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Optimization of drug-free nanostructured lipid carriers (NLC) for *Helicobacter pylori* eradication

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BACKGROUND

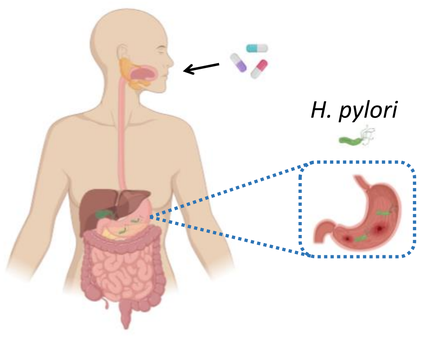
Helicobacter pylori (Hp) is a human pathogen ubiquitously distributed among population[1]. Hp causes several gastric disorders, such as gastritis and 80% of the global gastric cancer burden (5th most common and 3rd deadliest cancer worldwide) is attributed to infection by this bacterium[1,2]. Current treatments for Hp infection are failing, mainly due to the increase of antibiotic resistance[3]. Nanostructured lipid carriers (NLC) are a class of lipid nanoparticles that have been studied for Hp eradication. Previous studies established that drug-free NLC had both *in vitro and in vivo* effect against Hp without affecting the normal gut microbiota[4,5,6]. However, although these NLC have demonstrated bactericidal activity, complete Hp eradication was not achieved, and the underlying mechanism of action of these nanoparticles is still not known. Thus, NLC physicochemical characteristics need to be fine-tuned in order to achieve complete Hp eradication and, therefore, prevent reinfection.

AIM

This work aims to optimize NLC formulation by identifying which are the determinant physicochemical factors for the bactericidal activity.

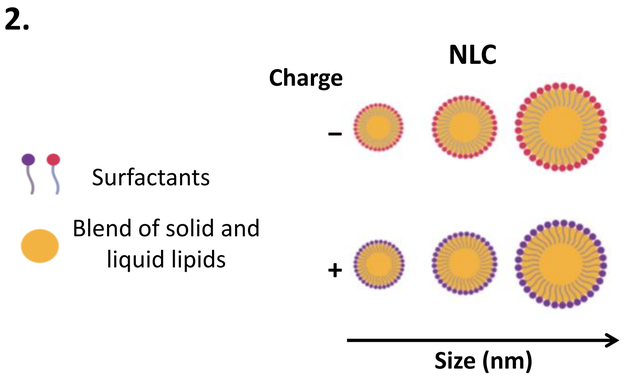
RATIONALE

1.



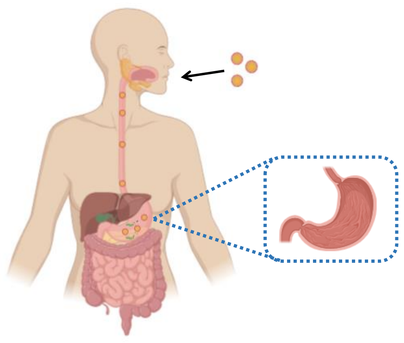
Hp infection treatments rely on antibiotics and proton pump inhibitors. The efficiency of these treatments is declining due to increasing antibiotic resistance.

2.



NLC physicochemical characteristics will be changed in order to discover which are the main factors influencing the bactericidal effect.

3.

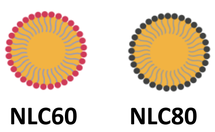


It is expected the obtention of an optimal NLC formulation, able to achieve Hp eradication, and the clarification of its mechanism of action.

NLC CHARACTERIZATION AND OPTIMIZATION

Three Formulations:

Anionic



Tween® 60 Tween® 80

Precirol®ATO5 and Miglyol®812

Cationic

CTAB - Cetyltrimethylammonium bromide

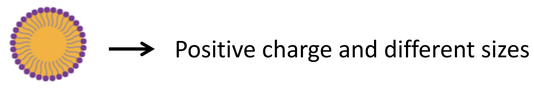
Precirol®ATO5 and Miglyol®812

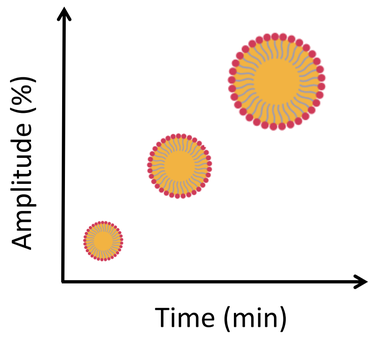
i)

ii)

Size: Dynamic light scattering
Charge: Electrophoretic light scattering
Concentration: Nanoparticle tracking analysis

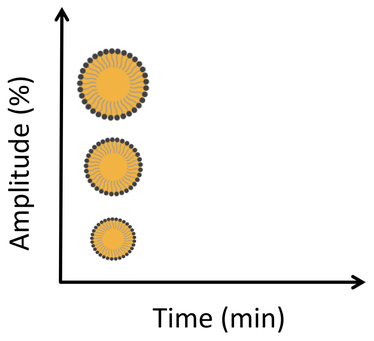
iii)





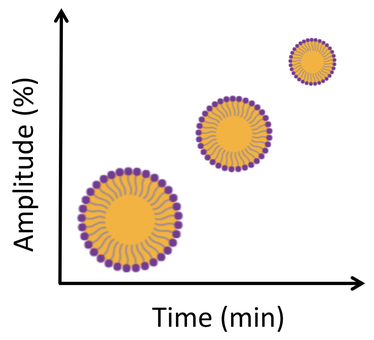
Size Range: 178 – 486 nm

Size (nm)	PdI	Zeta Potential (mV)
178 to 486	0.18 to 0.25	-25 to -30



Size Range: 190 – 228 nm

Size (nm)	PdI	Zeta Potential (mV)
190 to 228	0.18 to 0.22	-25 to -30



Size Range: 107 – 200 nm

Size (nm)	PdI	Zeta Potential (mV)
107 to 200	0.18 to 0.25	25 to 76

Figure 1. Schematic representation of NLC production, characterization and optimization. **i)** The nanoparticles were prepared by hot homogenization followed by ultrasonication. Part of the obtained formulations was dialyzed during 24h at room temperature. **ii)** NLC were characterized in terms of size, surface charge and concentration. **iii)** Optimization of NLC by changing the sonication parameters. Differently charged NLC showed an opposite tendency in size variation.

BACTERICIDAL ACTIVITY

NLC tested:

NLC	Size (nm)	PdI	Zeta Potential (mV)	Stock Concentration (particles/ml)
60/60D	268.2	0.2	-26.7	$1.26x10^{14}$
80/80D	207.9	0.2	-23.9	$1.35x10^{14}$
80/80D	227.5	0.2	-26.9	$1.33x10^{14}$

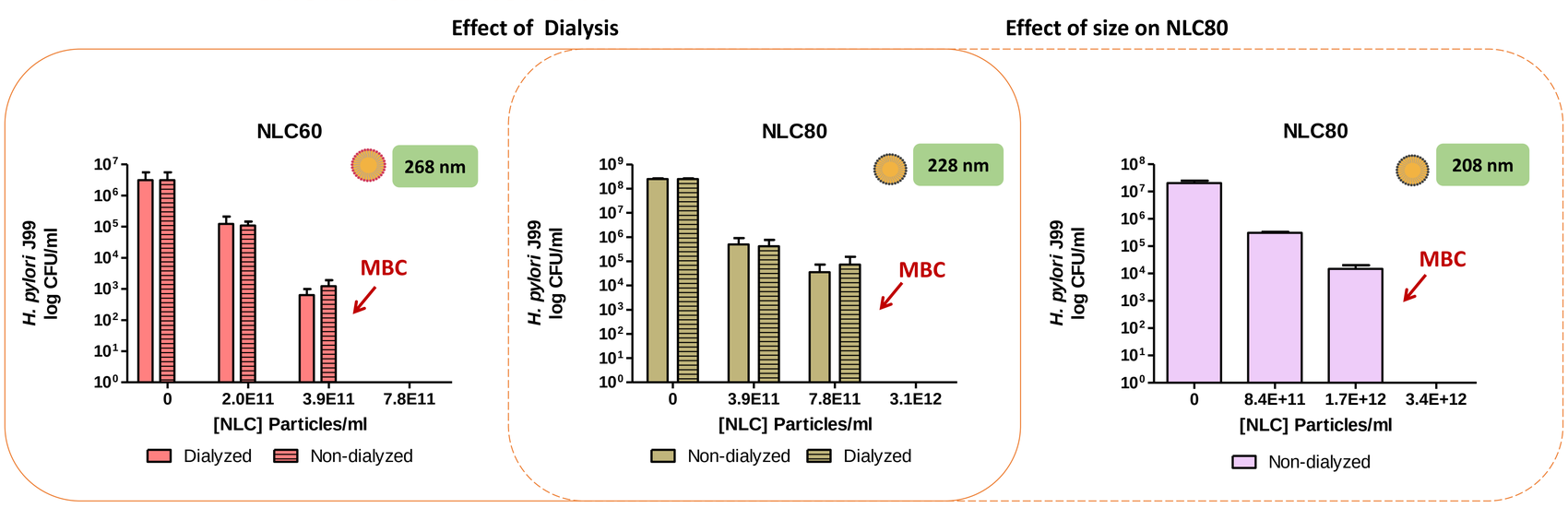


Figure 2. Determination of viable *H. pylori* J99 exposed to increasing concentrations of NLC. Bacteria viability was assessed by colony forming units (CFU). The statistical analysis was performed using a Two-way ANOVA considering statically significant differences at $p<0.05$. Data is expressed as mean ± standard deviation. Data representative of one experiment (n=1), with each condition done in triplicate.

MBC – Minimum Bactericidal Concentration

Smaller size, less bactericidal activity

CONCLUSIONS AND FUTURE WORK:

NLC optimization:

- NLC60 tended to increase in size with higher time and amplitude of sonication;
- In NLC80 the predominant factor for size change was the amplitude;
- Cationic NLC CTAB decreased in size with higher sonication settings;
- Further NLC optimization is ongoing.

Bactericidal Activity:

- Both NLC60 and NLC80 were effective against Hp;
- Smaller NLC80 had less effect showing that size influence the bactericidal activity;
- Dialysis didn't affect the bactericidal activity, what indicates that the NLC have an effect *per se*;
- Other optimized NLC formulations will be tested in Hp and other gut bacteria.

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The images in this presentation were created with BioRender.com

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