Interactions of biomimetic liposomes with enterocytes explored by 3D Raman imaging

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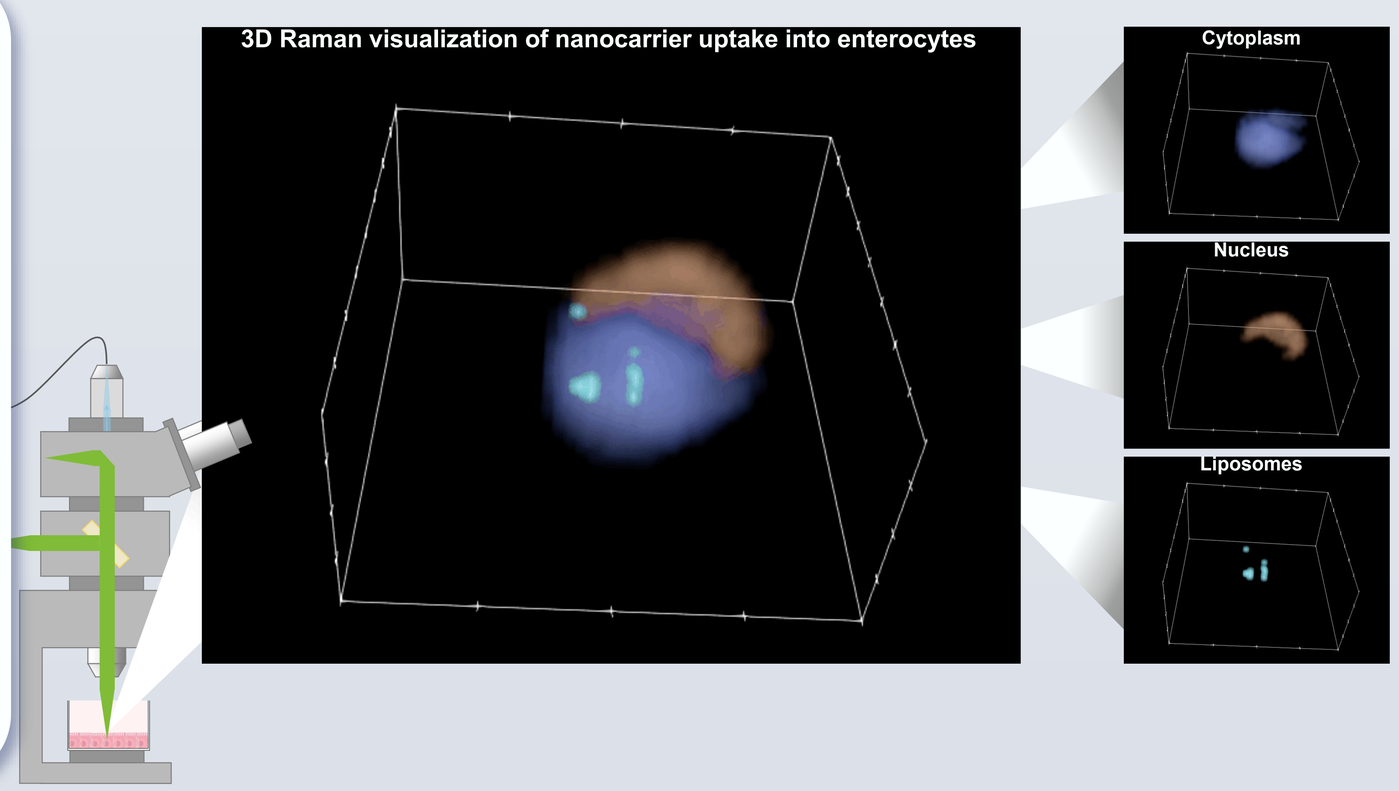
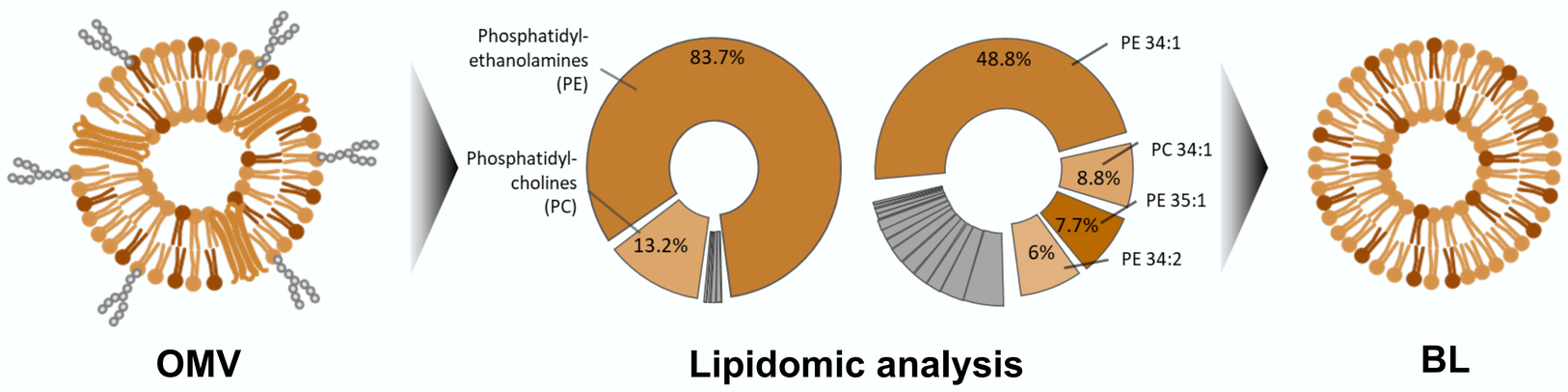
Introduction

Outer membrane vesicles (OMVs) are nanoscale, phospholipid-based structures, actively secreted by bacteria, playing crucial roles in both inter- and intraspecies communication. Certain OMVs exhibit remarkable stability in harsh gastrointestinal conditions and possess the ability to cross biological barriers, such as the intestinal epithelium, highlighting their potential as carriers for oral drug delivery. In this study, we present a bottom-up approach for the fabrication of biomimetic liposomes (BLs), imitating the lipid composition of native OMVs. Using 3D Raman imaging, we systematically compared the interactions of natural OMVs and their synthetic BL counterparts with the intestinal epithelium, providing insights into biocompatibility, stability and their epithelial uptake.



Lipidomic analysis

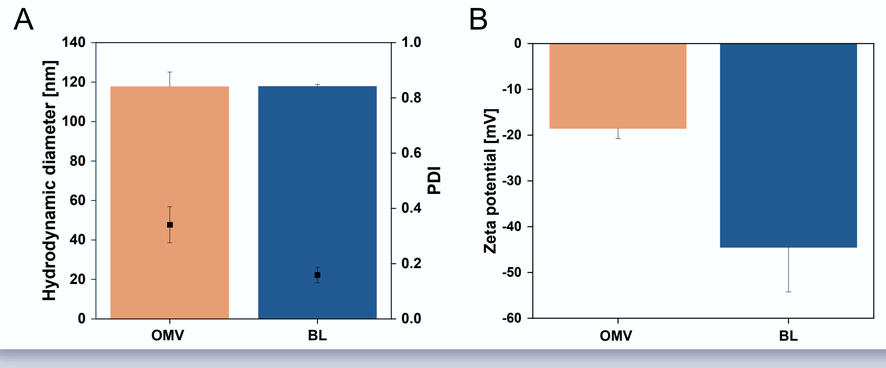
Lipidomic analysis was performed on OMVs, and the resulting data were used to guide the design of BLs. More than 95% of lipid group proportions were successfully replicated, and over 70% of specific lipid species proportions were accurately reproduced.



Physicochemical characterization

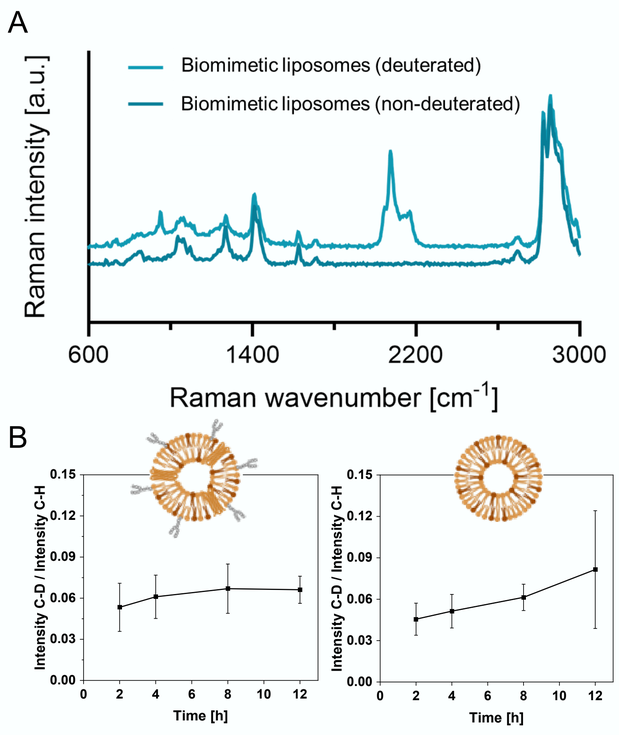
BLs were prepared using dual asymmetric centrifugation followed by extrusion. The process yielded unilamellar vesicles with hydrodynamic diameters of 117.71 ± 7.39 nm, comparable with bacterial OMVs (117.87 ± 1.00 nm) (A).

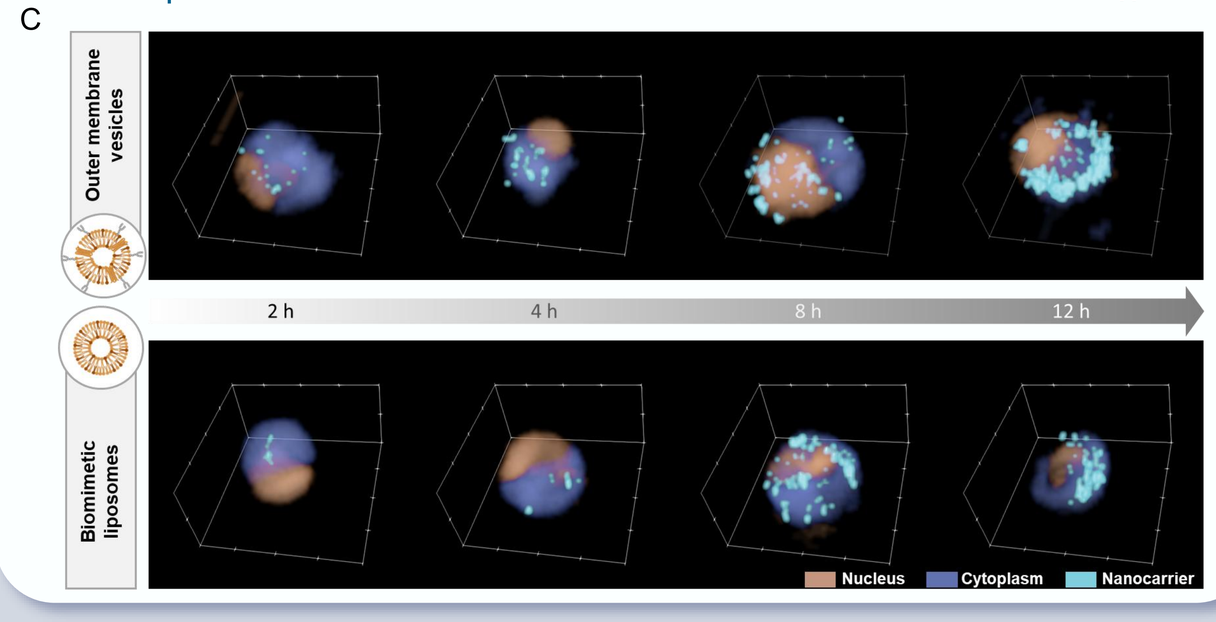
Notably, BLs exhibited a significantly lower polydispersity index (PDI) and zeta potential, reflective of a more uniform size distribution and enhanced electrostatic repulsion, resulting in an overall improved colloidal stability (A&B).



Cellular uptake by 3D Raman imaging

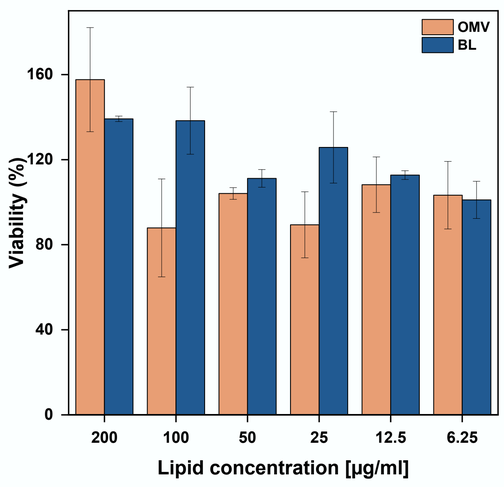
Deuteration of BLs enabled visualization and relative quantification of OMVs and BLs by confocal Raman microscopy.(A). After a 12-hour incubation period, BLs exhibited a more rapid cellular uptake compared to native OMVs (B). Both carriers demonstrated a time-dependent accumulation in regions close to the cell nuclei of enterocytes (C).
A semi-quantitative volumetric analysis, normalized to the overall cell volume, confirmed these observations and revealed a higher intracellular abundance of BLs compared to natural OMVs.





Biocompatibility

Biocompatibility of BLs was evaluated using an MTT assay with human intestinal epithelial cells (Caco-2). Viability was normalized to untreated controls and when compared to OMVs, BLs displayed a comparable bio-compatibility, highlighting their drug delivery potential.



Conclusion

- Fully synthetic BLs, inspired by the complex lipid composition of OMVs were successfully manufactured in a bottom-up approach.
- Confocal Raman microscopy and 3D imaging enabled semi-quantitative analysis of the time-dependent vesicle-uptake of enterocytes.
- BLs revealed superior biocompatibility, vesicular stability and an improved cellular uptake in the intestinal environment.

ACKNOWLEDGEMENTS
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