Abstract:

A method of wound healing comprising a scaffold comprising a hydrophilic-lipophilic balanced (HLB) scaffold comprising a nano fiber administered or laden with polyglycolic acid-co-lactic acid, polyvinyl alcohol and a drug. Other embodiments and advantages are also disclosed.

Title: WOUND HEALING NON IMMUNOGENIC NANO SCAFFOLDS

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This disclosure relates to healthcare, and in particular to electro spun Hydrophilic-Lipophilic Balance (HLB) based biodegradable, non-immunogenic scaffolds administered with antibiotics.

BACKGROUND OF THE INVENTION

Healthcare, in general, has long benefited from the use of nonwoven structures for wound healing applications due to their absorbent properties, facile processing schemes, and relative cost-effectiveness. Typically, nonwoven materials may be used in traditional wound healing approaches and have also been studied for the development of advanced wound care materials that offer both functionality and innovation in wound healing and tissue engineering applications. For example, various materials have been developed to provide wound dressings incorporating bioactive molecules, inorganic materials, and/or antimicrobial treatments to assist in dermal wound healing. Such modification of nonwoven structures may be generally performed by incorporating the active material during the fiber production process, or as a post-processing treatment after structural formation of the nonwoven has been achieved.

SUMMARY OF THE INVENTION

Embodiment of the present disclosure are related to an electro spun Hydrophilic-Lipophilic Balance (HLB) based biodegradable, non-immunogenic scaffolds having a coaxial structure with a nano fiber core, a first covering with a hydrophilic material and a second covering with a hydrophobic material, the nano fiber core administered with a drug, for example an antibiotic or an anti-inflammatory drug, wherein the drug may be effectively used in inhibiting the growth of Gram Positive MRSA resistant bacteria and showing activity on Gram Negative bacteria, and may be used as a potential functional wound biodegradable drug delivery carrier system showing a sustained release over a long period of time.

BRIEF DESCRIPTION OF THE DRAWINGS

For a better understanding of the nature and desired objects of the present invention, reference is made to the following detailed description taken in conjunction with the accompanying drawing figures wherein like reference character denote corresponding parts throughout the several views. Objects, features, and advantages of embodiments disclosed herein may be better understood by referring to the following description in conjunction
with the accompanying drawings. The drawings are not meant to limit the scope of the claims included herewith. For clarity, not every element may be labeled in every Figure. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments, principles, and concepts. Thus, features and advantages of the present disclosure will become more apparent from the following detailed description of exemplary embodiments thereof taken in conjunction with the accompanying drawings in which:

**Figure 1A** shows an exemplary embodiment of a coaxial structure of the scaffold;

**Figure 1B** shows an exemplary embodiment of a coaxial structure of the scaffold wherein the scaffold is deposited on a base material;

**Figure 2** Scanning electron microscopic (SEM) image of Poly (vinyl)alcohol(a). Poly (lactic-co- glycolide)- Poly (vinyl) alcohol [b], and Poly (lactic-co- glycolide)-Poly (vinyl)alcohol -Line/olidi |5mg/ml [c].

**Figure 3** shows an exemplary embodiment of an Atomic Force Microscopic (AFM) Phaseimages of Poly (vinyl) alcohol [a], Poly (lactic-co- glycolide) - Poly (vinyl) alcohol [b], and Poly (lactic-co- glycolide)-Poly (vinyl) alcohol -Linezolid (15mg/ml) [c];

**Figure 4** shows and exemplary embodiment of an In vitro release behavior of (PLGA (65:35)-PVA-Linezolid (5, 10, 15 mg/10ml) nanofiber scaffolds);

**Figure 5** shows an exemplary embodiment of the effect of HLB balanced scaffolds on skin morphology during Burn wound healing, a (i). Normal skin, a (ii). Burnt Skin (Day-0; immediately after burn wound induction), (b) Burn wound alone, (e) Burn wound + Standard treated group, (d) Burn wound + PVA Scaffold treated group, (e) Burn wound + PLGA-PVA scaffold treated group,(f) Burn wound + PLGA-PVA-Linezolid (5mg) scaffold treated group, (g) Burn wound + PLGA-PVA-Linezolid (10mg) scaffold treated group, (h) Burn wound + PLGA-PVA-Linezolid (15mg) scaffold treated group;

**Figure 6** shows the histopathology micrographs of the skin specimen during the administration of Oxazolidinone/Linezolid scaffold at different days of burn wound healing;

**Figure 7** shows the immunohisto chemistry results for the expression of COX-II in the skin specimen during wound healing; and

**Figure 8** shows the Immunohisto chemistry results for the expression of iNOS in the skin specimen during wound healing.

**DETAILED DESCRIPTION OF THE INVENTION**

Hereinafter, various exemplary embodiments of the present disclosure will be described with reference to the accompanying drawings. It should be noted that all of these drawings and description are only presented as exemplary embodiments. It is to note that
based on the subsequent description, alternative embodiments may be conceived that may have a structure and method disclosed as herein, and such alternative embodiments may be used without departing from the principle of the disclosure as claimed in the present disclosure.

It may be appreciated that these exemplary embodiments are provided only for enabling those skilled in the art to better understand and then further implement the present disclosure, not intended to limit the scope of the present disclosure in any manner. Besides, in the drawings, for a purpose of illustration, optional steps, modules, and units are illustrated in dotted-line blocks.

The terms "comprise(s)," "include(s)", their derivatives and like expressions used herein should be understood to be open, i.e., "comprising/ including, but not limited to." The term "based on" means "at least in part based on." The term "one embodiment" means "at least one embodiment"; and the term "another embodiment" indicates "at least one further embodiment." Relevant definitions of other terms will be provided in the description below.

Embodiments of the present invention may be relate to development of co-axially electro spunHLB balanced biodegradable, non-immunogenic scaffold loaded with Oxazolidinone and/or Linezolid based drugs as antibiotics. Embodiment of the present invention may be effectively used in inhibiting the growth of Gram Positive bacteria with a positive response on Gram Negative bacteria. Embodiments of the present invention in one example may be used as a potential functional wound, such as burn wounds or incision wounds, bio-resorbable drug delivery carrier system.

According to an embodiment of the invention, healthcare in particular related to burn wound care or incision wound care may include removal of nonviable tissue, impediment of infection and facilitation of wound healing, while controlling pain and maximizing outcome. Though embodiments of the invention specifically mention burn wounds, all other types of wounds may be cured by the HLB scaffold. In an example embodiment, an accident related wound wherein skin is bruised may be advantageously treated with a HLB scaffold. According to some embodiment, wound infections may be mostly caused by bacteria like methicillin-resistant *Staphylococcus aureus (MRSA)* and *Pseudomonas aeruginosa* which releases various proteases, adhesion and colonization proteins that result in damaging adherent tissues. According to some additional embodiment, cleaning the wound and applying various topical anti-microbial agents and wound dressings may be an effective solution in preventing microbial infections around the wound. In certain embodiments,
suitability of a wound dressing may depend much on the type of wound, for example the suitability of a burn wound dressing much depends on the type of burn wound itself.

In some embodiments, conventional dressings techniques may not be efficient enough as wound dressings due to its inefficiency to induce haemostasis, such as for burn wounds, adherence and in-holding of a moist environment around the wound. In some other embodiments, since microbial infections serve as one of the major problem associated with wounds, developing bio-engineered smart scaffolds for wound care and incorporation of functional moieties like antimicrobials and wound healing agents may be better alternatives to heal wounds. In some other embodiments, advances in nano-technology has made it possible to design a nano fiber based wound dressing, where an electro spun nano fibrous layer may be deposited on a basic support fabric material. In some embodiments, a key advantage of nano fiber based dressings is that they may include haemostasis. In some other embodiments, nano fiber based dressings may have the ability to uptake exudates. In some other embodiment, nano fiber based dressings may have a semi-permeable nature. In some other embodiments, nano fiber based dressings may have better comfort and functional ability that may be advantageous over materials that are currently available to heal burn wounds.

Embodiments of the present invention related to a hydrophilic-lypophilic balanced (HLB) scaffold comprising a coaxial structure. In a further embodiment, the coaxial structure of the scaffold may include a core nano fiber that may be administered or ladened with a drug. In a further embodiment the core is then covered with a first material, such as hydrophilic material. In a further embodiment the core is then subsequently covered with a second material, such as a hydrophobic material. In a further embodiment, the coaxial structure of the HLB scaffold may be configured to hold the release of the drug over time thereby prolonging the bio availability and decreases the burst release of the drug.

In one embodiment, the HLB scaffold may be administered with a poly (glycolic-co-lactic acid)(PLGA) and a polyvinyl alcohol (PVA) with a PLA:PGA ratio in the range of 50-80: 20-50. In a specific embodiment, it is noticed that the PLA:PGA ratio in the range of 60-70:30-40 showed improved holding capacity and constant release behaviour with higher efficiency or efficacy for wound healing. In a further embodiment, the drug may be an antibiotic and/or an analgesic. In a further embodiment, the antibiotic may consist one from the group of Oxazolidinone and/or Linezolid.

In one embodiment, the HLB scaffold may include a biodegradable polymer material. In a further embodiment, the core nanofiber may be deposited on a base material.
In a further embodiment the base material may either be one of an elastic supporting fabric or a non-elastic supporting fabric. In a further embodiment, the nano fiber may be semi-permeable and/or pourous and/or semi-pourous and/or non-pourous.

In a further embodiment, the drug inhibits the growth of Gram Positive bacteria. In yet a further embodiment, the drug inhibits the growth of Gram negative bacteria. In a further embodiment, the drug exhibits a sustained controlled release from the polymer composite. In a further embodiment, the drug regulates expression of inducible Nitric Oxide Synthase (iNOS) and/or Cyclo-Oxygenase-II (COX-II) inflammatory mediators in a wound site, thereby regulating inflammation. In yet a further embodiment, the wound is a burn or an incision or any other open wound that is susceptible to infection.

Reference is now made to Figure 1A, which shows an exemplary structure of the scaffold. Scaffold 100A has a core 105 comprising a nano fiber that is surrounded by a first material 107 covering the core 105, wherein the first material 107 is a hydrophilic material. A second material 109 covers the first material 105, wherein the second material 109 is a hydrophobic material. The nano fiber core 105 is administered or laden with a drug, which may be an antibiotic or an anti-inflammatory material or a combination thereof. The scaffold 100A is administered with a polyglycolic acid-co-lactic acid (PLGA) and a polyvinyl alcohol (PVA) with a PLA:PGA ratio in the range of 50-80: 20-50. In a specific embodiment, it is noticed that PLA:PGA ratio in the range of 60-70:30-40 provided higher efficiency or efficacy for wound healing. The antibiotic drug used in the nano-fiber core may consist one from the group of Oxazolidinone and/or one from the group of Linezolid. It should be obvious to one skilled in the art that the antibiotic used in this case may be replaced by other antibiotic, anti-inflammatory or other drugs used to heal different types of open wounds that are susceptible to infection. The core 105 nanofiber may be a semi-permeable and/or a porous and/or a semi-porous and/or a non-porous.

Reference is now made to Figure 1B, which shows an exemplary structure of the scaffold. Scaffold 100A has a core 105 comprising a nano fiber that is surrounded by a first material 107 covering the core 105, wherein the first material 107 is a hydrophilic material. A second material 109 covers the first material 105, wherein the second material 109 is a hydrophobic material. The nano fiber core 105 is administered or laden with a drug, which may be an antibiotic or an anti-inflammatory material or a combination thereof, as disclosed above. The core 105, hydrophilic material 107 and hydrophobic material 109 forming the scaffold 100a may be placed on a base material such as a polymer and/or the
base material may either be one of an elastic supporting fabric or a non-elastic supporting fabric.

Figure 2 shows an exemplary embodiment of a Scanning electron microscopic (SEM) image of Poly (lactic-co-glycolide)-Poly (vinyl) alcohol - Linezolid nano fiber scaffold. The illustration in Figure 2 shows nano fibers in a range of 130-200nm. Both the core and shell polymer blends may be electro spun with the help of co-axial spinneret containing inputs for two solutions. By controlling the temperature voltage and the distance between the spinneret tip and collector, uniform, ultrafine nano fibers ranging from 100-200 nm may be obtained. The morphology of electro spun nano fibers may be characterized using Field Emission Scanning Electron Microscopy (FE-SEM) Inspect F50 make of FEI. The images shown in Figure 2 were recorded at an operating voltage of 20kV, at different magnifications with a resolution of 1.2 nm at 30kV, and 3 nm at 1 kV, and the diameter distribution was calculated manually.

According to embodiments of the present invention, Di chloro methane (DCM) was selected as a solvent because DCM has a proven record of excellence as a solvent for PLGA, while addition of PVA may improve electro spinability of the solution blend. Electro spinning of these solutions was carried out at an applied voltage range of 13 to 20 kV. Morphology of electro spun PVA, PLGA-PVA, Linezolid-loaded PLGA-PVA nano fiber mats and their diameter distributions are shown in Figure2. The incorporation of Linezolid into the PLGA-PVA nano fibers not only significantly varied their average diameter, but also narrowed the diameter distribution of the electro spun nano fibers. The average diameter distribution of the nano fibers was estimated to be in the distribution range of 100 to 250 nm. Bio degradable electro spun fibers for drug delivery may typically reduce the diameter size and distribution of hydrophobic electro spun fibers of PLGA. The addition of PVA increased PLGA-containing Linezolid solution viscosity. This may be due to interaction between PVA and PLGA. Thus, diameter of the Linezolid-loaded PLGA-PVA nano fibers increases with distribution in the range of 100 to 250 nm.

Figure 3 shows an exemplary embodiment of an Atomic Force Microscopic (AFM) topographic image of Poly (vinyl) alcohol [a], Poly (lactic-co- glycolide) - Poly (vinyl) alcohol [b], and Poly (lactic-co- glycolide)-Poly (vinyl) alcohol -Linezolid (15mg/ml) [c]. Atomic force microscopy (AFM) measurements were conducted using an SPM. Freshly prepared samples were kept under high vacuum to avoid surface contamination and then stuck on a glass slide for imaging. Each sample was imaged at multiple locations. All the samples imaging was done in air at room temperature and finally images were analyzed
using Gwyddion (2.39) software. The availability of multiple block co-polymers can cause nano/micro scale phase separation within the bulk as well as surface of the electro spun polymeric micro/nano fibers. The presence of such separated phases can be visualized using AFM if the blended polymers are present in insufficient concentration. Such phase separation properties often result in deterioration of mechanical properties and hence can be undesirable. In the embodiments of the present disclosure the possibility of phase separation in the binary block copolymer was analyzed using the tapping mode of AFM. Tapping mode AFM experiments were performed to enable phase imaging of the nano fibers. The AFM images of PVA, PLGA-PVA, PLGA (65:35)-PVA-Linezolid, were recorded and the overall contrast is the indication of the homogenous distribution of the PLGA with PVA and hence miscibility.

Figure 4 shows an exemplary embodiment of \textit{in vitro} release behavior of a specific combination of PLGA (65%)-PVA (35%)-Linezolid for concentrations of 5mg/10ml (triangles connected line), 10mg/10ml (squares connected line), 15 mg/lOml (diamond connected line) nano fiber scaffolds. All the three different drug loaded scaffolds, i.e. 5mg/10ml, 10 mg/lOml and 15mg/10ml, used herein showed an initial burst release of Linezolid in Phosphate buffer saline (pH 7.4) release medium within the first 24 hours, which was then followed by controlled release of Linezolid until 200 hours with a subsequent sustained maintenance dose release behavior of the drug Linezolid up to 720 hours. As can be seen from the graph in Figure 4, in summary, irrespective of the percentage of the drug used, in the initial phase, there is a burst or steep increase in percentage of cumulative release of the drug in the first 24-30 hours, which is then followed by a release of the drug which then stabilizes over time until the study time of 720 hours. Therefore, even for period's beyond 720 hours it may be predicted that the drug will have a stabilized release.

Figure 5 shows a specific embodiment of the effect of HLB balanced scaffolds on skin morphology during burn wound healing. In Figure 5, a(i) indicates normal skin which is typically normal untreated skin and a (ii) indicates burnt skin on Day-0, i.e., burn wound immediately after induction (Day 0). Figure 5(b) indicates the burn wound alone. Figure 5(c) indicates the burn wound and a standard treated group. Figure 5(d) indicates the burn wound and PVA Scaffold treated group. Figure 5(e) indicates the burn wound and PLGA-PVA scaffold treated group. Figure 5(f) indicates the burn wound and PLGA-PVA-Linezolid (5mg) scaffold treated group. Figure 5(g) indicates the burn wound and PLGA-PVA-Linezolid (10mg) scaffold treated group. Figure 5(h) indicates the burn wound and PLGA-PVA-Linezolid (15mg) scaffold treated group. Figure 5 also shows
pictorially; wound morphology variations with respect to wound contraction on day 4, day 8, day 15 and day 21 respectively for each of the composition of drug usage.

Drugs from the group of Oxazolidinone or Linezolid were incorporated at three different concentrations namely 5mg, 10mg and 15mg respectively during the preparation of PLGA-PVA scaffold and the resulting scaffold were applied on the thermal burn wound. The results were shown in Figure 5f (PLGA-PVA-drug [5mg]), Figure 5g (PLGA-PVA-drug [10mg]) and Figure 5h (PLGA-PVA-drug [15mg]) respectively. Figure 5f(i), 5f(ii), 5f(iii) and 5f(iv) shows the pictures of skin morphology after treatment with PLGA-PVA-drug [5mg] at Day 4, Day 8, Day 15 and Day 21 respectively. Similarly 5g(i), 5g(ii), 5g(iii) and 5g(iv) shows the pictures of skin morphology after treatment with PLGA-PVA-drug [10mg] at Day 4, Day 8, Day 15 and Day 21 respectively. Figure 5g(i), 5g(ii), 5g(iii) and 5g(iv) shows the pictures of skin morphology after treatment with PLGA-PVA-drug [15mg] at Day 4, Day 8, Day 15 and Day 21 respectively.

Figure 6 shows the histopathology photographs of the skin specimen during the administration of drug (from the group of Oxazolidinone or Linezolid) scaffold at different days of burn wound healing. Figure 6a(i) shows the histo pathology of normal untreated skin whereas Figure 6a(ii) shows the histopathology picture of experimental burn wound immediately after the induction (Day 0). Figure 6b shows the histopathology pictures of the control group (Burn wound untreated group) at different days [Day 4: Figure b(i); Day 8: Figure b(ii); Day 15: Figure b(iii) and Day 21: Figure b(iv)].

Figure 6c shows the skin histopathology pictures after treatment with standard ointment (Burnol). [Day 4: Figure c(i); Day 8: Figure c(ii); Day 15: Figure c(iii) and Day 21: Figure c(iv)]. The effect of PVA alone scaffold on the skin histopathology is shown in Figure 6d [Day 4: Figure d(i); Day 8: Figure d(ii); Day 15: Figure d(iii) and Day 21: Figure d(iv)]. Figure 6e shows the skin histopathology pictures after treatment with PLGA-PVA scaffold at different days [Day 4: Figure d(i); Day 8: Figure d(ii); Day 15: Figure d(iii) and Day 21: Figure d(iv)].

The drug (from the group of Oxazolidinone or Linezolid) were incorporated at three different concentrations namely 5mg, 10mg and 15mg respectively during the preparation of PLGA-PVA scaffold and the resulting scaffold were applied on thermal burn wound. The skin histopathology results are shown in Figure 6f (PLGA-PVA-drug [5mg]), Figure 6g (PLGA-PVA-drug [10mg]) and Figure 6h (PLGA-PVA-drug [15mg]) respectively. Figure 6f(i), 6f(ii), 6f(iii) and 6f(iv) shows pictures of skin histopathology after treatment with PLGA-PVA-drug [5mg] at Day 4, Day 8, Day 15 and Day 21 respectively. Similarly 6g (i),
6g (ii), 6g (iii) and 6g (iv) shows pictures of skin histopathology after treatment with PLGA-PVA-drug [10mg] at Day 4, Day 8, Day 15 and Day 21 respectively. Figure 6g (i), 6g (ii), 6g (iii) and 6g(iv) shows pictures of skin histopathology after treatment with PLGA-PVA-drug [15mg] at Day 4, Day 8, Day 15 and Day 21 respectively.

Figure 7 shows the immuno histo chemistry results for the expression of COX-II in the skin specimen during wound healing. Figure 7a shows the COX-II expression in normal skin. Figure 7b shows the expression of COX-II in burn wound control skin (Day 21). Figure 7c, 7d and 7e shows the photographs of COX-II expression during standard (for example Burnol) treatment, PVA alone scaffold and PLGA-PVA scaffold respectively on day 21 after burn wound induction. Figure 7f, 7g and 7h shows the expression of COX-II in the skin specimen after treatment with PLGA-PVA-drug [5mg], PLGA-PVA-drug [10 mg] and PLGA-PVA-drug [15mg] respectively on day 21 after the induction of burn wound, where the drug used is from the group of Oxazolidinone or Linezolid.

Figure 8 shows the immuno histo chemistry results for the expression of iNOS in the skin specimen during wound healing. Figure 8a shows the iNOS expression in normal skin. Figure 8b shows the expression of iNOS in burn wound control skin (Day 21). Figure 8c, 8d and 8e shows the photographs of iNOS expression during standard (Burnol) treatment, PVA alone scaffold and PLGA-PVA scaffold respectively on day 21 after burn wound induction. Figure 8f, 8g and 8h shows the expression of iNOS in the skin specimen after treatment with PLGA-PVA-drug [5mg], PLGA-PVA-drug [10 mg] and PLGA-PVA-drug [15mg] respectively on day 21 after the induction of burn wound, wherein the drug is from the group of Oxazolidinone or Linezolid.

The optical densities of bacterial solutions was used to evaluate antibacterial activity of the Linezolid-loaded nanofiber mats against the common Gram positive and Gram negative bacterial strains like, *Staphylococcus aureus* (Grampositive, facultative anaerobic, ATCC 653S), *Pseudomonas aeruginosa* (Gram-negative, aerobic, ATCC 15442). *E.coli* (Gram-negative, facultatively anaerobic, ATCC 25922), *Bacillus Subtilis* (Gram positive, facultative aerobe, ATCC 23857), *Salmonella Typhimurium* ATCC 14028. The testing suspensions were prepared as follows; A 50-mg fragment of each nano fiber mat was introduced into 5 mL of the diluted bacterial solution. To prevent interference from the color of Linezolid, each nanofiber mat was incubated separately in a broth solution. The mixtures were cultured at 37°C in a shaking incubator for 24 hrs. The turbidity of the medium represents growth of the bacterial cells, and was measured using a spectrophotometer at a
600nm wavelength after a given time (24 hrs). The antibacterial efficiency of the Linezolid-loaded nano fiber mats was then calculated from the following equation below,

\[
\text{Antibacterial efficiency} = \left(1 - \frac{OD_2}{OD_1}\right) \times 100
\]

where, \(OD_1\) and \(OD_2\) represent the optical densities of the bacteria in the medium and the bacteria in the solutions containing the different nano fiber mats, respectively, for certain incubation times.

The familiar Gram positive and Gram negative bacterial strains like, \textit{Staphylococcus aureus} (Grampositive, facultative anaerobe, ATCC 6538; \textit{s.aureus}), \textit{Pseudomonas aeruginosa} (Gram-negative, aerobic, ATCC 15442), \textit{E.coli} (Gram-negative, facultative anaerobe, ATCC 25922), \textit{Bacillus subtilis}(Gram positive, facultative aerobe, ATCC 23857) and \textit{Salmonella Typhimurium} ATCC 14028 were grown in tryptic soy broth (TSB) overnight at 37°C.20(VL) of each culture were then spread onto tryptic soy agar (TSA) plates. Square fiber mats (8 mm diameter) were applied fiber side down onto TSA plates pre-spread with bacterial cultures. All Linezolid loaded nano fiber samples of different polymers were separated on the agar so as to not interfere with each other. Each plate was then sealed with para film and placed in an incubator at 37°C for 18hrs. The samples were then examined for bacterial inhibition zones around the fiber matrixes. Table 1 below indicates the Bacterial growth inhibition Assay on Liquid Culture.

As seen from graphs 1 and 2, advantageous the drug used herein has a positive effect on Gram negative bacteria which is not observed in the regular usage of the drug and other drugs used for treatment of wounds, specifically burn or incision wounds.

In one embodiment, advantages mentioned above with respect have facilitated development of nano fiber based dressings that may accommodate antibiotics and or analgesics (through co-spinning), wherein these dressings may possess multi-layering capacity and do not leave scars on the skin, i.e. the healing is scar free. In some embodiment, these nano fiber based dressings may operate in moist environment, and may not require frequent changing and thus reduces pain and scars that may be extremely beneficial for burn victims. In some embodiments, nano fiber based dressings may possess certain limitations such as (i) delicacy and a need for tangible support and (ii) may be expensive compared to conventional dressings.

In some embodiments, biodegradable polymeric nano fibrous scaffolds loaded/laden with antimicrobials/ antibiotics exclusively for burn wound care may be used successfully
not only for local controlled delivery but may also act as a synthetic bio-resorbable skin substitutes.

In one specific embodiment PLGA (65:35) for nano fibers fabrication may be used, and PLGA-PVA blending (HLB balancing) may be achieved. In a further embodiment, PLGA-PVA scaffold as a controlled delivery vehicle for a sparingly water soluble antibiotic may be produced. In a further embodiment, Co-axial emulsion electro spinning is performed in order to obtain core-shell architecture. In a further embodiment Linezolid an oxazolidinone class of antibiotic in the nanofiber form is used. In a further embodiment, use of Linezolid for local sustained delivery to heal burn wounds is illustrated in the Figures and the accompanying description. In a further embodiment, use of minimal dose of Linezolid in comparison to oral dosage regimen is illustrated.

In an embodiment of the present disclosure linezolid loaded PLGA-PVA nanofiber scaffold not only delivers the antibiotic in a controlled fashion, but also acts as a wound cover because of its mimicking structural resemblance with ECM, which will facilitate necessary support for any broken/open structure of the skin and helps in aiding the attachment and proliferation of the cells like fibroblasts. In a further embodiment, PLGA owing to its biodegradable and biocompatible features metabolizes In vivo and aids in hypoxia, which may be a demanding feature for Burn wound healing, and this property of PLGA supports in fast regeneration, and thus finally Scar free tissue development will be achieved. In a further embodiment, conversely the demand of surface hydrophilization and moisture uptake of the wound bed will be taken care by PVA, owing to its viscoelastic behavior (hydrophilic).

In one embodiment, encapsulation of Linezolid in a more of a hydrophobic core with PLA:PGA ratio as disclosed above, which will hold the release of drug ,and thus bio availability of the drug can be prolonged, which will reduce the dosage intervals , and ultimately the toxicity of the drug can be minimized. In a further embodiment, Linezolid is used as it is effective against resistant strains like MRSA & VRSE, etc., and may be a good choice for targeting burn wounds, as burn wounds are open, acute wounds, which will suffer from the anomalies with S.aureus and Pseudomonas, etc., which will produce infections that demand quick recovery, otherwise resulting in an increases in mortality. In a further embodiment, stabilization and dose reduction of Linezolid by nano based delivery by topical, targeted, local drug delivery may be achieved.

Core shell nano fibers prepared by Co axial electro spinning of PLGA-PVA-Linezolid 0/W/O emulsion as a core and 10% PVA solution as a shell fabricated with 3
different loading concentrations of the drug (as disclosed above), which is a HLB balanced nano architecture showed rapid wound closure and improved wound healing performance.

In some embodiments, various methods and techniques may be used to show that nanofiber dressings incorporated with molecules may be used as drug delivery system for wound care. However these methods suffer from a number of drawbacks not limited to the list provided below:

1) Solely Lypophilic base material; or
2) Blend of both hydrophilic and hydrophobic combination; or
3) The drug molecule which is encapsulated is either selective to gram positive or gram negative bacteria; or
4) Having a scaffold to enhance the uptake of exudates with a suitable medical textile; or
5) Poor adhesion property to the base support; or
6) Expensive compared to conventional dressings.

Therefore, there exists a need to develop a method that ameliorates some or all of these disadvantages by

(i) selectively encapsulates a drug with a hydrophobic base
(ii) co-axially spun by a hydrophilic outer layer
(iii) drug delivered in nano scale with a carrier material
(iv) Showing extended drug release mechanism for a sustained period
(v) Showing a broad spectrum antibiotic response, specific to gram+ve, gram-ve bacteria

Embodiment of the present invention related to electro spin PLGA nano fiber scaffold containing Oxazolidinone or Linezolid and the additive PVA that may prevent the most vulnerable resistant wound infections and may serve as a functional wound dressing material. In a further embodiment, the enhanced solubility and sustained controlled release of Oxazolidinone or Linezolid from composite nanofiber scaffold has made it possible to use this matrix as a potential functionalized burn wound biodegradable drug delivery carrier system, and effectively heal burn wounds.

One embodiment of the invention include providing an electro spin nano fibrous scaffold as a carrier for delivery of FDA approved antibiotic, wherein the antibiotic comes under a unique class of synthetic antibiotics such as "Oxazolidinones or Linezolid". Another embodiment may be a design of an electro spin nano fibrous scaffold which may be configured to release the medicament in a controlled and orderly manner. In a further
embodiment, a supplementary function of the said scaffold may be to make available a biodegradable, non-toxic electro spin scaffold as a wound dressing that will be adhered to the wound surface and need not to be removed from the wound until the wound has completely healed. In some embodiment, the biodegradable, non-toxic electro spin scaffold as a wound dressing that will be adhered to the wound surface may dissolve when the wound heals.

In one embodiment, a complementary aim of the development is to deliver "Oxazolidinones or Linezolid" in a topical form so as to produce local benefit to the burn wounds and also to give additional benefits for burn wound healing. In a further embodiment, a contraption of the present invention is to present a method of synthesizing an electro spin scaffold that includes an antibiotic. In a further embodiment, advantage of this creation is to make available a synergistic combination of Oxazolidinone or Linezolid - PLGA for augmented accumulation of lactic acid, which is beneficial for wound healing. A further embodiment may be to check the efficacy of a co-axially electro spin HLB based biodegradable, non-immunogenic scaffold loaded with the Oxazolidinone or Linezolid antibiotic on the growth inhibition of gram positive and gram negative bacteria. Yet a further embodiment of the contraption is to present a burn wound healing antibiotic encumbered scaffold successful against MRSA, which reduces the impediment of infections, a major concern and principle menace for burn victims.

In one embodiment, biological rarity of Oxazolidinone loaded Hydrophilic-Lypophilic balanced (HLB) nano scaffolds involving a co-axial architecture of drug-PLGA-PVA nanofibers demonstrates a pronounced antibacterial efficacy, highlighting a molecular mechanistic role of Linezolid on Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Enterococcus* (VRE) and various multi drug resistant gram positive bacteria such as *Streptococci sp.*, *Staphylococci sp.* and *Enterococci sps.* In a further embodiment, it may be noteworthy that HLB Oxazolidinonenano scaffold also show profound effect in gram negative bacteria such as *Pseudomonas* and *E.coli* that otherwise may be found to be inactive to bulk Oxazolidinone delivery.

In one embodiment, a criticality of nanofiber based dressing may include highlights such as the Hydrophilic-Lypophilic balance being achieved by a combination of 90:10 ratio of PLGA (ration disclosed previously): PVA, co-axially electro spin nanofibers on cotton fiber as substrate. In a further embodiment, *In vivo* studies using burn wound model reveal that treatment with PLGA-PVA-Oxazolidinone/Linezolidshow a marked increase in wound closure compared with other burn wound control group. In a further embodiment,
Histopathological evaluation of test samples and control burnt skin tissues showed improved proliferation of fibroblasts, increased number of blood vessels and pronounced re-epithelialization process. In a further embodiment, skinhydroxyproline level may be found to be significantly increased (p<0.001) after treatment with PLGA-PVA-Oxazolidinone/Linezolidnano scaffold as compared to the burn wound control group. In a further embodiment, immunohistochemistry shows a marked decrease in iNOS and COX-II expression after the treatment with the PLGA-PVA-Oxazolidinone/Linezolid scaffold. In a further embodiment, overall results show that Oxazolidinone loaded co-axially electro spun HLB based biodegradable, non-immunogenic scaffold treatment for 21 days could significantly (p<0.01) heal the wound in vivo animal models as illustrated in Figure 4.

In one embodiment, a co-axially electro spun biodegradable polymer based wound dressing scaffold loaded with an Oxazolidinone antibiotic for burn wound care is provided. In a further embodiment, the wound dressing may include core-shell architecture of electro spun nano fibers. In a further embodiment, the core may be a HLB balanced drug loaded polymer emulsion layer and the shell is a hydrophilic polymeric coating layer. In a further embodiment, the shell layer may include biocompatible polymer. In a further embodiment, the bio compatible polymer may be a hydrophilic polymer. In a further embodiment, the hydrophilic polymers may be selected from a group consisting of Chitosan, gelatin, collagen, poly vinyl alcohol (PVA), polyethylene oxide (PEO), or a combination thereof. In a further embodiment, the hydrophilic polymer selected may be a poly vinyl alcohol. In a further embodiment, the outer layer may provide a moist environment on a surface of a wounded area in order to accelerate the wetting of bunt skin and also provides good adhesion of the scaffold owing to its visco-elastic nature.

In one embodiment, the core layer may include of a combination of hydrophilic polymer and hydrophobic polymer and an antibiotic. In a further embodiment, the hydrophobic polymers may be selected from a group consisting of polyamides, poly caprolactone (PCL), poly (lactic acid)(PLA),poly(lactic-co-glycolic acid) (PLGA) or a combination thereof. In a further embodiment, the hydrophobic polymers may be poly(lactic-co-glycolic acid)(PLGA). In a further embodiment, a 10% weight ratio of hydrophilic polymer may be added to 90% of poly (lactic-co-glycolic acid) drug loaded blend. In a further embodiment, the blending not only decreases the initial burst release of the drug but may also result in prolonging the drug release rate.

In one embodiment, the release rate of the drug may depend on the type of polymer used. In a further embodiment, the release rate of the drug may depend on the amount of
drug loaded. In a further embodiment, the release rate of the drug may depend on the extent of cross linking of the polymer and the addition of another polymer and additives if any.

In one embodiment, a method of synthesizing an electro spun nano fibrous wound dressing may include a core-shell architecture. In a further embodiment, the outer layer of the nano fiber may be a polymeric layer that may act as a support layer for the inner core layer. In a further embodiment, the inner core layer may be a polymeric emulsion layer. In a further embodiment, the core layer is a PLGA based nano fibrous layer loaded with Oxazolidinone/Linezolid class of antibiotic.

In one embodiment, the electro spun nano fibrous scaffold may be fabricated by the electro spinning process. In a further embodiment, the electro spinning process may be carried out using an electro spinning device. In a further embodiment, the electro spinning device may have a voltage in a range of 0-50kV and a syringe pump with dual syringe capacity. In a further embodiment, the syringe pump may be controlled to provide a flow rate in a range of 0.1-300ml/h. In a further embodiment, the electro spinning may be carried out at temperature of 20 - 25°C. In a further embodiment, the steps of forming the shell solution preparation may include a preparation of 10% poly vinyl alcohol solution in water.

In one embodiment, a step forming a core solution blend may further includes a preparation of poly (lactic-co-glycolide) solution in Di Chloro methane (DCM) solvent. In a further embodiment, a drug solution may be in a 4:1 ratio of ethanol: water. In a further embodiment, the prepared poly (lactic-co-glycolide) solution and drug solution were blended together with the help of 10% PVA to get OAV/O emulsion. According to another embodiment, a polymer emulsion of 10% PVA was added to improve the viscosity and to achieve proper mechanical strength of the core layer. In a further embodiment, both the core and shell polymer blends may be electro spun with the help of co-axial spinneret containing inputs for two solutions. In a further embodiment, by controlling the temperature voltage and the distance between the spinneret tip and collector, uniform, ultrafine nano fibers ranging from 100- 200 nm may be obtained.

In one embodiment, a method of anti- bacterial activity testing with an electro spun scaffold including a bio degradable polymeric biomaterial loaded with an antibiotic may be provided. In a further embodiment, the method includes contacting the bacterial cells with the engineered scaffold in the presence of culture media. In a further embodiment, a method of delivering the agent to an animal model is provided. In a further embodiment, the method includes administering an engineered scaffold consisting of an anti- bacterial agent effective against MRSA resistant strains, etc. that constitute the prevalent strains for morbidity of the
burn patients. In a further embodiment, a morphological and histopathological benefit achieved with the prepared scaffold compared to the marketed standard burn wound healing ointment product is provided. In a further embodiment, a beneficial improvement is shown as per the immunohistochemical changes of iNOS and COX-II inflammatory mediator's expression.

In one embodiment, safety and beneficial actions of the PLGA emerge from the encapsulation of varied number of hydrophilic/hydrophobic chemical entities for oral, parenteral, transdermal drug delivery as well as for wound repair and tissue regeneration. In a further embodiment, none of the prior arts provides a co-axially electro spun nano fibers incorporated with an antibiotic effective for burn wound healing. In a further embodiment, Linezolid in a nano formulation form is not provided. In a further embodiment, Linezolidnano delivery in topical form obviates existing limitations and also provides additional benefits for wound healing processes.

In one embodiment, the Core shell nanofibers prepared by co-axial electro spinning of PLGA-PVA- Oxazolidinone/Linezolid 0/W/O emulsion as a core and 10% PVA solution as a shell fabricated with 3 different loading concentrations of the drug, which is HLB nano architecture shows rapid wound closure and improved wound healing performance. In a further embodiment, encapsulation of Oxazolidinone/Linezolid in a more of a hydrophobic core with specific PLA: PGA ratio of 65:35, but other ratios in the range disclosed above may be advantageously used, which will hold the release of drug, and therefore bio availability of the drug may be prolonged.

In some embodiments, the biological rarity of Oxazolidinone loaded Hydrophilic-Lipophilic balanced (HLB) nano scaffolds involving a co-axial architecture of drug-PLGA-PVA nanofibers demonstrates a pronounced antibacterial efficacy, and anti-inflammatory activity highlighting the molecular mechanistic role of Linezolid on Methicillin-resistant Staphylococcus aureus (MRSA) and Vancomycin Resistant Enterococcus (VRE) and various multi drug resistant gram positive bacteria such as Streptococci sp., Staphylococci sp. and Enterococci sp. In a further embodiment, it is noteworthy that HLB Oxazolidinonenanoscaffold may also show profound effect in gram negative bacteria such as Pseudomonas and E.coli which otherwise was found to be inactive to bulk Oxazolidinone delivery. In a further embodiment, the criticality of the nanofiber based dressing highlights the hydrophilic-lipophilic balance achieved by a combination of 90:10 ratio of specific ratio PLGA (65:35): PVA, co-axially electro spun nano fibers on marketed cotton gauze as substrate. In a further embodiment, in vivo findings using burn wound model revealed that
treatment with PLGA-PVA-Oxazolidinone / Linezolid showed a marked increase in the wound closure compared with the burn wound control group. In a further embodiment, histopathological evaluation of the test and control burnt skin tissues shows improved proliferation of fibroblasts, increased number of blood vessels and pronounced re-epithelialization process. In a further embodiment, skin hydroxy proline level was found to be significantly increased \( (p<0.001) \) after the treatment with PLGA-PVA-Oxazolidinone nano scaffold compared to the burn wound control group. In a further embodiment, immuno histo chemistry findings showed a marked decrease in the iNOS and COX-II expression after the treatment with the test scaffold. In a further embodiment, results show that Oxazolidinone loaded co-axially electro spun HLB based biodegradable, non-immunogenic scaffold treatment for 21 days significantly \( (p<0.01) \) heal the burn wound in \textit{in vivo} animal models.

Although embodiments of the invention have been described using specific terms, such description is for illustrative purposes only, and it is to be understood that changes and variations may be made without departing from the spirit or scope of the following claims.

\textbf{DISCLAIMER}

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Claims:

1. A hydrophobic-lypophilic balanced (HLB) scaffold comprising a co-axial architecture, the coaxial structure of comprising a core nano fiber administered or laden with a drug and first covered with a hydrophilic material and then covered with a hydrophobic material, where in the co-axial structure of the HLB scaffold is configured to hold the release of the drug over time thereby prolonging the bioavailability of the drug.

2. The HLB scaffold as claimed in claim 1, wherein the scaffold comprises a poly (glycolic-co-Lactic acid) (PLGA) and polyvinyl alcohol (PVA) in addition to the core nano fiber.

3. The scaffold as claimed in claim 2, wherein the PLGA has a PLA: PGA ratio in the range of 50-80:20-50.

4. The scaffold as claimed in claim 1, wherein the drug is an antibiotic and/or an analgesic.

5. The scaffold as claimed in claim 4, wherein the antibiotic consists one from the group of Oxazolidinone and/or a Linezolid.

6. The scaffold as claimed in claim 1, wherein the scaffold comprises a biodegradable polymer material.

7. The scaffold as claimed in claim 1, wherein the core nano fiber is deposited on a base material.

8. The scaffold as claimed in claim 7, wherein the base material is either one of an elastic supporting fabric or a non-elastic supporting fabric.

9. The scaffold as claimed in claim 1, wherein the nano fiber is semi-permeable and/or porous and/or semi-porous and/or non-porous.
10. The scaffold as claimed in claim 4, wherein the drug inhibits the growth of Gram Positive bacteria.

11. The scaffold as claimed in claim 4, wherein the drug inhibits the growth of Gram negative bacteria.

12. The scaffold as claimed in claim 6, wherein the drug exhibits a sustained controlled release from the polymer composite.

13. The scaffold as claimed in claim 9, wherein the drug regulates expression of inducible Nitric Oxide Synthase (iNOS) and/or Cyclo-Oxygenase (COX-II) inflammatory mediators expression in a wounded area, thereby regulating inflammation.

14. The scaffold as claimed in claim 13, wherein the wound is a burn or an incision.
Figure 5
Figure 6
A. CLASSIFICATION OF SUBJECT MATTER
A61L2/00, A61F13/00, A61K9/00, C08J3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L, A61F, A61K, C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search Serins used)
Patseeer, IPO Internal Database
Keywords: scaffold, coaxial, nano, hydrophilic, lipophilic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Gupta et al.: &quot;Nanofibrous scaffolds in biomedical applications&quot;, Biomaterials Research 2014, 18:5. Entire document</td>
<td>1-14</td>
</tr>
</tbody>
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*Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search: 04-09-2017
Date of mailing of the international search report: 04-09-2017

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