Antimicrobial peptide LL37 loaded PLGA nanoparticles promote wound healing activity

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INTRODUCTION
Dermal wounds remain one of the most prevalent and economically burdensome healthcare issues in the world. [1] One of simple and pragmatic solutions to faster wound healing processes reported recently was the application of exogenous lactate that accelerates vasculogenesis, activation of procollagen factors and recruitment of endothelial progenitor cells in wounds. Utilization of poly (lactic-co-glycolic acid) (PLGA) is one of the strategies to supply lactate sustainably. [2] Moreover, PLGA is biodegradable, biocompatible, have versatile degradation kinetics and have been approved by European Medical Agency and FDA. [3]

LL37 is the only antimicrobial peptide of the cathelicidin family identified in humans and is part of the innate immune system. [4] LL37 exerts different immunomodulatory functions like broad antimicrobial activity, antiviral and antifungal activity, endotoxin-binding properties, modulation of pro-inflammatory response, chemotactic, influence of cell proliferation and differentiation, promotion of wound healing and angiogenesis, induction of gene expression, etc. [6] The major difficulties associated with LL37 administration for wound healing are its immediate degradation in wound environment, requirement of large dose and dosage [7] or gene therapy.

PLGA nanoparticles (NP) have been successfully proved as efficient carrier for large biomolecules. [3, 8] Hence, we hypothesized that the administration of LL37 encapsulated in PLGA polymer (PLGA-LL37 NP) could deliver the LL37 efficiently and accelerate wound closure due to the encapsulated LL37 and released lactic acid. Thus, we present the PLGA-LL37 NP for the active healing of dermal wounds and study their mechanisms of action.

METHODS
PLGA-LL37 NP were prepared by W/O/W emulsion-solvent evaporation method with a few modifications from the literature. All the materials used were sterilized either by autoclaving or membrane filtration and the procedures were performed under sterile hood. The physico-chemical properties (size, zeta potential), release profile were studied. Cytotoxicity (MTT and LDH), migration (24h) and proliferation assay (72h) were performed on HaCat cells.

The effects of LL37, PLGA NP and PLGA-LL37 NP on wound healing were evaluated in a splinted mouse full thickness excisional model. The wounds were treated with only vehicle, 5 µg LL37, 5 mg PLGA NP and 5 mg of PLGA-LL37 NP containing 5 µg LL37. A group treated with electroporation of a plasmid encoding LL37 (50 µg) was included to compare the therapeutic potential of PLGA-LL37 NP. Wound area was tracked over a period of 19 days and animals were sacrificed on days 5 and 10 for histology (hematoxylin and eosin (H&E) and Masson's Trichrome (MT) green, Immunohistochemistry of CD31 (IHC), biochemical analysis (Sircol collagen assay and MPO assay) and qPCR of IL6, VEGF expression at mRNA level).

RESULTS
PLGA-LL37 NP have enhanced the LL37 and stability and offered protection against invivo environment. Thus some of the limitations of LL37 administration have been overcome. The size, PDI, zeta potential and encapsulation efficiency of PLGA-LL37-NP were found to be 304.3 ± 10.0 nm, 0.18 ± 0.01, −21.9 ± 2.5 mV and 70.0 ± 3.3% respectively. PLGA-LL37 NP displayed a sustained release of LL37 over a period of 8 days. The LL37 release followed a biphasic release profile and sustained drug release was observed due to degradation of PLGA. The cell viability and mortality results of MTT and LDH assay have clearly shown that, with the given concentration of LL37, PLGA NP and PLGA-LL37 NP there was no statistically significant cytotoxic effect on HaCat cells. LL37 showed a faster migration than PLGA-LL37 NP in first few hours where later PLGA-LL37 NP also showed an equivalent migration effect. No formulation showed a significant effect on proliferation of HaCat cells.

We found while measuring the wound area that the PLGA-LL37 NP showed a twofold higher wound healing activity compared to that of only PLGA or LL37 and by the 10th post wounding day, PLGA-LL37 NP showed nearly complete healing. In H&E and MT stained sections and sircol assay, PLGA-LL37 NP treated group showed complete re-epithelialization of the wound with well-formed and differentiated epithelium and significant increased deposition of connective tissue. PLGA-LL37 NP treated groups evidently showed greater angiogenesis activity (CD31 and VEGF expression) and showed...
immunomodulatory activities by up-regulating the expression of the IL6 at mRNA level compared to all the other groups on both day 5 and day 10 of post wounding.

**Figure 1.** Representative pictures of wounds of different groups closure over time

<table>
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<th>Day</th>
<th>Untreated</th>
<th>LL37</th>
<th>PLGA NP</th>
<th>PLGA LL37 NP</th>
<th>Electropermeation</th>
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**Figure 2.** a) Histology (HE, MT staining) b) Sircol assay

**CONCLUSION**

We demonstrated that PLGA-LL37 NP are capable of protect and maintain the release of LL37 and lactic acid and significantly accelerated the wound closure by comprehensive healing which included regulation of inflammatory/immuno modulatory responses (IL6), expedited re-epithelialization, improved granulation tissue formation and angiogenesis. Thus, PLGA-based drug delivery systems are promising particularly for the wound healing activity because of its innate lactic acid activity and sustained drug release.

**Figure 3.** q-PCR evaluation of IL6 and VEGF mRNA expression on day 10 of post wounding

**ACKNOWLEDGEMENTS**

The financial support from the European Commission and Marie Curie Actions is greatly acknowledged. Kiran Kumar Chereddy is an early stage researcher (ESR) of FP7 Marie Curie NANODRUG network.

**REFERENCES**


