Lipid Nanoparticles Loaded With Resveratrol And Glycyrrhetinic Acid As New Tool For Wound Healing

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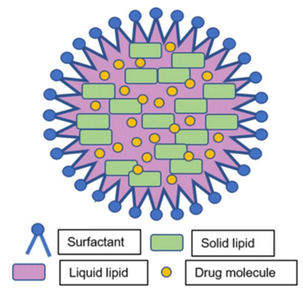
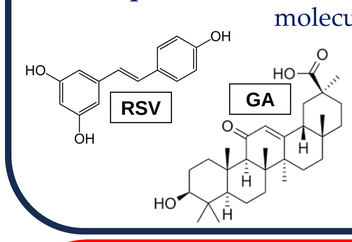
Introduction

Wound healing is a dynamic and intricate process vital for maintaining the body's homeostasis and safeguarding against microbial infections. The **skin** and **mucous membranes** serve as a first line of defense, and their integrity is crucial. In healthy individuals, a series of well-coordinated events, including **inflammation**, generation of **pro-oxidative species**, cell proliferation, and **tissue remodeling**, work in concert to **restore the damaged tissues** comprehensively. However, even **minor disruptions within this cascade can lead to delays in wound healing** or, in severe cases, irreversible tissue damage. Efforts to promote wound healing have explored the potential of **natural compounds**, particularly **polyphenols** and **triterpenoids**, due to their robust **antioxidant** and **anti-inflammatory properties**, as well as their **antimicrobial abilities,** with minimal side effects. Nevertheless, these promising molecules are hindered by their less-than-ideal physicochemical properties, including **low water solubility** and **susceptibility to degradation.**





Aim of work

This work focuses on a groundbreaking approach based on the development and characterization of a **novel drug delivery system** in form of **multicomponent lipid nanoparticles (LNPs)**. These LNPs consist of a complex mixture of **PEGylated lipid**, **Glyceryl monoester**, and **Menthol**, purposefully designed to encapsulate two pivotal wound-healing agents: **Resveratrol (RSV)** and **Glycyrrhetinic Acid (GA)**. By leveraging this innovative drug delivery system, these actives could be protected from degradation and their efficacy might result maximized, thereby rendering **RSV-GA-LNPs** highly valuable tools for **wound management**.

Preparation of LNPs

Actives loaded in the lipid mix:
GA (2.73 ± 0.23%w/w)
RSV (4.56 ± 0.04%w/w)

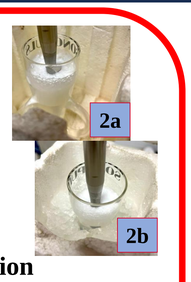
Aqueous phase
Citrate buffer pH 5.5
with **1% w/v of Pluronic F-127**
Pre-heated at 80±0.5°C

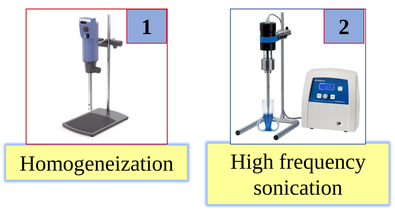
Fused lipid mix

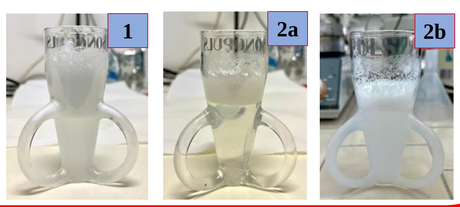
1) Homogeneization (19000 rpm, 1 min) → hot microemulsion

2a) Hot high frequency sonication → hot nanoemulsion

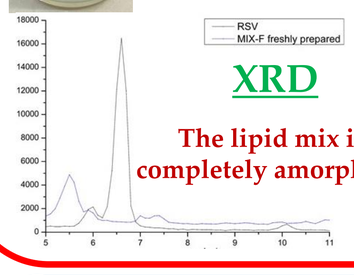
2b) Cold high frequency sonication → LNPs solidification





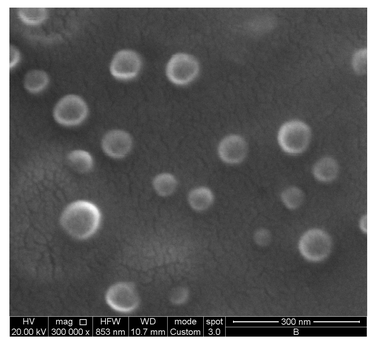


XRD



The lipid mix is completely amorphous

SEM



Quantitative evaluations

RSV	GA
DR% = 96.82 ± 1.34	DR% = 99,6 ± 1.29
DL% = 4.20 ± 0.01	DL% = 2,71 ± 0.11
LE% = 95.17 ± 0.25	LE% = 97.15 ± 0.19

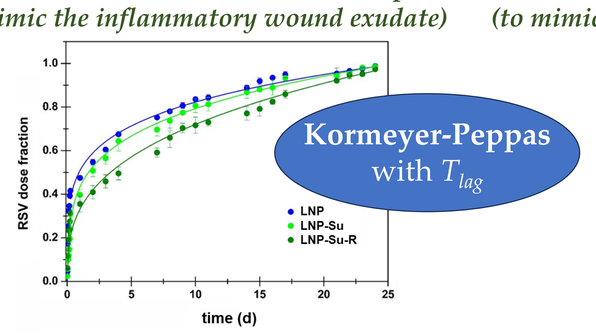
DLS *and* Z-Potential

Intensity (nm) = 162.86 ± 3.12
PDI = 0.267 ± 0.010
Z-potential (mV) = -21.40 ± 7.33

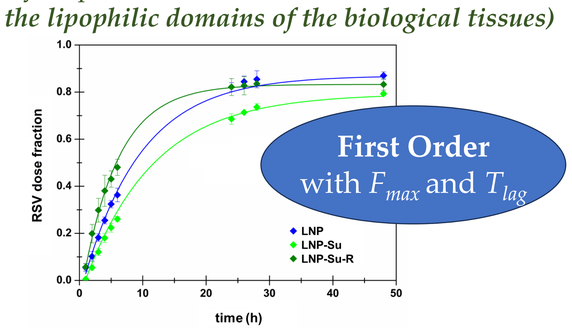
RSV Release study

Different chemical environments simulating the wound conditions

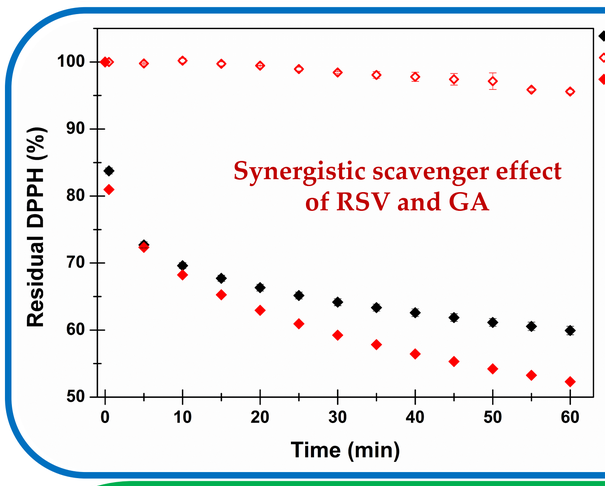
Hydrophilic environment: **Citrate buffer pH 5.5**
(to mimic the inflammatory wound exudate)



Hydrophobic environment: **1-Octanol**
(to mimic the lipophilic domains of the biological tissues)



The release kinetic is not affected by the freeze-drying process

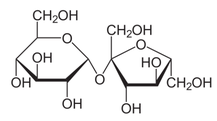


DPPH assay

Antioxidant activity
expressed as residual DPPH% as a function of time

Cryopreservation

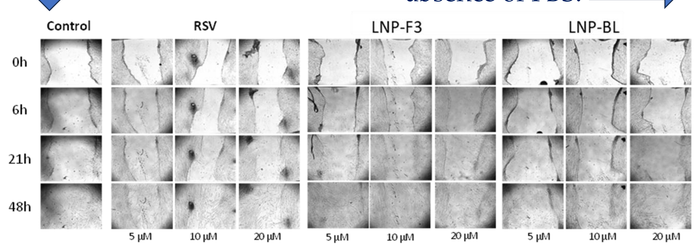
Fully achieved by adding sucrose (LNP:Su=1:8 w/w)
The presence of sucrose in the final dry powder could also:
- ❑ Display some healing effects
- ❑ Minimize the oedema due to enhanced osmotic pressure

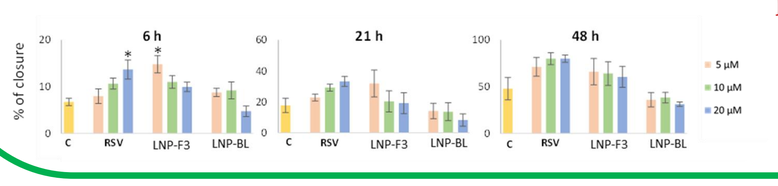


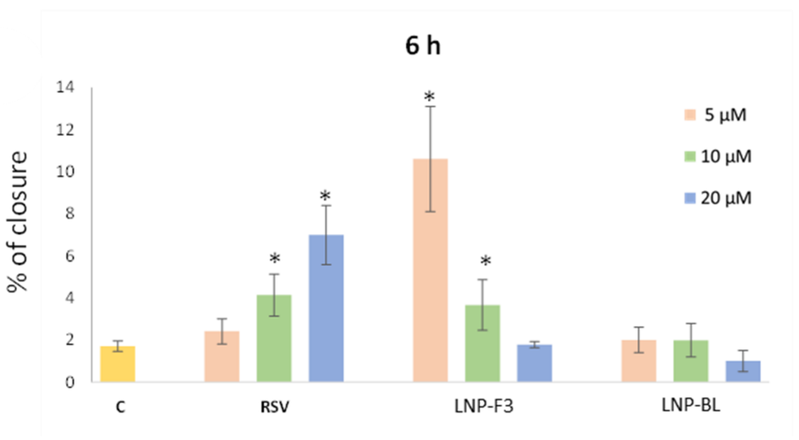
Scratch wound healing assay
(Normal fibroblasts; IMR-90 cells)

To observe the longer time points a medium containing FBS was used. However, the presence of FBS stimulates cells proliferation thus impairing the ability of evaluating cells migration.

As the best performance was observed after 6 h of experiment, the latter was repeated in absence of FBS.



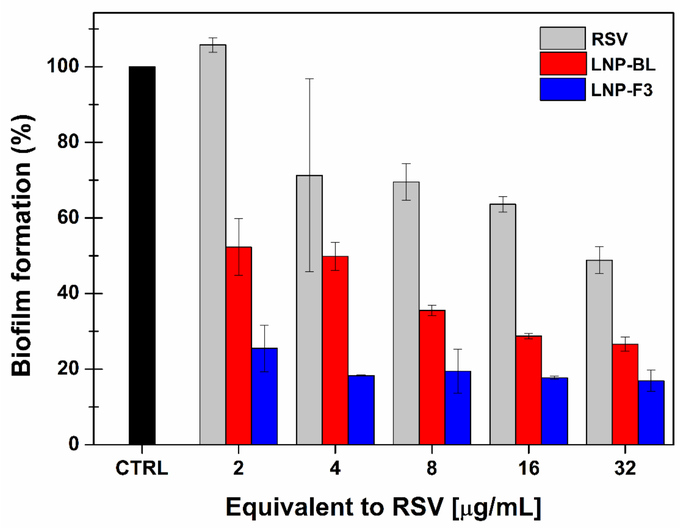




Free RSV stimulates cells migration in a dose-dependent manner.

After embedding into the LNPs RSV effects are greatly magnified and a reverse trend could be observed due to the well-known dual RSV action

Inhibition of biofilm formation
(S. aureus; ATCC 12973)



Synergistic antibiofilm effect of RSV, GA and Menthol

Conclusions

To summarize, the proposed **multicomponent LNPs loaded with RSV and GA** were designed for **wound healing** purposes. The careful choice and balancing of each lipid component has allowed to obtain a stable and workable mixture characterized by *ad hoc* properties. The LNPs preparation was optimized to allow **proper nanometric size**, **low polydispersity**, and **high encapsulation efficiency,** also after freeze drying, by the aid of a cryoprotectant. The LNPs successfully **released RSV** in both **hydrophilic** and **hydrophobic environments**, thus being suitable for wound application. Furthermore, the **biological evaluations** showed **enhanced cell migration**, leading to suitable **wound healing effects at extremely low RSV doses (RSV 5 µM correspond to LNPs concentration equal to 22 µg/mL)**. The latter dose also exhibited promising **antibiofilm** properties against ***S. aureus***. Overall, the LNPs should be potentially useful to promote injured tissue regeneration, both directly (wound closure) and indirectly (scavenging and antibiofilm actions), resulting a promising tool for wound management.

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