water and no chemical interaction between

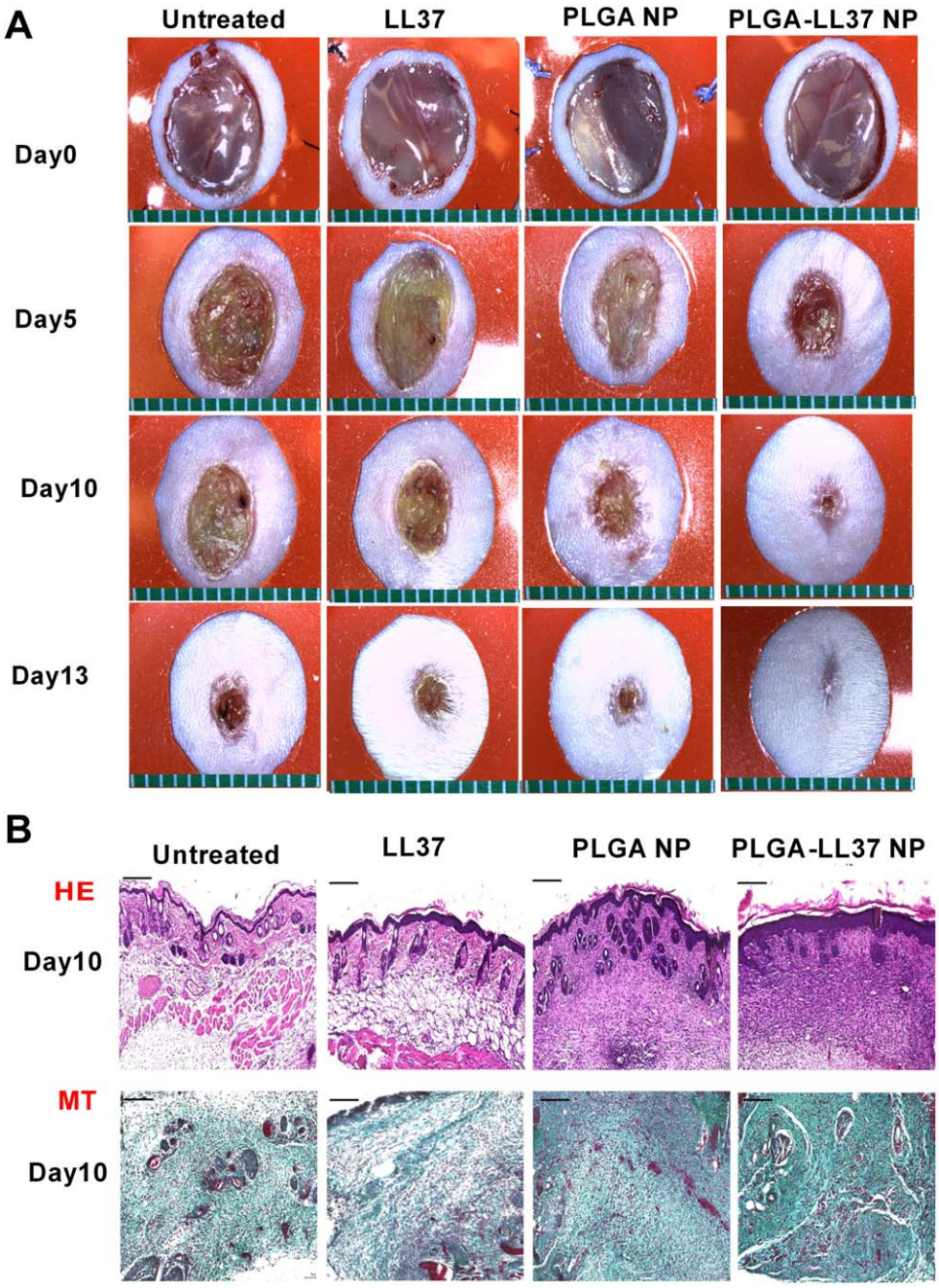


Figure 5. PLGA-LL37 NP accelerated wound healing and increased wound reepithelialization and collagen content of granulation tissue. (A) Representative images of wounds of four tested groups: Untreated, LL37, PLGA-NP and PLGA-LL37 NP (Ruler units in mm, $n = 10$, mean ± SD). (B) Wound sections ($n = 3$) were stained with hematoxylin and eosin (HE) and Masson's trichrome (MT). Representative images of sections are presented for all four groups. Scale bar = 200 μm).[99]

encapsulated CC and PLGA polymer matrix.[84] Drug release profile of CC from PLGA NP also showed a biphasic phase that includes the initial burst release and followed by the sustained release.[85,86] PLGA NP provided a solution for the efficient delivery of CC for wound healing.[40,87] PLGA-CC NP were capable of control and maintain the release of CC and lactate and significantly accelerated the wound closure by comprehensive healing which included down-regulation of inflammatory responses, expedited reepithelialization and improved granulation tissue formation. Thus, PLGA-based drug delivery systems served dual role as a carrier by delivering the loaded drug and as a therapeutic agent through lactate activity (Figure 3).[88]

PLGA nanoparticles encapsulating biomolecules

Many cellular mediators and biomolecules like host defense peptides, eicosanoids, cytokines, and growth factors are involved in wound healing process. Rapid degradation by enzymes and short half-life time in vivo are the challenges associated with the administration of biomolecules and the other biological wound supplements. In

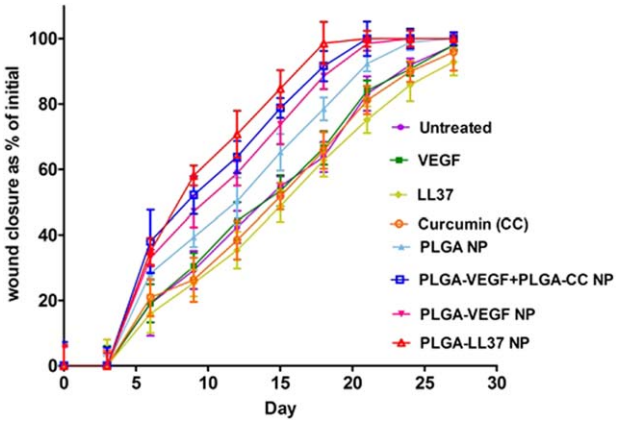


Figure 6. Drug loaded PLGA NP accelerated wound healing in diabetic mice. Quantitative representation of wound areas as a function of time ($n = 10$, mean ± SD).

addition, repeated administrations should be practiced to achieve the effective therapeutic levels.[89] Hence, the strategy of polymeric encapsulation and local application could solve some of the foresaid problems.

PLGA-VEGF nanoparticles

Growth factors, which stimulate fibroblasts and keratinocytes via transmembrane glycoproteins, have been studied extensively more than any other biological wound healing products. A review by Werner et al. discussed the regulation of wound healing by growth factors and cytokines.[11] VEGFa is a powerful therapeutic tool for proangiogenic therapy in many clinical settings. The multi-active wound healing mechanisms of VEGFa reported so far were multiple activities including deposition of collagen, angiogenesis and reepithelialization.[90,91] But to acquire therapeutic effect larger amount of VEGF and frequent dosing[92] or gene therapy[93] would be needed, suggesting the requirement of efficient drug delivery systems for VEGF.

PLGA NP have been proved to be efficient carriers for VEGF and can accelerate angiogenesis.[94] Exploiting the dual roles of PLGA in wound healing, PLGA-VEGF NP were formulated and evaluated for diabetic wound healing. PLGA-VEGF NP were able to supply lactate and VEGF sustainably to wounds. Moreover, PLGA-VEGF NP significantly accelerated the wound healing compared to the control groups. PLGA-VEGF NP induced higher reepithelialization and granulation tissue formation. Immunohistochemistry (CD34+) studies revealed that PLGA NP and PLGA-VEGF NP treatments significantly promoted angiogenesis and blood capillary infiltration in wound sections than the other groups (Figure 4A and B). In conclusion, PLGA based VEGF delivery systems can promote wound healing because of its lactate and the encapsulated VEGF activities.[95]

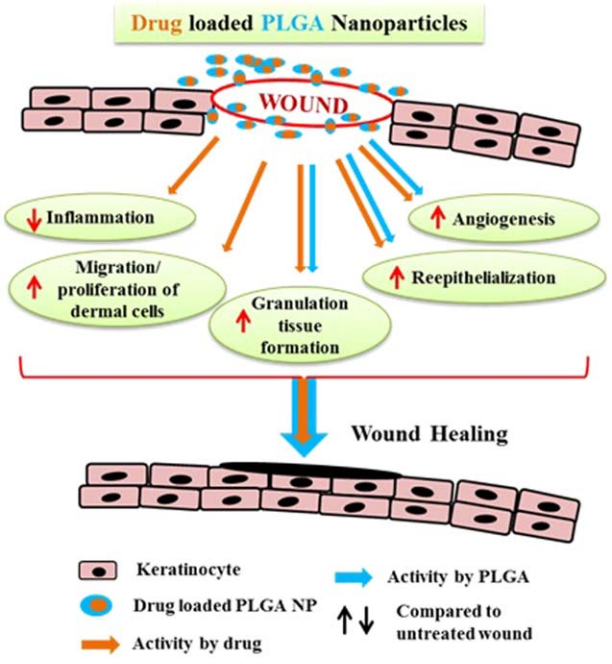
PLGA-LL37 nanoparticles

Host defense peptide LL37 belongs to antimicrobial peptides/host defense peptides which exerts broad antimicrobial activity, antiviral and antifungal activity, endotoxin-binding properties, modulation of proinflammatory response, chemotaxis, influence on cell proliferation and differentiation, promotion of wound healing and angiogenesis, and so forth.[96] The major difficulty associated with LL37 administration for wound healing is its instability in wound environment and thus the treatment may require high dose and repeated doses[97] or gene therapy[98] to produce the therapeutic effect.

It has been demonstrated that the encapsulation of LL37 in PLGA NP significantly enhanced wound healing activity as compared to PLGA or LL37 alone (Figure 5A). The acceleration of wound healing by PLGA-LL37 NP and PLGA NP became highly significant from days 7 than LL37 and untreated groups. This could be attributed to the lactate release from PLGA and its effects on wound healing. In PLGA-LL37 NP treated group, the epidermal and subepidermal layers were well organized (Figure 5B). The extent of collagen deposition was significantly higher in PLGA-LL37 NP and PLGA NP groups and the deposited collagen showed a compact and denser alignment compared to the other groups (Figure 5B). The healing effects of PLGA-LL37 NP included higher reepithelialization, granulation tissue formation and immunomodulation. The results demonstrated that PLGA NP can promote wound healing activities with its innate lactate activity and sustained release of bioactive LL37.[99]

Comparison of activity of drug loaded PLGA nanoparticles in diabetic wound healing

By performing in vivo evaluation of all three types of drug loaded PLGA nanoparticles (PLGA-VEGF NP, PLGA-VEGF+PLGA-CC NP, PLGA-LL37 NP) and five controls



Scheme 1. Schematic representation of possible mechanisms of action of drug and PLGA (lactate) in wound healing processes.

Table 1. PLGA based drug delivery systems for wound healing activity

Drug delivery system	Loaded drug	Class of drug	Benefits after encapsulation	Ref.
PLGA microparticulate scaffolds	Cytomodulin	Peptide	Increase aqueous solubility and stability, protection from photo-degradation and physiological pH, sustained and controlled supply	53
PLGA microspheres	Chlorhexidine, PDGF	Hydrophobic, growth factor	Simultaneous and controlled drug release and kinetics	55
PLGA-Alginate microspheres	rhEGF	Growth factor	Efficient delivery, protection from in vivo enzymes and pH degradation, sustained and controlled supply	54
PLGA microparticulate knitted mesh integrated with collagen-chitosan scaffold (CCS)	Collagen–chitosan scaffold	Protein (collagen)	Improved mechanical properties of CCS and angiogenesis and in situ tissue regeneration	55
Double-layer PLA-PLGA microparticles	Peroxisome proliferator-activated receptor β/δ (PPARβ/δ) agonist GW501516	Hydrophobic	Increase aqueous solubility, sustained and controlled supply	57
PLGA microspheres incorporated into polyvinyl alcohol-gelatin slurry and casted into multiphase hydrogel	Gentamicin and serratiopeptidase	Antibiotics	Efficient delivery, prolonged local bioavailability and enhanced pharmacological response, reduced dose and systemic toxicity	58
Thermosensitive gel loaded with PLGA microspheres	KSL-W	Cationic antimicrobial peptide	Efficient delivery, protection from in vivo degradation, sustained and controlled supply	59
Thermosensitive PEG-PLGA-PEG hydrogels	TGF-β1 pDNA	Nucleic acid	Efficient and sustained delivery, protection from in vivo degradation	62
Sandwich-structured PLGA-collagen nanofibrous membranes	Vancomycin, gentamicin, and lidocaine	Antibiotics	Efficient delivery, prolonged local bioavailability and enhanced pharmacological response	45
PLGA ultrafine fibers	Fusidic acid	Hydrophobic	Efficient delivery, sustained and controlled supply	47
PLGA membranes	(1,3)-(1,6)-β-D-glucan	Natural immune stimulatory polysaccharide	Local sustained drug delivery and wound dressing	47
	Epigallocatechin-3-O-gallate	Polyphenol	Local sustained drug delivery and wound dressing	47
	Metformin	Biguanid	Local sustained drug delivery and wound dressing	51,52
PLGA nanoparticles	VEGF and bFGF	Growthfactors	Increase stability, protection from invivo degradation and physiological pH, sustained and controlled kinetics	66

Table 1. Continued.

Drug delivery system	Loaded drug	Class of drug	Benefits after encapsulation	Ref.
	siRNA and DNA	Nucleic acids	Protection from enzymes and local environment, increased cellular permeability and molecular integrity	60
	Gentamycin	aminoglycoside antibiotic	Enhanced tissue biocompatibility, reduced systemic toxicity	71
	Nitric oxide (NO) releasing PLGA-polyethylenimine NP	Active NO	No burst release, sustained and controlled release	72
	Curcumin	Polyphenol	Increase aqueous solubility and stability, sustained and controlled delivery, combined effect with PLGA	88
	Host defense peptide	Peptide	Protection from in vivo enzymes and degradation, sustained delivery, combined effect with PLGA	99
	VEGF	Growth factor	Sustained delivery and enhanced bioactivity, combined effect with PLGA	95

(untreated, VEGF, LL37, CC, PLGA NP) we attempted to present a comprehensive comparative validation of their potential to promote wound healing (Figure 6, unpublished data). Compared to all the control groups, wounds treated with drug loaded PLGA NP showed a significantly faster wound closure. From day 6 onwards drug loaded PLGA NP exhibited higher healing and by day 13 wound closure was much evident. The healing percentage of different treatments at day 9 were, untreated 29% vs. LL37 25% vs. CC 26% vs. VEGF 30% vs. PLGA NP 39% vs. PLGA-VEGF+PLGA-CC NP 52% vs. PLGA-VEGF NP 47% vs. PLGA-LL37 NP 58%. Untreated, LL37, CC and VEGF groups showed much lower healing of wounds. Wounds that either untreated or treated with LL37 or CC or VEGF alone showed poor healing even at day 18 (untreated 64%, LL37 62%, CC 65% and VEGF 66%) and wounds completely recovered after 28 days. It is worth mentioning that compared to untreated negative controls, from day 7 onwards, PLGA NP and drug loaded PLGA NP treatment displayed its beneficial add on effect of sustained lactate release to the wounds by significant accelerated healing. This is in strong commitment with in vivo lactate release studies and other wound healing assays reported in our previous work.

CONCLUSIONS AND PERSPECTIVES

The current review emphasized the PLGA as a potential drug delivery system for wound healing applications and also combined therapeutic effects of PLGA (lactate) and loaded drug for wound healing (Scheme 1). Due to its versatile mechanical properties, different forms of PLGA based drug delivery systems were formulated and studied. PLGA carriers offer various benefits for the encapsulated drug (Table 1) such as protection from enzyme and pH degradation, enhanced bioavailability, solubility and stability and efficiently deliver different drugs to promote wound healing with a reduced dose. Also, PLGA carriers can increase the efficacy of wound treatments by releasing the therapeutic agent at a controlled and sustained rate. Moreover, therapeutic application of PLGA via lactate mechanism increased its use in wound treatments. Lactate, one of the byproducts of PLGA degradation showed positive effect on wound healing processes. Thus, combined therapeutic effects of lactate and a loaded drug can aid in active healing of wounds.

A few challenges associated with PLGA formulations present new opportunities to research and optimize for better utilization. PLGA carriers often yield in high encapsulation efficiencies but the drug loading is generally poor (~1%, which means that NP contain 1 mg active ingredient per 100 mg of polymer). This could be a problem in case of bulk production of PLGA-based carriers.[65] Aggregation of PLGA carriers and acidic environment created by byproducts should be prevented. Careful optimization of formulation, NP size, surface modification, addition of stabilizers and other copolymers could potentially overcome the aforementioned challenges and present PLGA-drug loaded carriers as a promising combination therapy for wound treatment.

Current active wound care primarily includes one or more of the following depending on the wound intensity: dressing, antimicrobial and stimulant for healing. Any therapy that presents all these three critical needs in one dosage form would greatly cut down the expenses and accelerate the healing of chronic wounds. Development of single multifunctional PLGA dosage form that possess the entire ideal

characteristics such as antimicrobial, protect hydration, allow exchange of gases and stimulate active healing would be a great achievement and it may cut down the expenses and accelerate the healing of chronic wounds.

Many researchers widely used rodents as animal wound models because of their availability and the fact that they are easy to maintain even at small animal facility. Major limitations, as rodents are loose skinned animals and their wound heals mainly by contraction, resulting in biased outcomes. In a translational point of view, pig is the most preferred large animal model that is commonly used in dermatology experiments due to its similarities with that of human skin. A thick epidermis and subdermal adipose tissue, dermal collagen and elastic content, distribution of hair follicles and blood vessels, similar physical and molecular responses to various growth factors and both heal primarily by reepithelialization are the close similarities that human and swine skin share.[100] To generate valuable conclusions, the effects of PLGA on wound healing should be extensively investigated in swine models.

Concerning translational approaches, FDA and EMA already approved PLGA as an excipient. In our current work, PLGA acts as a sustained source of lactate, which is having therapeutic activities as opposed to the normal properties of an excipient. So, it may require some more time to find PLGA as an approved therapeutic agent.

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