Nanoscale Lipid Drug Conjugates (NLDCs) of Diclofenac as an Efficient and Alternative Wound Healing Agents

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Abstract

This study evaluated nanoscale lipid-drug conjugates (NSDCs) effects on cold-induced burn wounds. NLDCs were physico-chemically characterized based on their critical quality attributes (hydrodynamic diameter size, polydispersity index, surface charge zeta-potential, morphology, drug release in biologically relevant media, etc.). *In vitro,* a scratch assay was performed to measure cell migration and wound closer. *In vivo*, a cold burn wound model was developed via direct exposure of the dorsal rat skin to liquid nitrogen. NLDCs were subcutaneously injected after 15 min of burn wound induction. The gross macroscopic examination of wound tissues was performed at different time points; days 1,4,8,16. Wound regeneration was observed in control (untreated) and treated groups (diclofenac and NLDCs). This regeneration was subsequently assessed by histological, gene expression, and immunohistochemical analyses at the early (day 8) and late (day 16) phases of wound healing. Scratch assay exhibited enhanced cell migration towards scratch area in the treated groups compared to the control. Macroscopic examination revealed early scab formations at day 8 in treated groups, while complete regeneration was only observed in the NLDC group at day 16. Histological findings showed enhanced regeneration of skin layers along with hair follicles in the NLDCs treated group, while increased neovascularization was noted in both treated groups. Gene expression profile of wound healing mediators illustrated downregulation of inflammatory (IL-1β, IL-6, and TNF α) and Pain (COX-2, YKL-40, and Substance-7) and upregulation of angiogenic and remodelling (VEGF, TGF-beta, FGF, PDGF, MMP-9, and EGF) markers at respective time points. In conclusion, NLDCs are potential anti-inflammatory, pain relieving, and wound healing agents. Prospects; We are optimistic that the NLDCs development and their scale-up production will pave the way towards clinical translation of indigenously developed nanomedicine in treating inflammation and wound complications.

Introduction

Diclofenac Sodium

Potent Non Steroidal Anti inflammatory Drug (NSAID) used as analgesic, anti inflammatory, pain relieving and wound healing agent post surgery

Diclofenac Sodium leads to severe side due to undesirable pharmacokinetics like

- Short half life
- Large volume of distribution
- Time dependent dosage





Nanoscale Diclofenac as wound healing agent

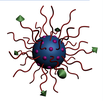
- Protect and effectively maintain moist environment
- Increased efficacy and sustained release
- Heals wounds with simultaneous reduction of inflammation

Nanoparticles lack in rate of clinical translation due to

- Limited drug loading
- Premature burst release

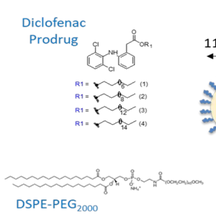
Prodrugs of diclofenac & lipid polymer

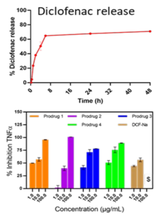
- Higher percent encapsulation efficiency
- Enhanced efficacy



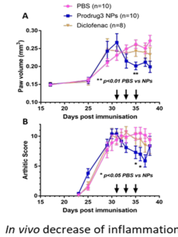


Background Studies



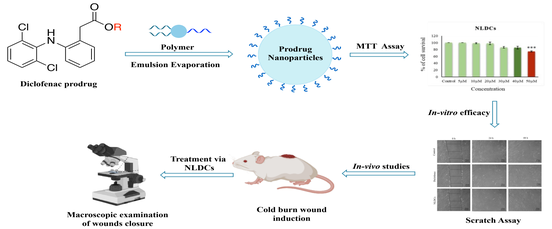


In vitro inhibition of TNFα



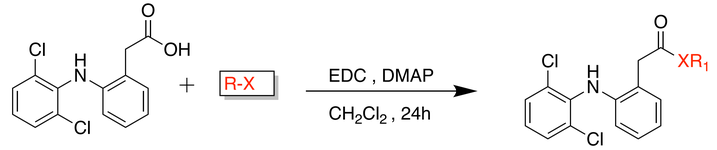
In vivo decrease of inflammation on CIA murine model

Objectives



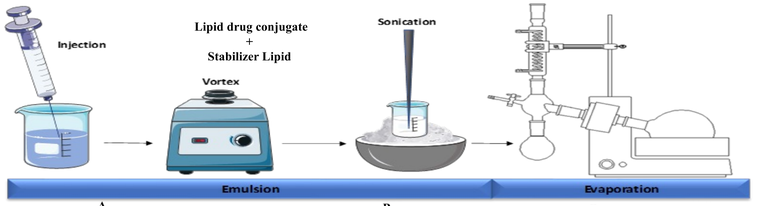
- To design potent anti-inflammatory, pain relieving, and wound healing agents.
- To design nanoparticles with sustained release avoiding exposure to high doses of antibiotics.
- To design NLDCs more beneficial for treating highly localized chronic wounds

Synthesis of Lipid Drug Conjugate



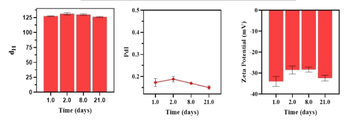
X = Nitrogen, Sulphur or Oxygen atom

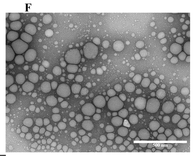
Formulation and Characterization of Nanoparticles



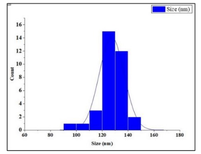
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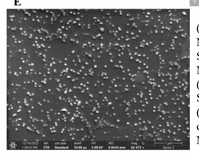
Nanoscale lipid-diclofenac conjugates critical quality attributes	
Size (nm)	130±2
PdI	0.186±0. 10
Zeta potential	32± 3
LDCs % EE	93.34





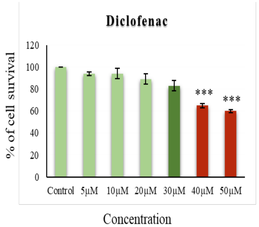


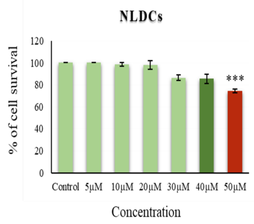




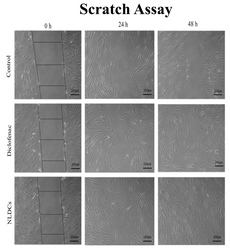
(A) Critical quality attributes of NLDCs; **(B)** Hydrodynamic diameter Size (d_H), PdI and Zetapotential of NLDCs; **(C)** %EE of LDCs in NLDCs; **(D)** Size distribution of NLDCs as per SEM analysis using image J software; **(E)** SEM images of NLDCs, scale bar of 1000 nm; **(F)** TEM micrgraph of NLDCs designed

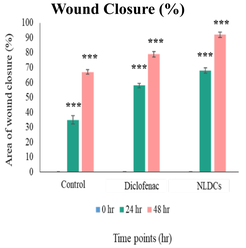
Cytotoxicity Assay (MTT)



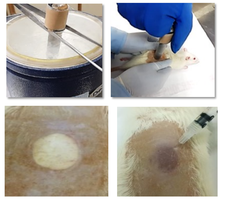


In Vitro efficacy of NLDCs Via Microscopic Analysis of Scratch Assay



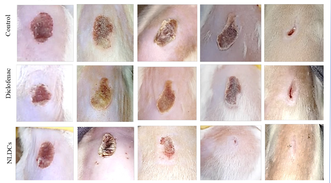


Second degree cold burn wound induction in rat model

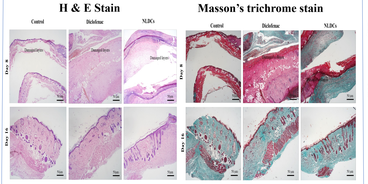


In-Vivo Efficacy of NLDCs designed for Wound Healing

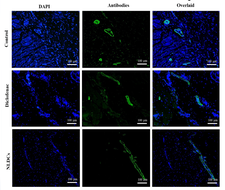
Temporal Macroscopic Examination of Healing



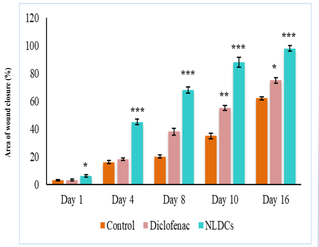
Histological Analysis of Wound Tissues



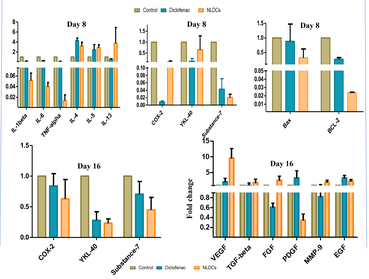
Immunohistochemical Analysis



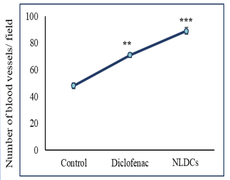
Statistical Analysis of the Wound Closure



Gene Expression Profile of Wound Healing Mediators



Quantification of Blood Vessels



Conclusion

The designed NLDCs were characterized for their particle size, polydispersity index and zeta potential. NLDCs were tested for preclinical efficacy in *in-vivo* mice model. Macroscopic evaluation of wounds followed by peripheral dosage of standard drug and NLDCs revealed wound closure with NLDCs to be significant compared to the one treated with diclofenac and untreated. The histological findings showed enhanced regeneration of skin layers along with hair follicles in the NLDCs treated group, while increased neovascularization was noted in both treated groups. The gene profile of wound healing mediators illustrated downregulation of *IL-1β, IL-6,* and *TNF α,* similarly, downregulation of Pain (*COX-2, YKL-40,* and *Substance-7*) and upregulation of angiogenic and remodeling (*VEGF, TGF-beta, FGF, PDGF, MMP-9,* and *EGF)* markers.

References

1. K. Sen, G. M. Gordillo, S. Roy, R. Kirsner, L. Lambert, T. K. Hunt, F. Gottrup, G. C. Gurtner and M. T. Longaker, *Wound Repair Regen,* 2009, 17, 763–771.
2. Hussain, S., Ur-Rehman, M., Arif, A., Cailleau, C., Gillet, C., Saleem, R., ... & Tsapis, N. (2023). Diclofenac prodrugs nanoparticles: An alternative and efficient treatment for rheumatoid arthritis?. *International Journal of Pharmaceutics*, *643*, 123227.