# LIPOSOMES DECORATED WITH NOVEL MUCOSAL PENETRATING PEPTIDES TO TARGET THE ESOPHAGUS

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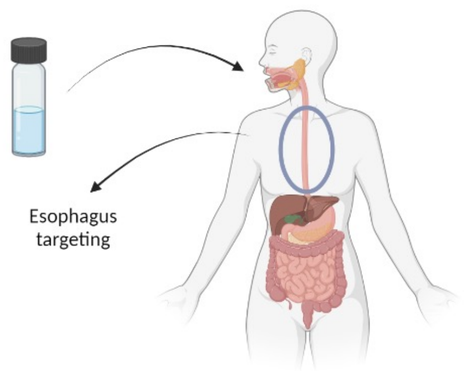
## Background

Targeting of drugs to the esophagus is essential for the treatment of many diseases such as infections, gastric reflux, esophagitis and cancer, that would all benefit from localized drug delivery. Nevertheless, the achievement of a sufficient retention of drugs in this section of the gastrointestinal tract (GI) is limited by the low residence time along with the barrier action exerted by the esophageal mucus. Despite the benefit of local drug release in the upper tract of GI goes beyond the treatment of local diseases, the research in this area is extremely poor[1]. Looking to other mucosae, nanocarriers and in particular liposomes seem to offer an advantage in term of mucus penetration and mucosal drug delivery[2]. The extent of interaction with mucus barrier as function of the physico-chemical properties (i.e. particle size, charge, transition temperature) and surface decoration of liposomes has been investigated in different mucus models. To favor the crossing of the mucus barrier an innovative approach may rely on the decoration of liposomes with peptides appositely selected to overcome both the mucus and epithelia barrier

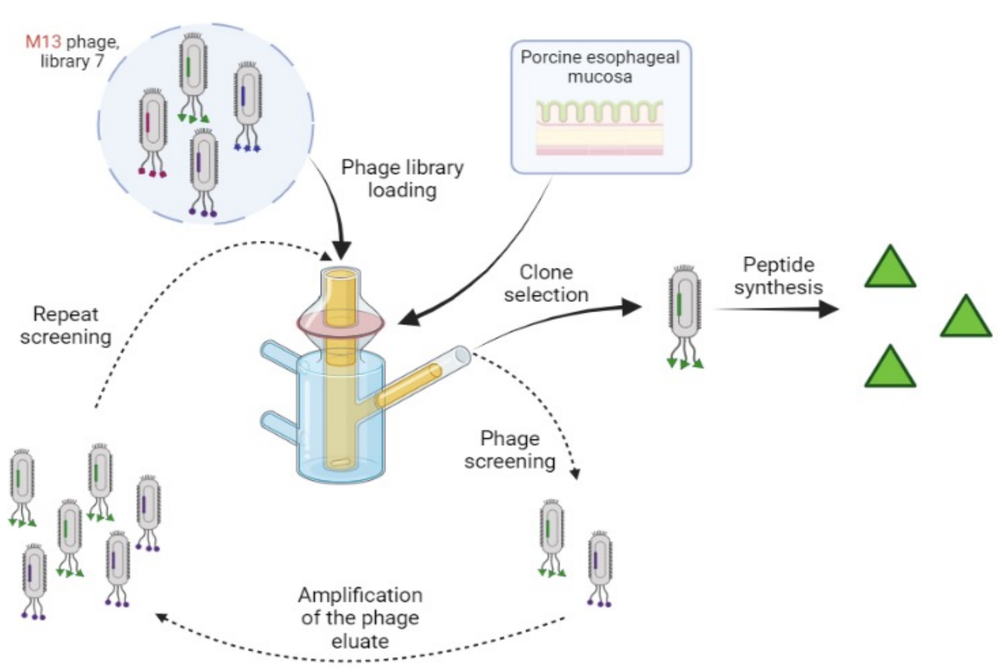



## Aim

**To evaluate the usefulness of screening mucosa penetrating peptides to be used as targeting moieties on liposomal carriers for the localization of drugs in the esophageal mucosa**

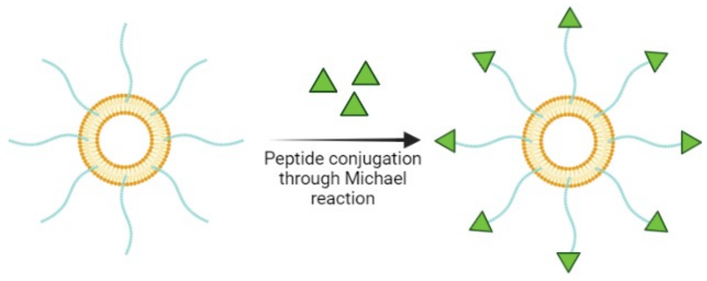
## Methods

### PHAGE DISPLAY SCREENING AND PEPTIDES SELECTION

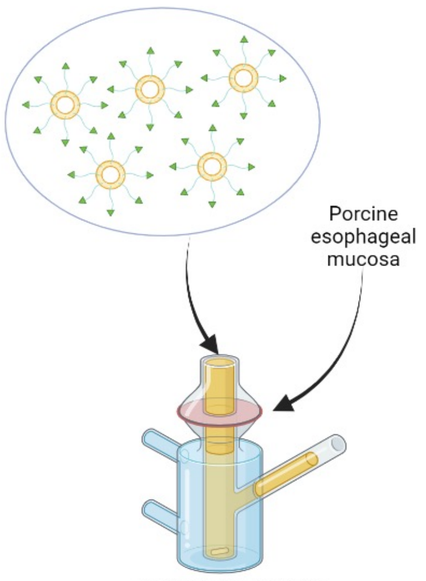



### LIPOSOMES PREPARATIONS

| | DPPC | DOPC | CHOL | DSPE-PEG 2000 | DSPE-PEG-mal | CUR (mg/mL) |
|---|---|---|---|---|---|---|
| L | 60 | 30 | 10 | - | - | 0.5 |
| L-PEG 1% | 60 | 30 | 9 | 1 | - | 0.5 |
| L-PEG-mal 1% | 60 | 30 | 9 | - | 1 | 0.5 |
| L-PEG-mal 5% | 60 | 30 | 5 | - | 5 | 0.5 |




### PENETRATION TEST




**PERMEABILITY AND MUCOSAL RETENTION ASSAY**
at 15min, 30min, 7 hours

## Results and discussion

### IDENTIFICATION OF POTENTIAL MUCOSA PENETRATING PEPTIDES

Table 1 - Peptide sequences expressed on permeated phages surface

| PEPTIDE | AA SEQUENCE | $M_W$(g/mol) |
|---|---|---|
| **P1** | NPLLLRG | 885.1 |
| **P2** | QWQGSVW | 993.1 |
| **P3** | SLENKGP | 847.0 |

Esophageal mucosa resulted overall highly permeable to phages.
Only three peptide sequences were found in more than 20% of clones.

### PHYSICO-CHEMICAL CHARACTERIZATION OF MUCOSA PENETRATING PEPTIDES DECORATED LIPOSOMES

Table 2 - Main physico-chemical properties of prepared liposomes.

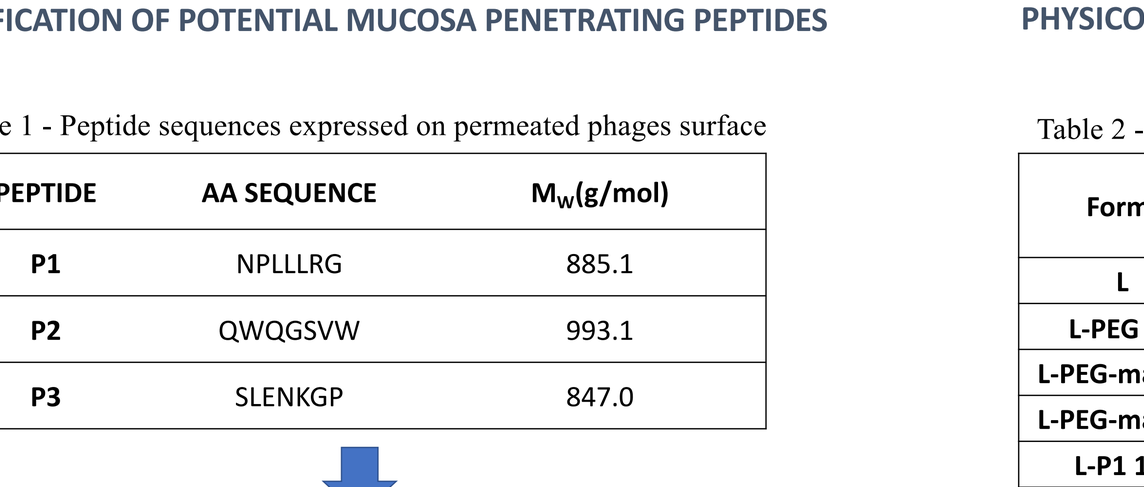
| Form. | D (nm) | ζ (mV) | CUR EE (%) | P CE (%) |
|---|---|---|---|---|
| **L** | 96±9 | -5.4±0.7 | 64.1±4.4 | - |
| **L-PEG 1%** | 108±0.5 | - | 72.1±6.4 | - |
| **L-PEG-mal 1%** | 79±1 | -12.4±2 | 60.0±3.0 | - |
| **L-PEG-mal 5%** | 87±1 | -13.0±1.8 | 70.0±1.0 | - |
| **L-P1 1%** | 98±1 | -11.6±0.4 | 70.4±2.7 | 88.7±8.9 |
| **L-P2 1%** | 93±1 | -12.8±0.7 | 71.9±0.4 | 77.8±4.4 |
| **L-P3 1%** | 91±2 | -12.0±1.1 | 66.7±4.6 | 82.7±10.1 |
| **L-P3 5%** | 98±1 | -12.2±0.8 | 60.0±0.20 | 55.3±2.0 |

DECORATION OF LIPOSOME SURFACE WITH PEPTIDE DID NOT CHANGED THE MAIN PHYSICO-CHEMICAL FEATURES

PEPTIDE **CONJUGATION EFFICIENCY (CE%)** QUITE HIGH

**DIAMETER < 100 nm** IN ALL CASES

### TRANSMUCOSAL PERMEATION EXPERIMENTS

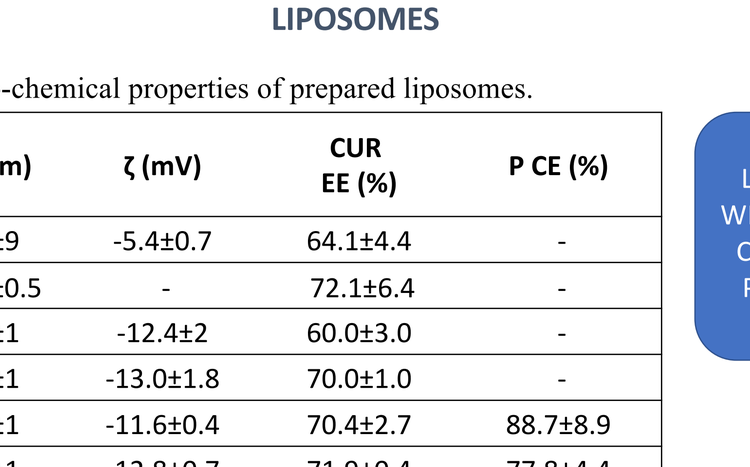



- After 7 hours, only **L-P3 liposomes** significantly enhanced CUR retention with respect to control liposomes ($p<0.05$). The increase of PEG concentration up to 5% molar in P3 decorated liposomes did not cause a further increase of CUR retention despite of the overall higher amount of peptide exposed.
- After 30 min application, the amount of CUR delivered by L-P3-1% and L-P1-1% was comparable and significantly higher than L-PEG-1%. In the case of L-P1-1% none significant variation of CUR retained amount in the mucosa was found at the two different times (30 min and 7 hours); conversely CUR retained amount doubled after 7 hours exposure to L-P3-1%. **These data suggest that P3 unlike P1 might favor both mucus and mucosa penetration enhancing the partitioning of CUR in the tissue.**

**L-P3** was then selected for the further studies

### CONFOCAL LASER SCANNING MICROSCOPY

**5 minutes application**




✓ *Control pegylated liposomes remained mainly entrapped in the mucus layer while the L-P3 1% penetrated deeply in the epithelium*

## Conclusions

This work is the first proof of concept about the possible use of mucosa penetrating peptides labelled liposomes for drug targeting to the esophagus

References:
1. Subramanian et al. J. Nanotechnol. 2022, 20, 362-385.
2. Zhang L. et al. Crit Rev Ther Drug Carrier Syst 2008, 25 (3), 259-304