Poster ID: 2027286

# Designing of nanostructured lipid carrier for topical gene silence therapy in chronic wound healing

**Milena Finazzi Morais; Maria Vitória Lopes Badra Bentley**

*School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil*

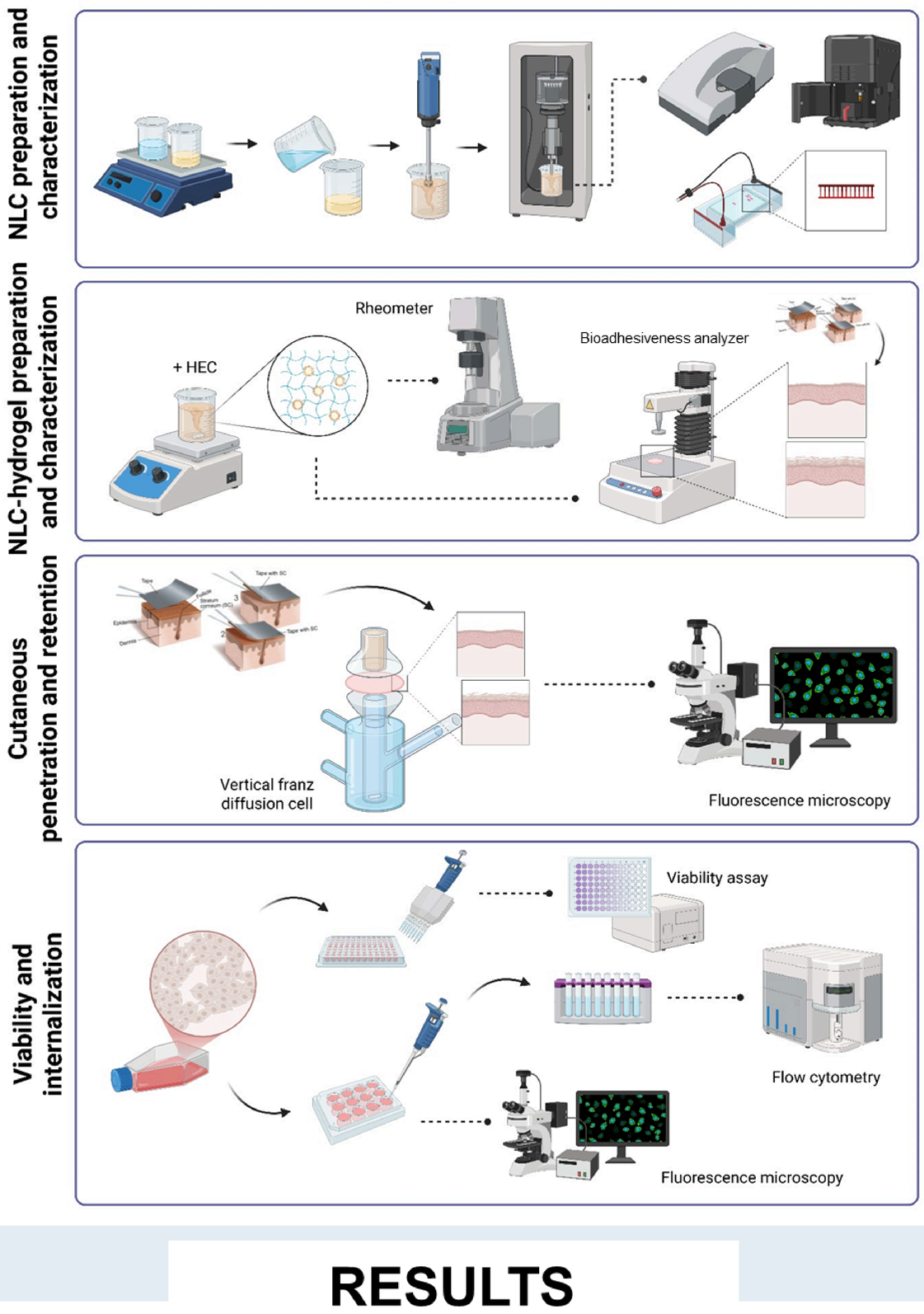
milenafm@usp.br
vbentley@usp.br

NanoGeneSkin Bentley's Laboratory

## INTRODUCTION

Chronic wounds are a global and increasingly recurring problem due to their association with primary chronic disorders, such as diabetes, which is experiencing a growing incidence. However, the multifactorial nature of chronic wounds makes them difficult to treat, becoming a burden on healthcare systems (1). Small interfering RNA (siRNA) has become a promising technology for the treatment of various diseases, including chronic wound healing. However, due to the physicochemical characteristics of these macromolecules, they exhibit limited skin penetration and cellular transfection, which hinders their therapeutic application. Nanostructured lipid carriers (NLCs) have already been shown to be potential siRNA delivery systems, facilitating its delivery not only into cells but also into the skin tissue (2). Thus, this study proposes the development of an NLC capable of enabling siRNA delivery into injured skin tissue, supported by the use of hydrogels, and into cells, aiming at an innovative topical therapy for chronic skin wounds.

## METHODS




## RESULTS

### Colloidal characteristics

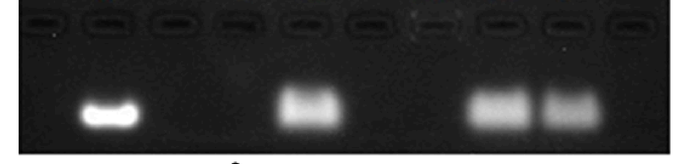
**NLC**
Size: 139.33 ± 0.95 nm
PdI: 0.144 ± 0.011
ξ potential: 25.77±1.11 mV
1.32 ± 0.09 × $10^{13}$ nanoparticles/mL

**NLC-siRNA**
Size: 165.42 ± 0.45 nm
PdI: 0.176 ± 0.016
ξ potential:19.27± 0.52 mV




**Fig. 1** Representative electrophoretic profile of siRNA in agarose gel

INCT NanoFarma – Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (#465687/2014-8)
/Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (#2014/50928-2; #2022/01969-4)




## RESULTS

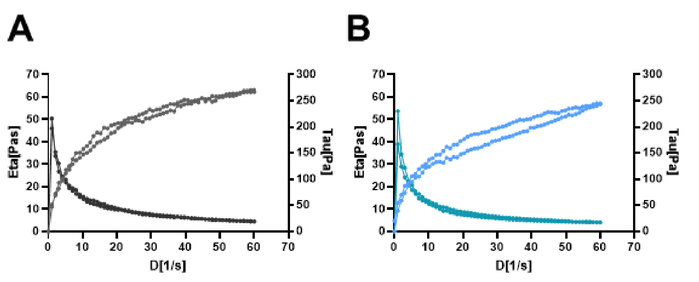



**Fig. 2** Reograms related to variations in shear stress as a function of shear rate and apparent viscosity. **[A]** Hydrogel control **[B]** NLC-hydrogel.

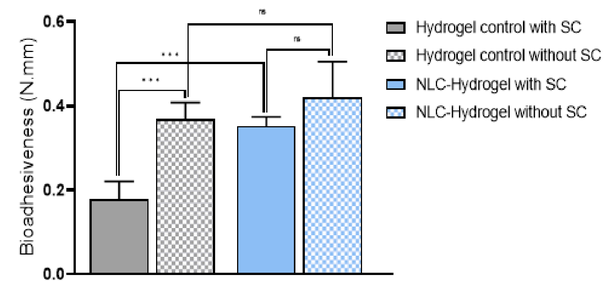



**Fig. 3** Graphical representation of the bioadhesiveness of hydrogels on pig membrane with and without the stratum corneum (SC).

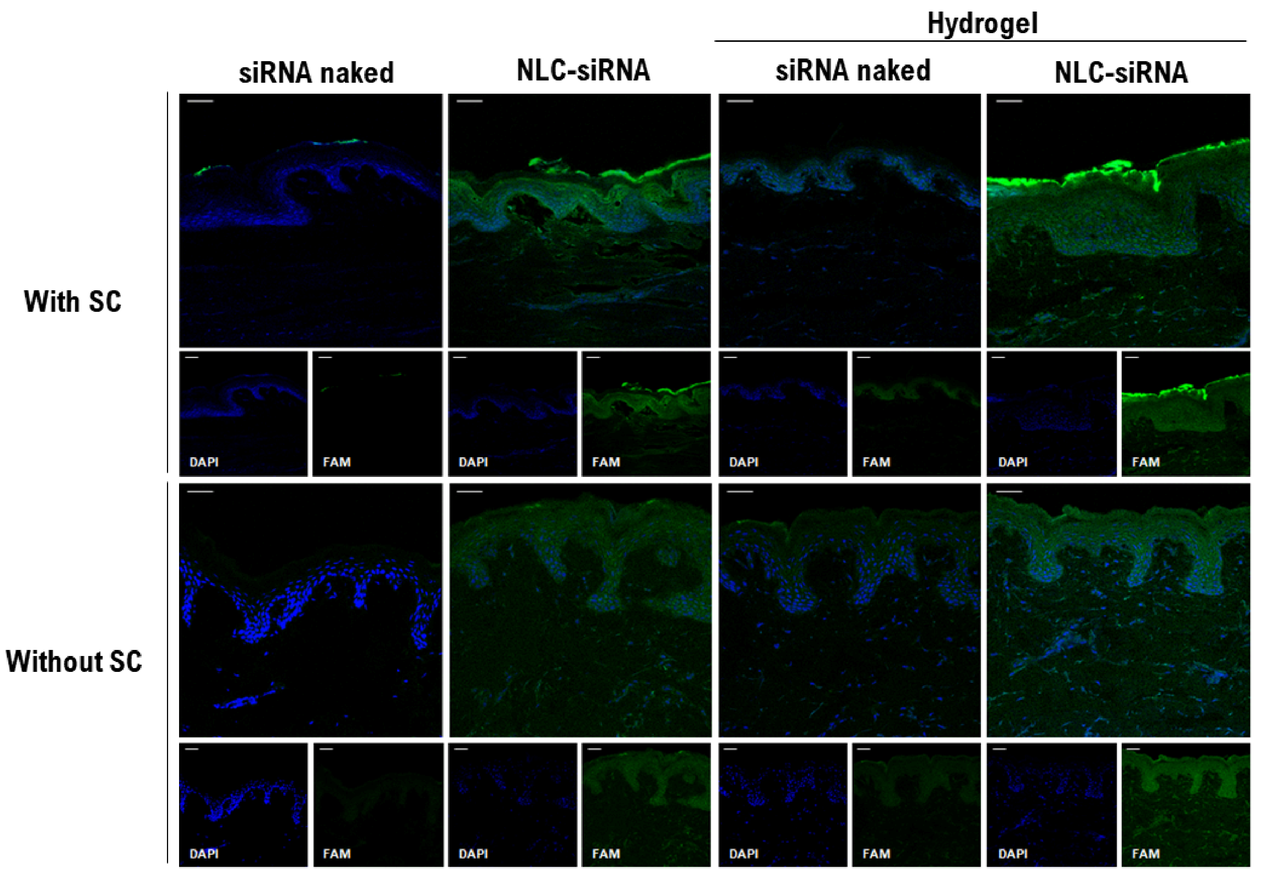



**Fig. 4** Representative CLSM images of porcine skin after 6 h of treatment: siRNA naked and NLC-siRNA (green - FAM) and nuclei (blue - DAPI). Scale bar: 100 µm.

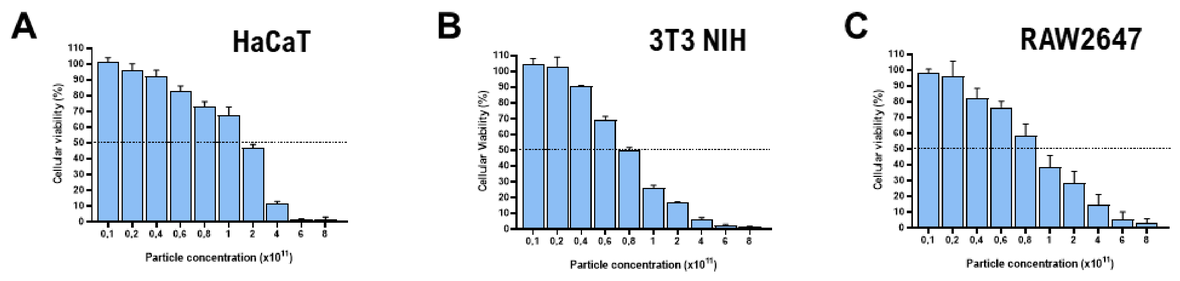



**Fig. 5** Cell viability of NLC in 24 h in the **[A]** human keratinocytes (HaCaT) **[B]** murine fibroblasts (3T3 NIH) and **[C]** murine macrophages (RAW264.7) lineages (Means ± SD n = 4).

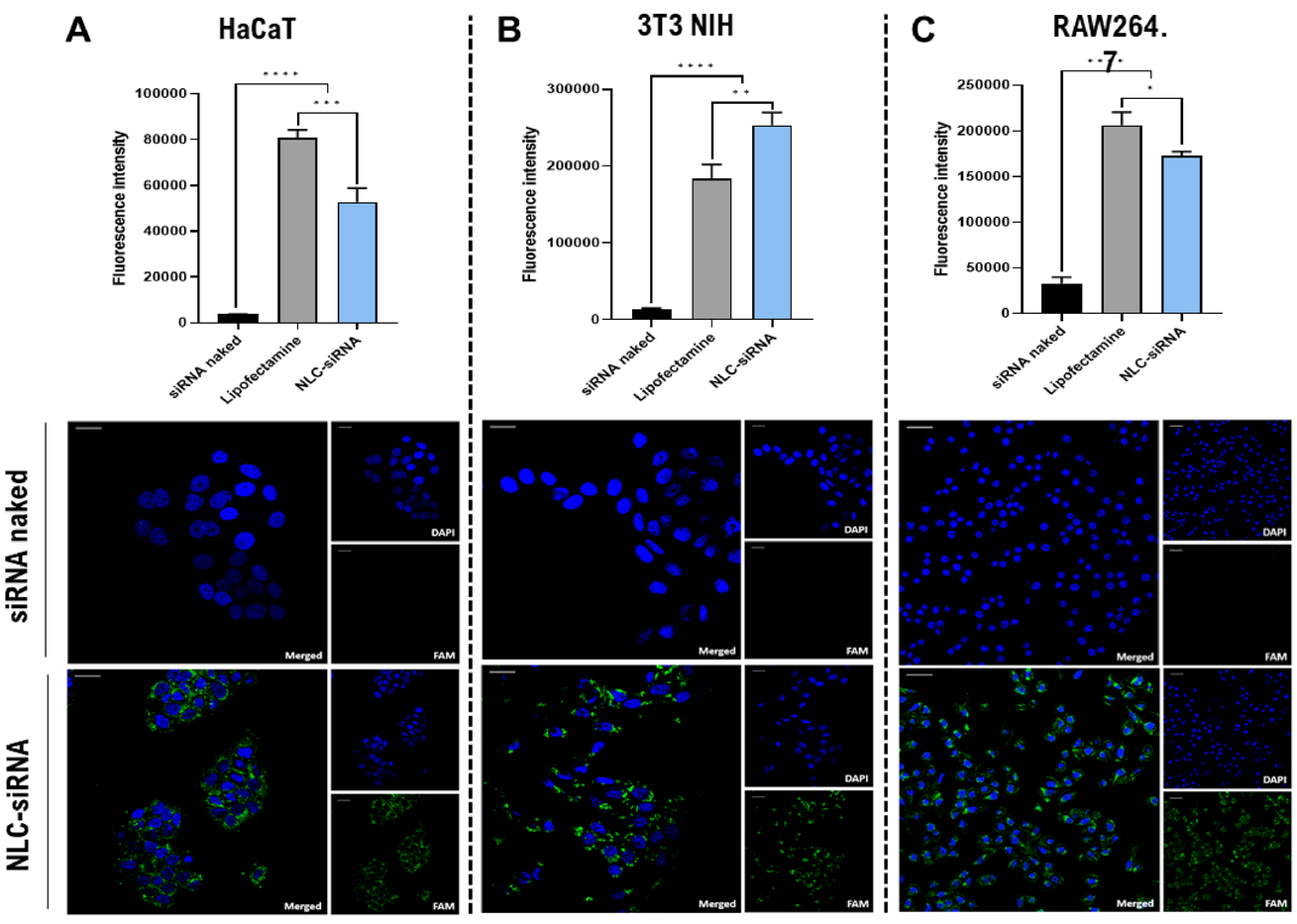



**Fig. 6** Cell internalization of siRNA FAM measured by flow cytometry (Means ± SD n = 4) and representative CLSM images in the **[A]** HaCaT **[B]** 3T3 NIH and **[C]** RAW264.7 lineages after 24 h of treatment. Nuclei (blue – DAPI) and siRNA/NLC (green – FAM). Scale bar corresponds to 20 µm.

## CONCLUSION

The developed NLCs exhibited important colloidal characteristics for the application of siRNA into the skin, include lesioned skin model, as evidenced by *in vitro* studies using vertical Franz diffusion cells. The NLCs combined with HEC hydrogel increasing of viscosity, bioadhesiveness and improved siRNA delivery into the skin. After evaluating its cytotoxicity, it was confirmed that the NLCs enabled the cellular internalization of siRNA in the main cells involved in the wound healing process. Thus, it can be concluded that a promising platform was developed for cutaneous and cellular delivery of siRNA, aiming at wound healing applications. These results encourage future *in vivo* studies to assess its potential use in the treatment of chronic skin wounds.

**References:** (1) Falanga et al., Nature Reviews Diseases Primers, 2022.; (2) Silvestrini et al., Advanced Drug Delivery Reviews, 2024.