

Recent Advances in the Use of Phospholipid Excipients

Workshop organized by the
Phospholipid Research Center Heidelberg/Germany



INTEGRATING
Delivery Science
ACROSS DISCIPLINES



Phospholipids in parenteral depot formulations: recent advances, current challenges and future opportunities

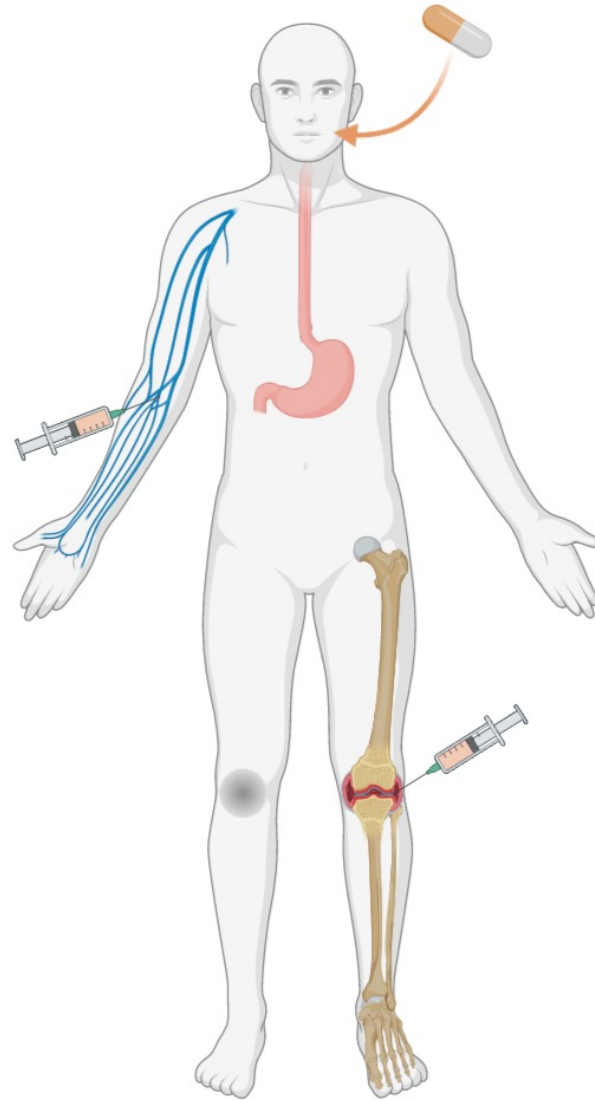
Prof. Dr. Paola Luciani

Full Professor of Pharmaceutical Technology

Department for Chemistry, Biochemistry and Pharmaceutical Sciences - University of Bern, Switzerland

How to reach a therapeutic objective:
pathology – age – sex and gender – possible disabilities

>50% of the
world's population
takes at least
one drug each day



Non-adherence is
estimated to be
around 50% for
chronic illnesses

The evolving therapeutic landscape

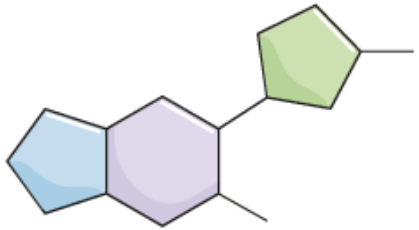
- Solubility
- PK

- Stability

- Intracellular delivery

- Viability
- Scalability

Small molecules



Proteins and peptides



Monoclonal antibodies



Nucleic acids



Live cells



Time

Why depots?

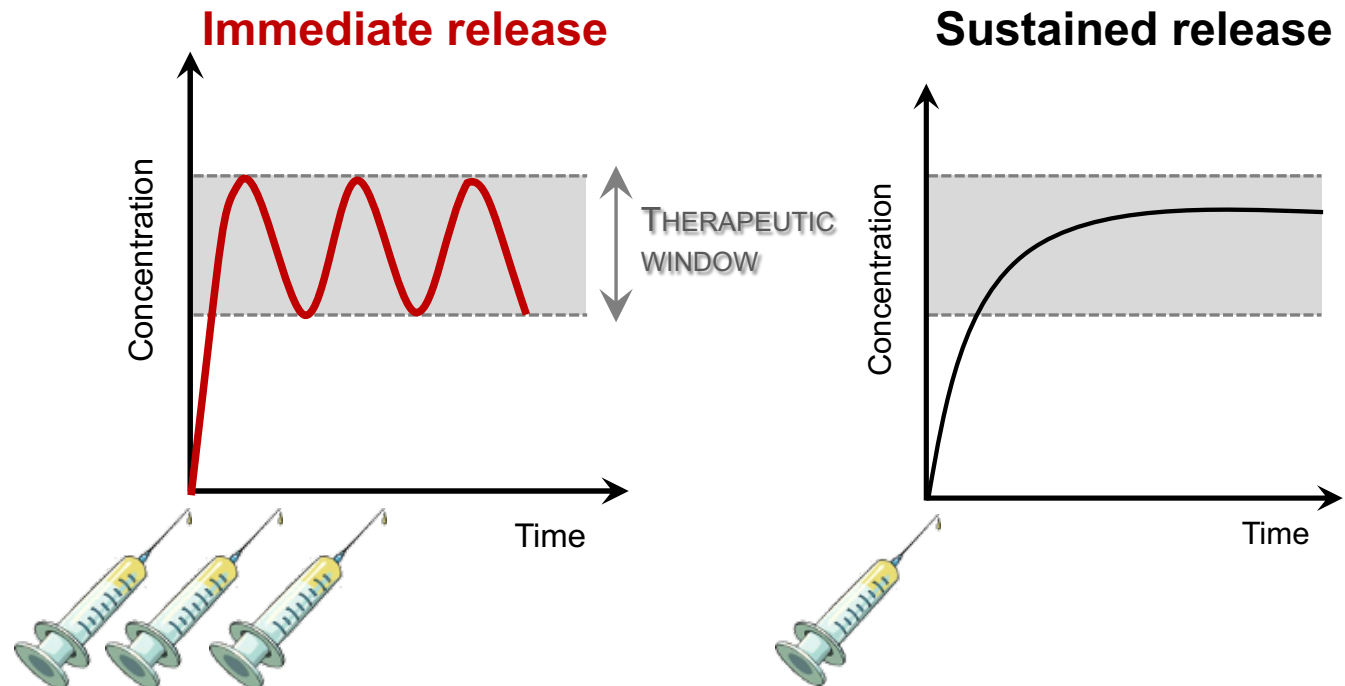
The **therapeutic utility** of many drugs can be enhanced by **prolonged administration** or by **local delivery**

DRUG:

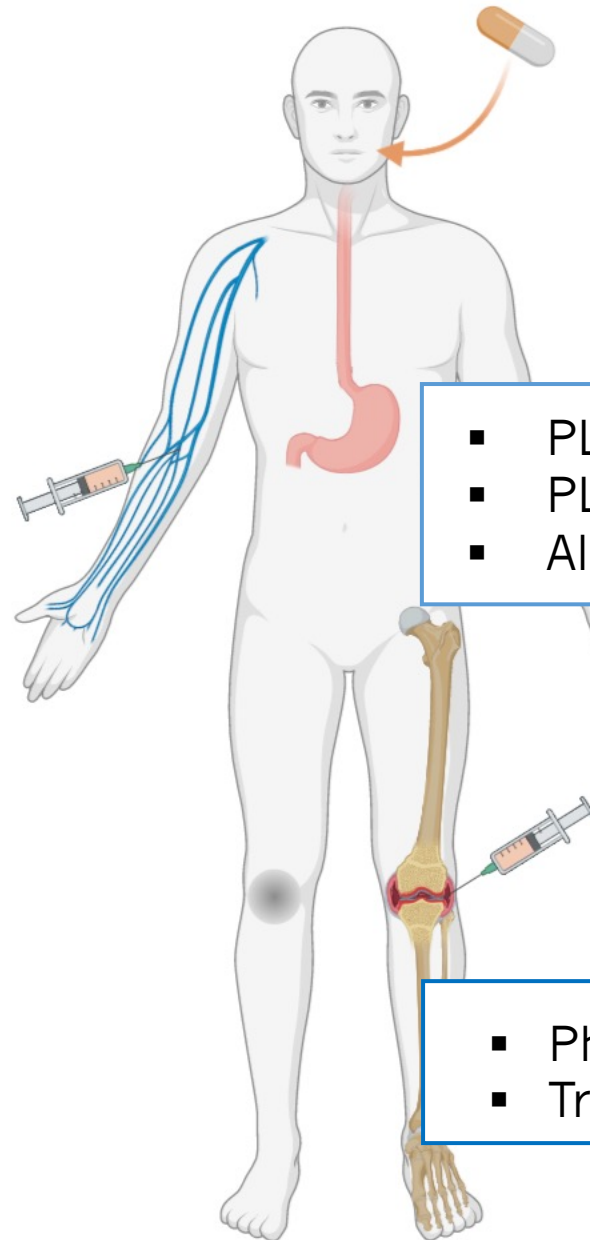
- Low oral bioavailability
- Extensive first-pass effect
- Chemical instability

PATIENT:

- Compliance
- Health costs
- Pharmacodynamics

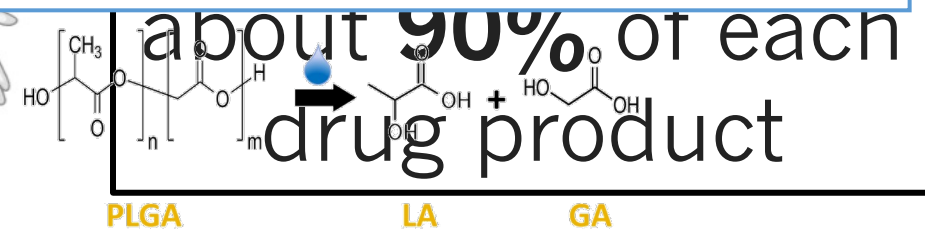


Pharmaceutical
excipients
are substances
other than the
pharmacologically
active drug
or prodrug



POLYMERS

- PLGA
- PLA
- Alginate
- Chitosan
- Sucrose acetate isobutyrate
- Cellulose derivatives...



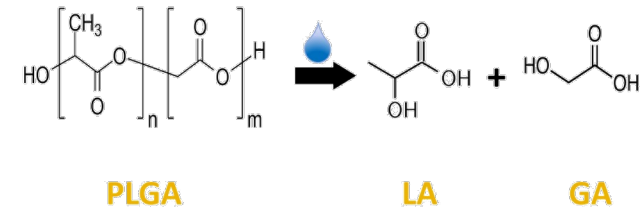
LIPIDS

- Phospholipids
- Triglycerides
- Cholesterol
- Cholesterol esters...

Pharmaceutical
excipients
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pharmacologically
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POLYMERS

- PLGA
- PLA
- Alginate
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LIPIDS

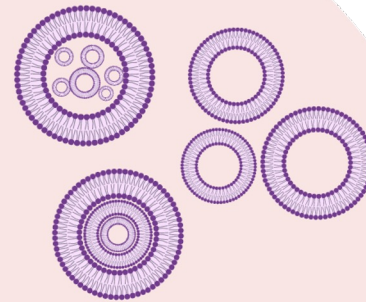
- Phospholipids
- Triglycerides
- Cholesterol
- Cholesterolesters...

Lipid-based long acting injectables

Solutions



Liposomal systems



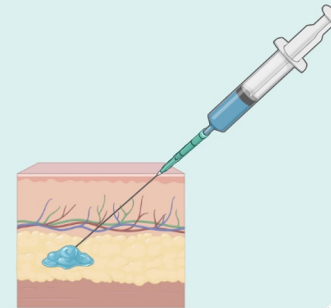
Vesicular phospholipid gels

Semisolid



FluidCrystal®

In situ forming
depots

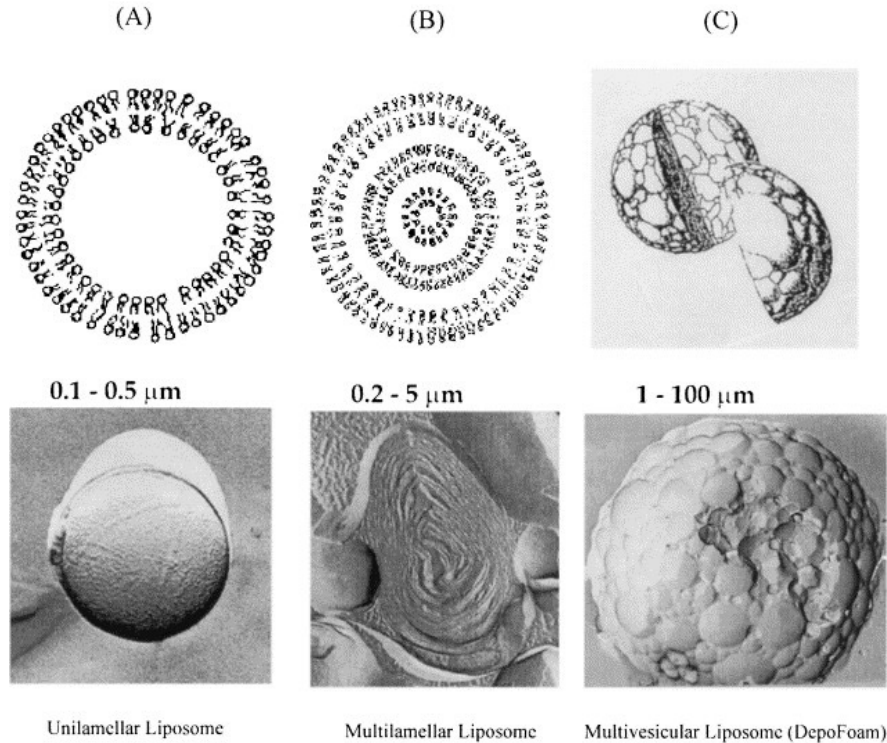


Breitsamer & Winter *Int J Pharm* (2019) 557:1-8
Rahnfeld, Luciani. *Pharmaceutics* (2020) 12:567

<https://www.camurus.com/science/technology/>

DepoFoam™

MVL (DepoFoam) versus conventional liposomes



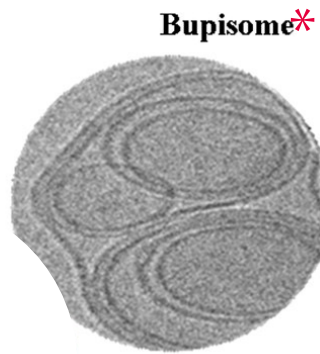
Typical 10-20 μm

- Encapsulation and release of small molecules as well as therapeutic proteins and peptides
- Intratechal, epidural and subcutaneous route
- *Downside*: complex manufacturing process by water-in-oil-in-water double emulsification with organic solvent

Phospholipids: PC (typically dioleoyl or dierucoyl) + PG
Cholesterol, Triglyceride (typically triolein)

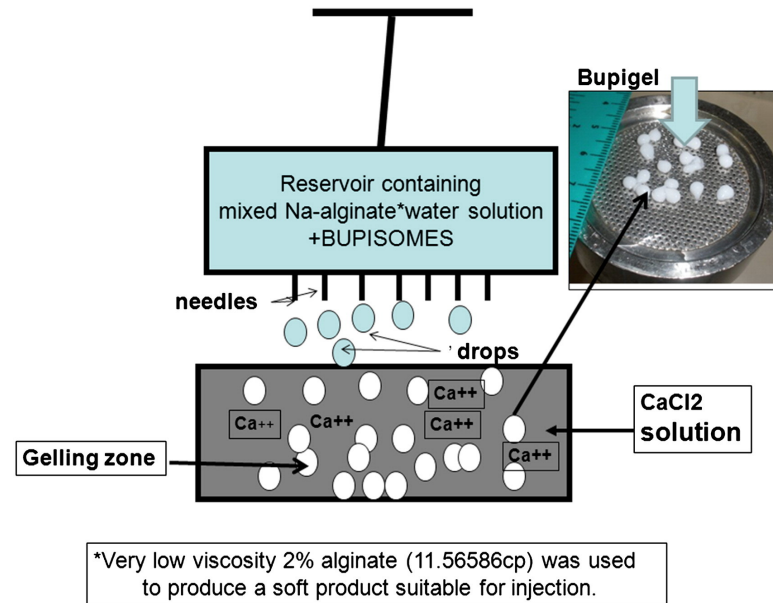
Bupisome and Bupigel

Bupivacaine in large multivesicular vesicles (LMVV)

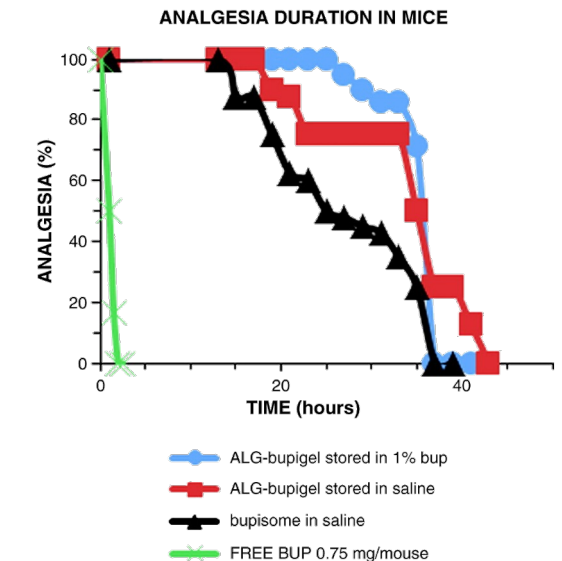
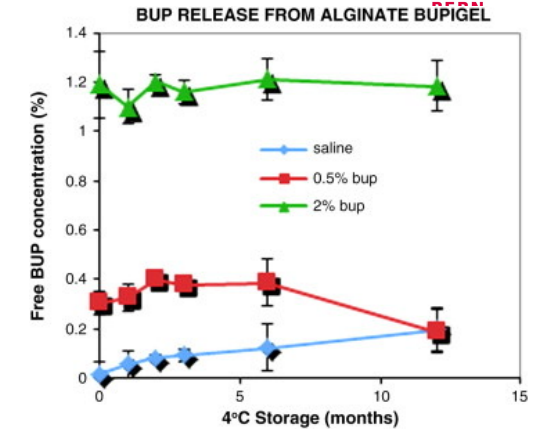


Bupi-LMVV in cross-linked hydrogel (alginate)

Fabrication of Bupigel

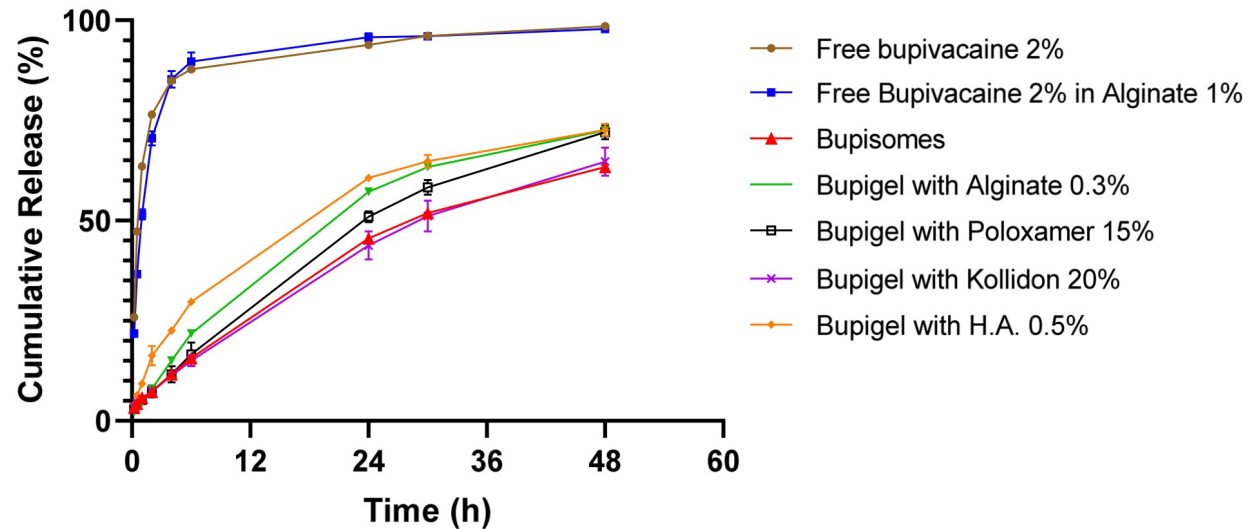


* HSPC100, C16-SPM, and cholesterol at 3/3/4 mole ratio + isoosmotic extraliposome BUP-containing medium

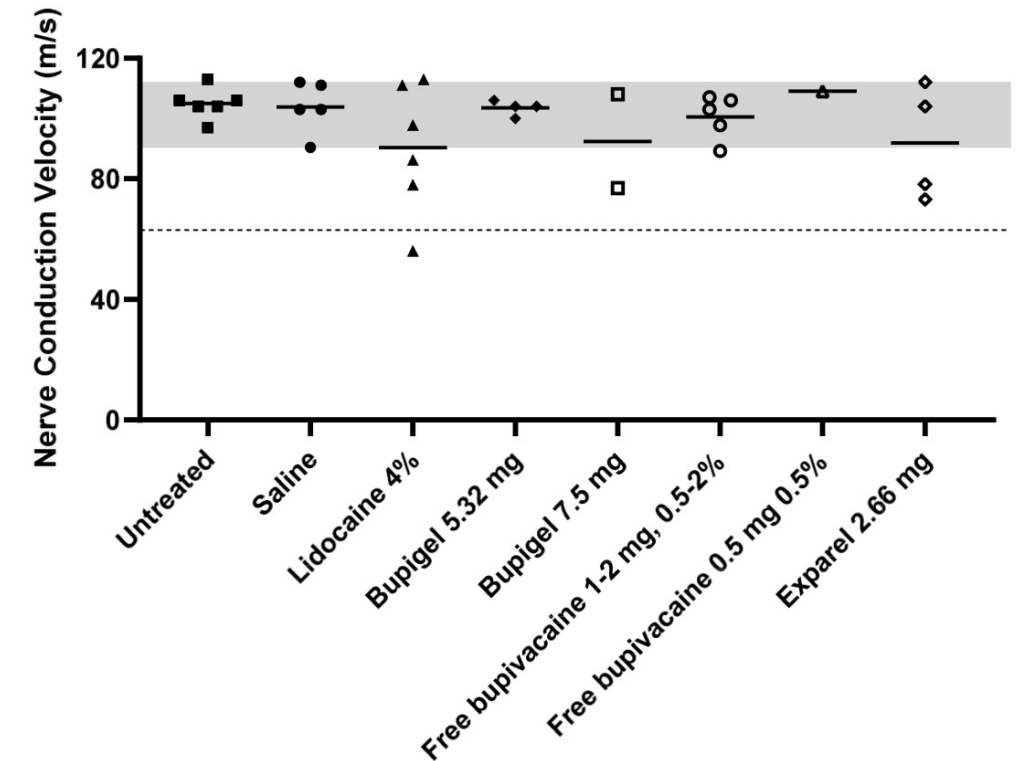


Bupigel in a rabbit functional model

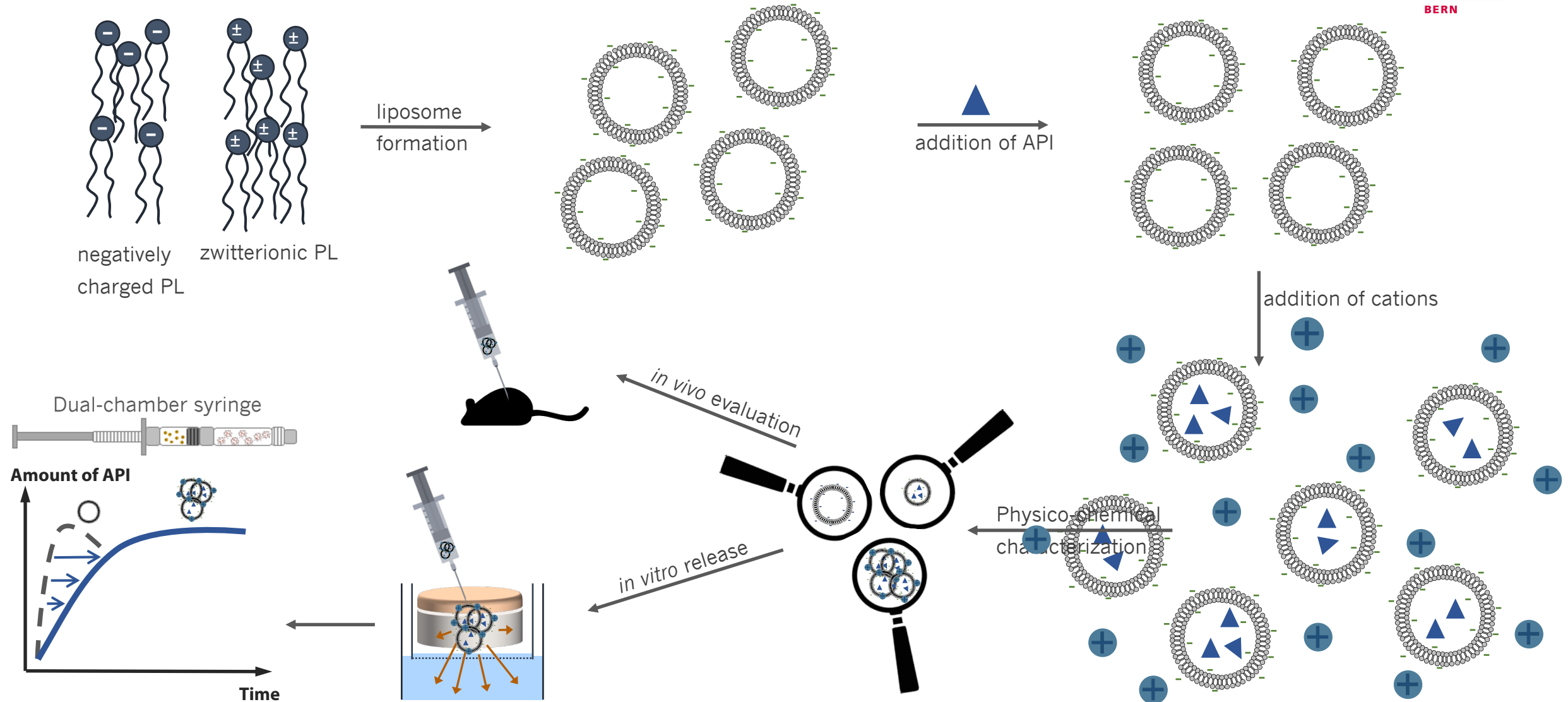
Bupigel neurotoxicity was tested on rabbit sciatic nerve using histopathology and electrophysiologic testing



Effect of different formulations on rabbit sciatic nerve conduction velocity



Cation-mediated liposomal aggregates



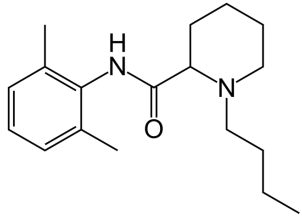
Characterisation workflow

In absence of drug

Lipid screening

Size distribution
Zeta potential

Aggregation studies:
turbidity, cryo-TEM,
fusion assay, DSC



In presence of drug

BUP
encapsulation

Shelf-life
liposomal BUP

Aggregation
studies

Rheology
Injectability

In vitro/ex vivo
release

In vivo
imaging and
PK

Rahnfeld et al. *Colloids Surf B Biointerfaces* (2018) 168:10-171
Aleandri et al. *Eur J Pharm Biopharm* (2022) 181:300-309

BUP: bupivacaine

Aggregation studies

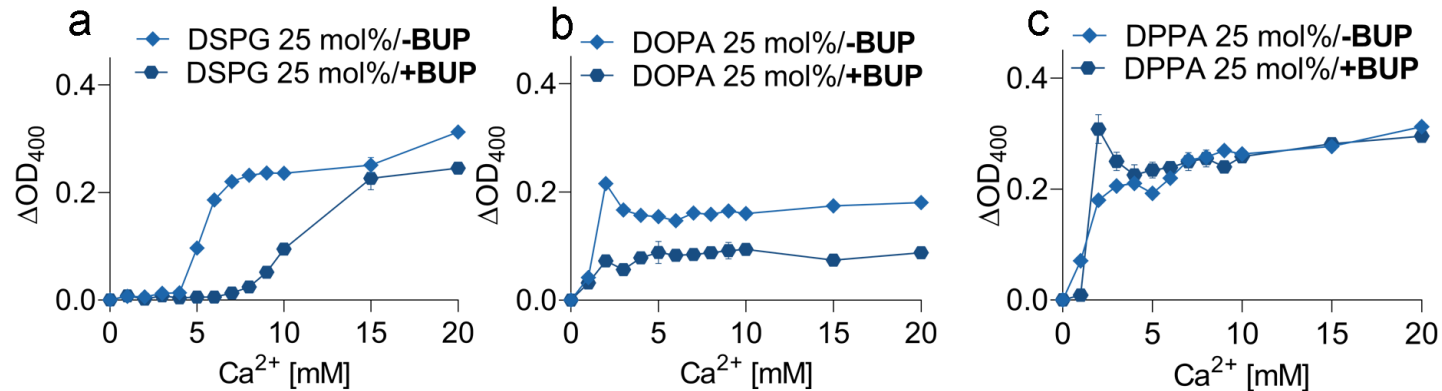
25 mol% NCP | 30 mol% CHOL | 45 mol% DPPC

DSPG

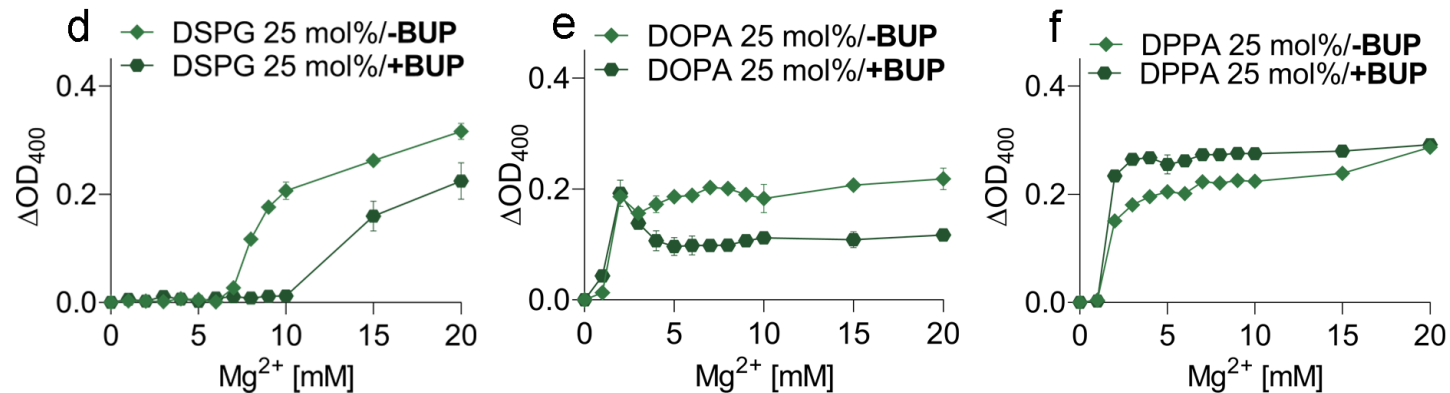
DOPA

DPPA

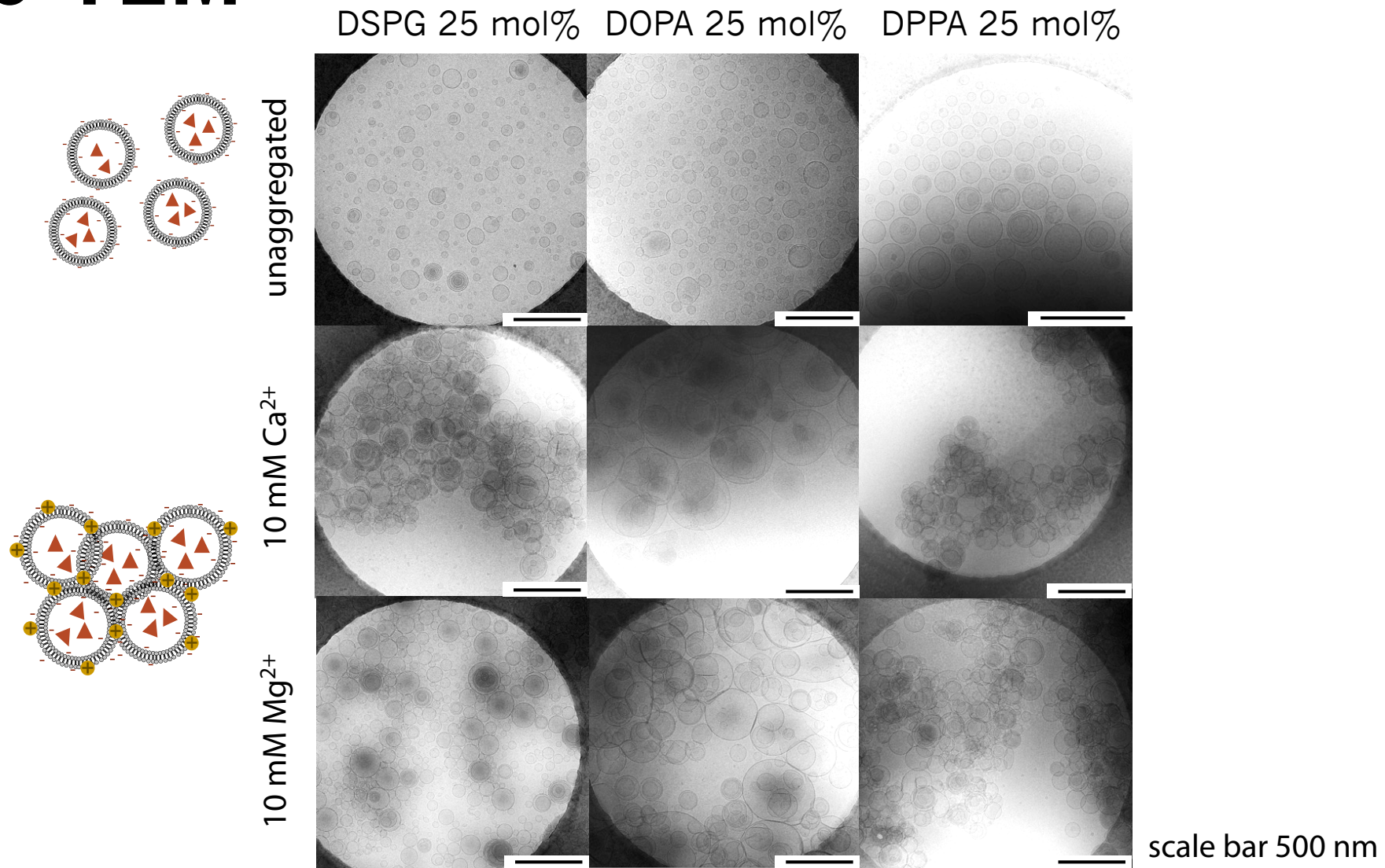
Ca²⁺-mediated
aggregation



Mg²⁺-mediated
aggregation

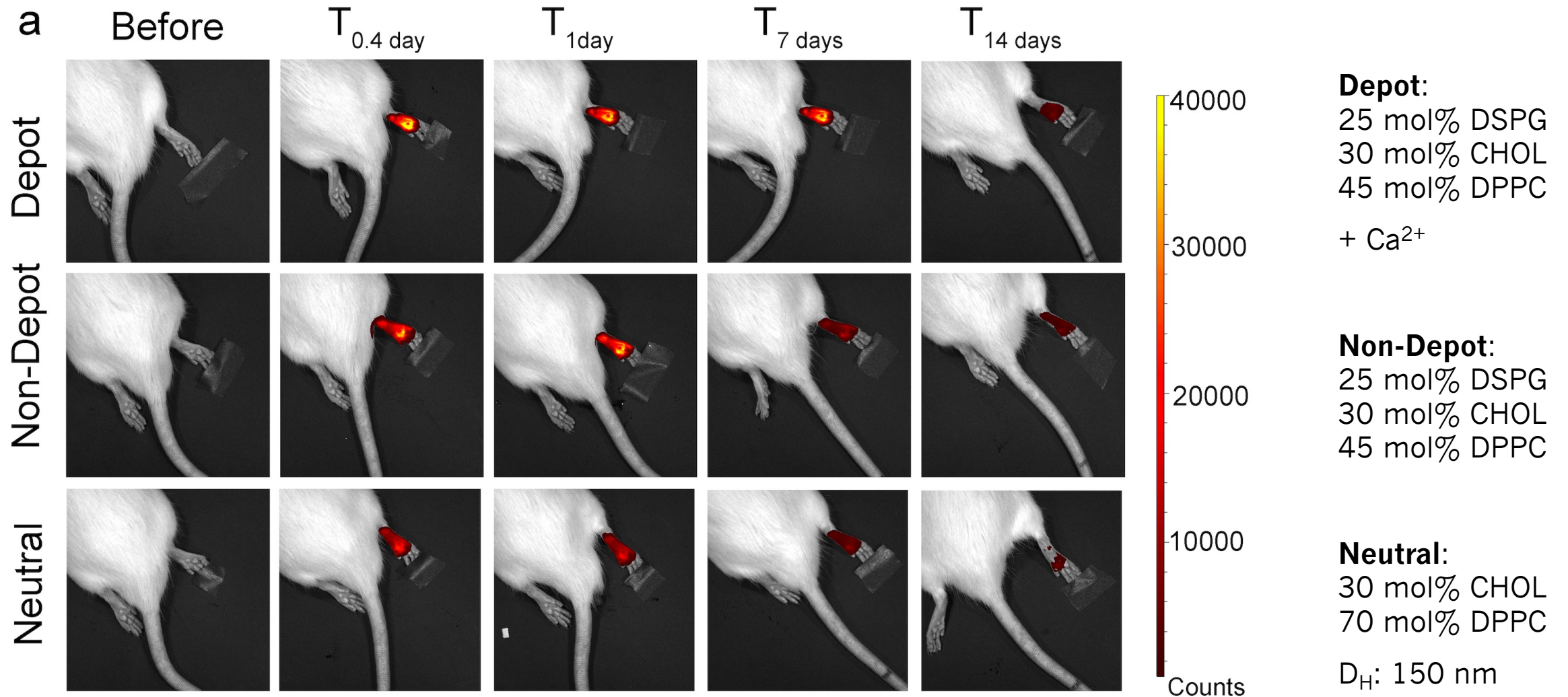


Cryo-TEM



Aleandri & Rahnfeld et al. *Eur J Pharm Biopharm* (2022) 181:300-309

In vivo imaging of fluorescent depots

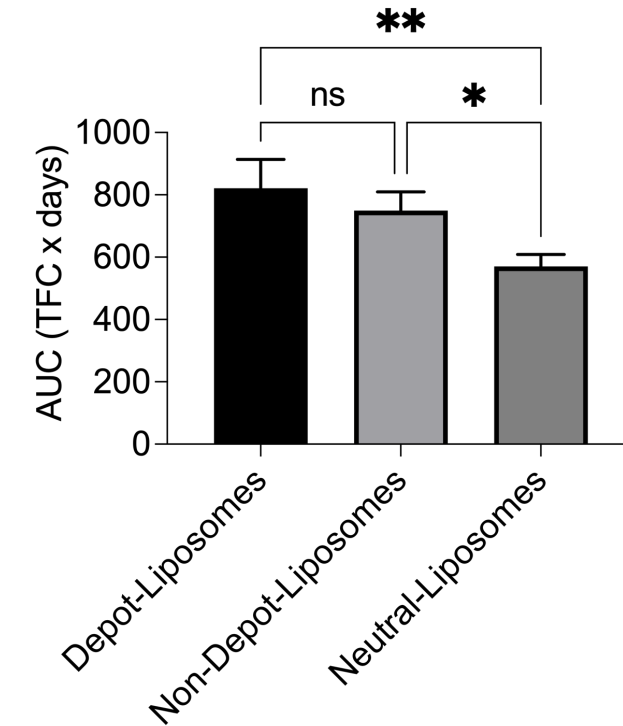
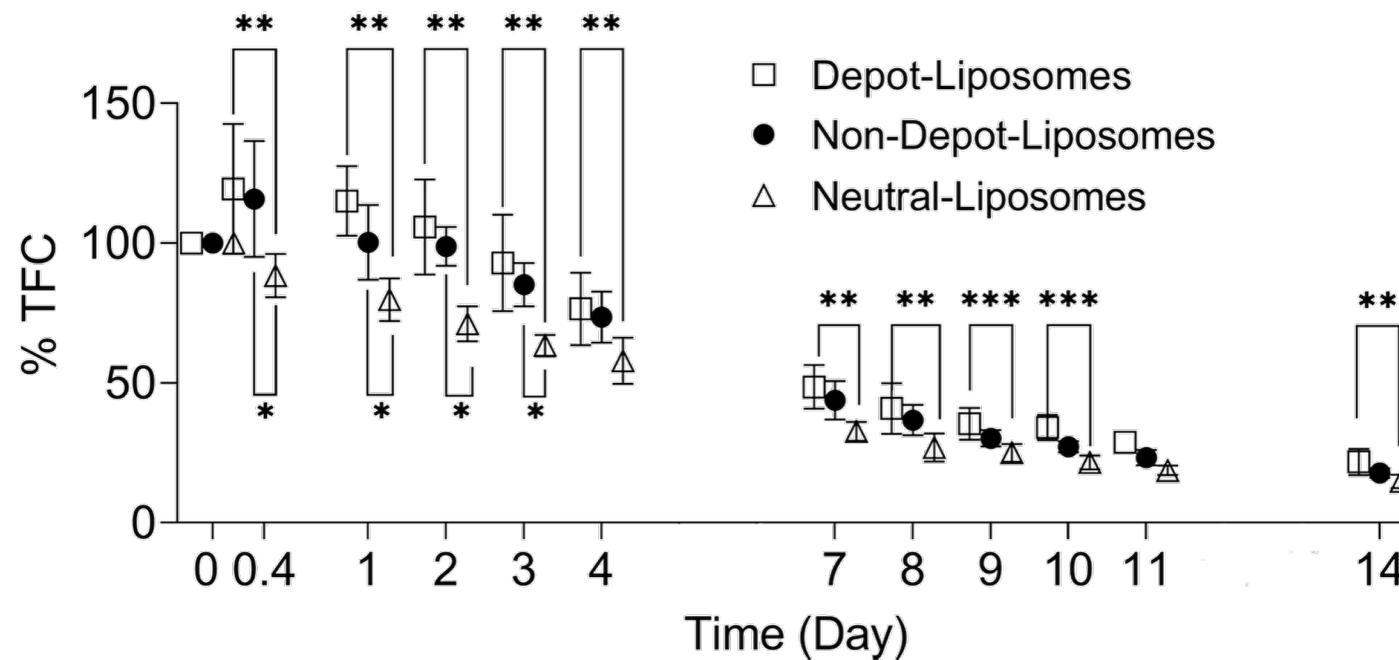


Aleandri & Rahnfeld et al. *Eur J Pharm Biopharm* (2022) 181:300-309

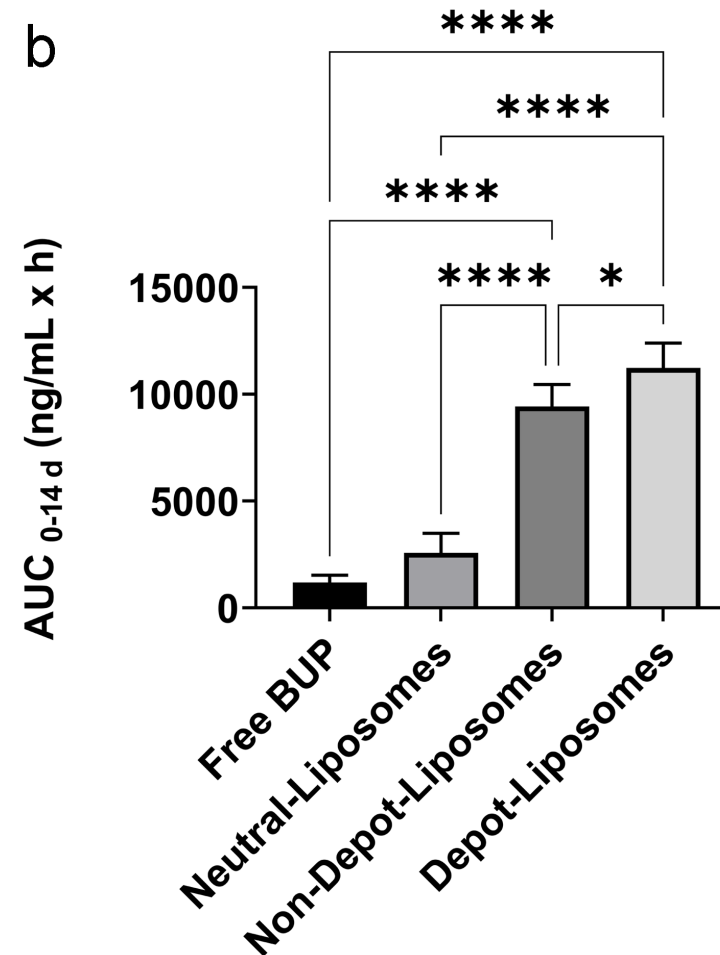
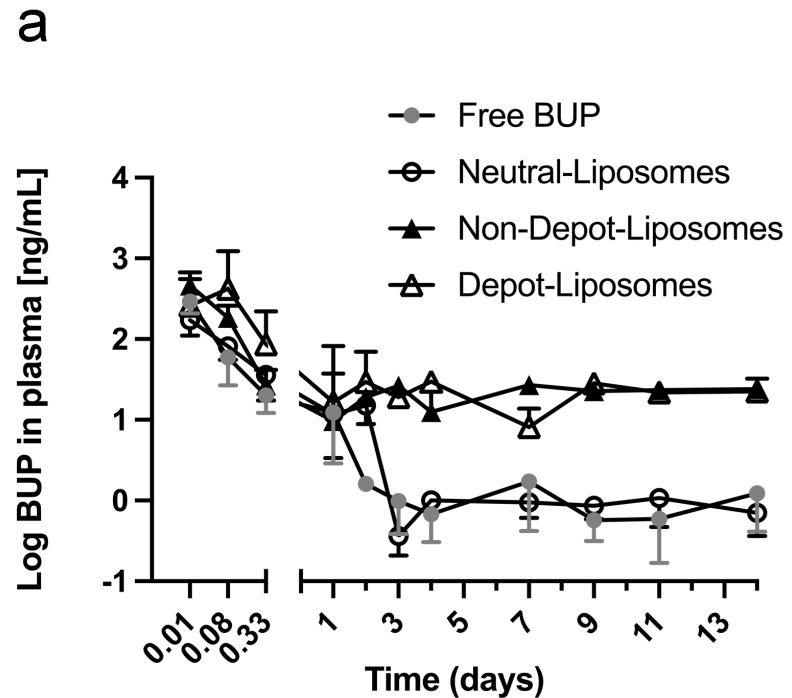
In vivo imaging of fluorescent depots

$$\% \text{ TFC} = (\text{TFC}_t - \text{TFC}_{\text{blank}}) / \text{TFC}_{t0}$$

TFC: Total Fluorescence Counts
(photons/second)



Pharmacokinetics



Depot:

25 mol% DSPG
30 mol% CHOL
45 mol% DPPC
+ Ca^{2+}

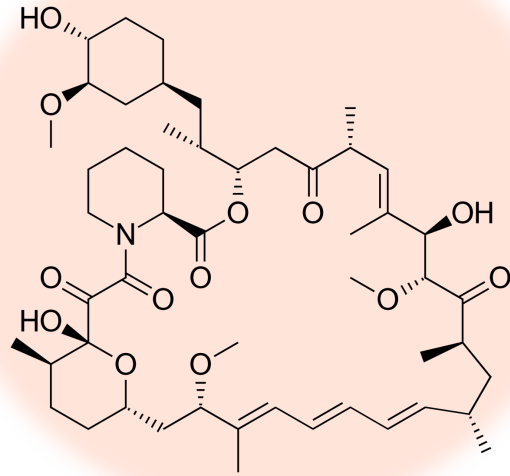
Non-Depot:

25 mol% DSPG
30 mol% CHOL
45 mol% DPPC

Neutral:

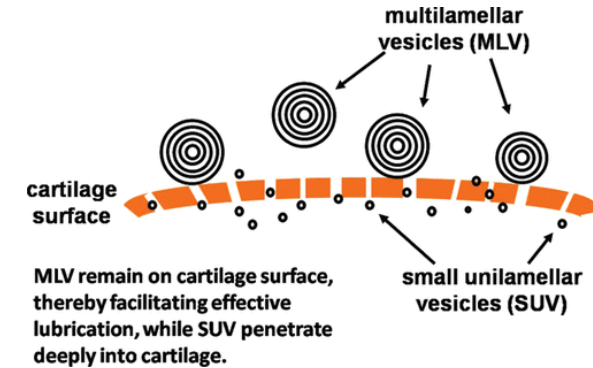
30 mol% CHOL
70 mol% DPPC

Liposomal aggregates to deliver rapamycin

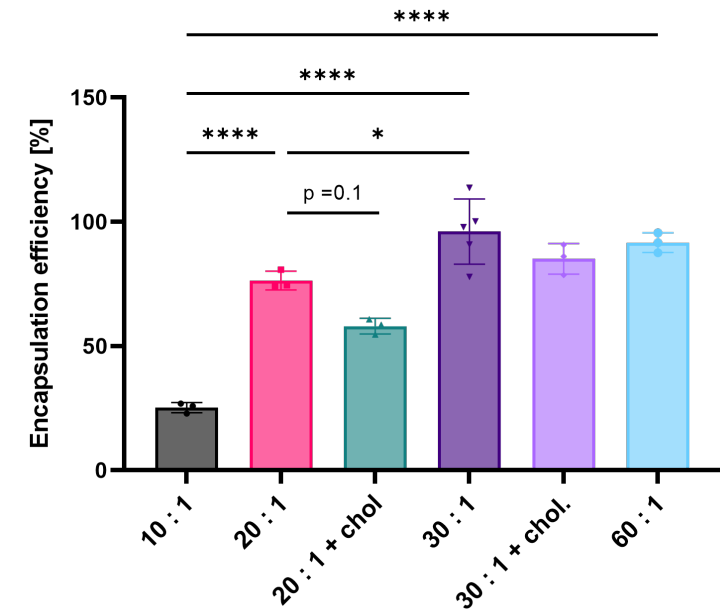


Rapamycin

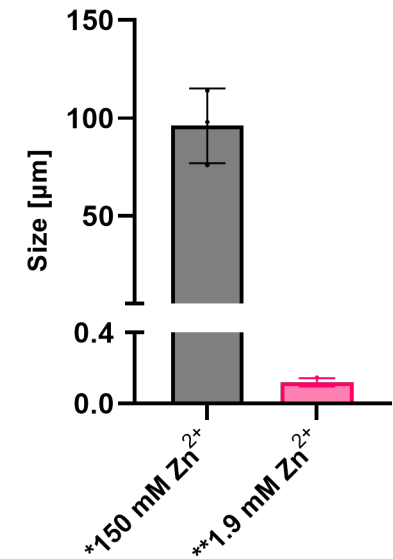
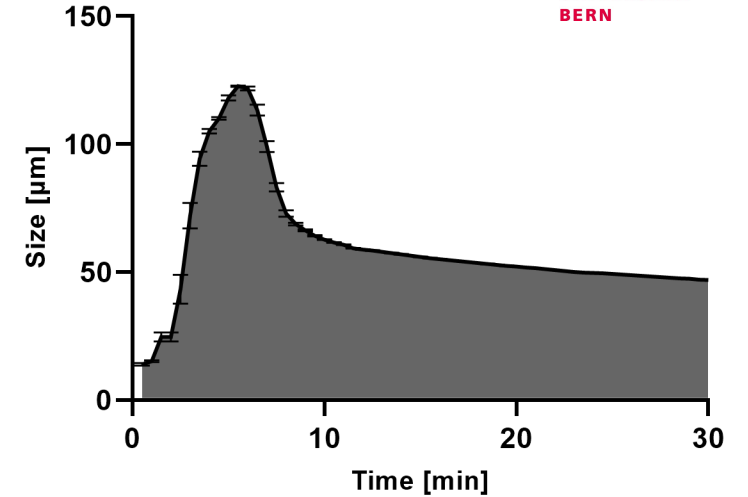
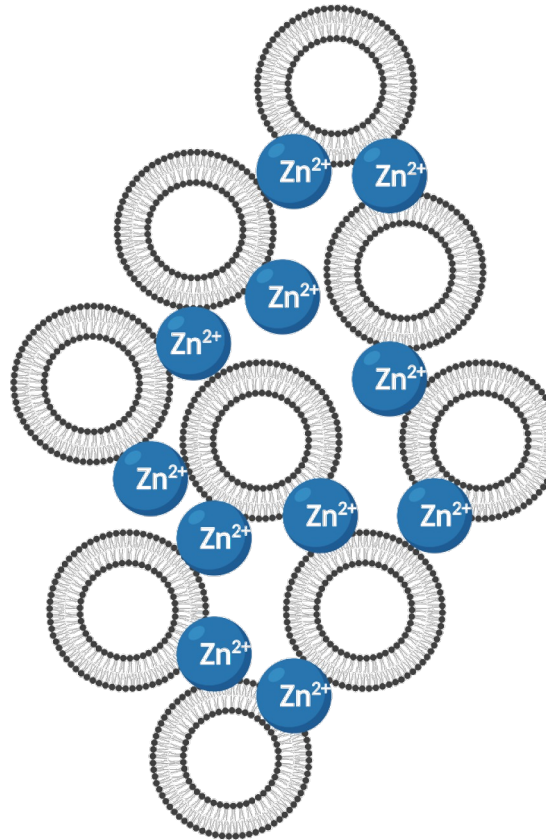
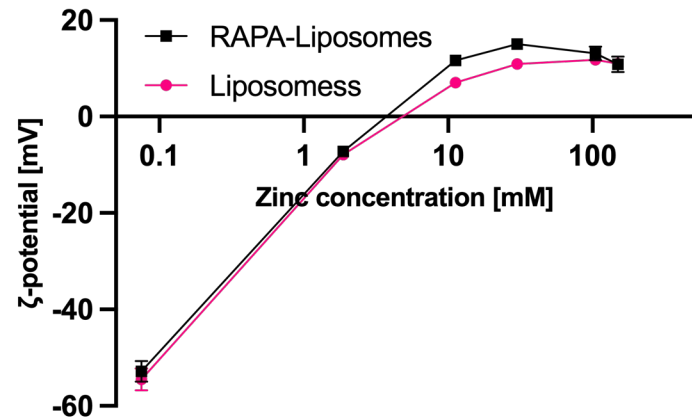
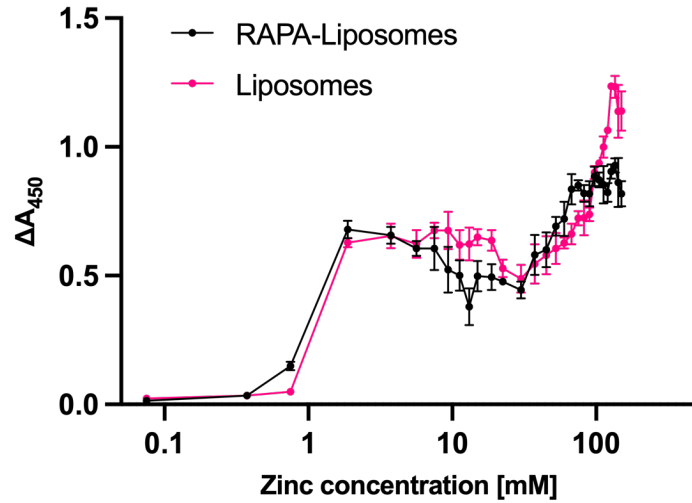
From subcutaneous to intrarticular administration



Sivan, ..., Barenholz, Langmuir (2010) 26:1107–1116



Rapamycin-loaded aggregated liposomes

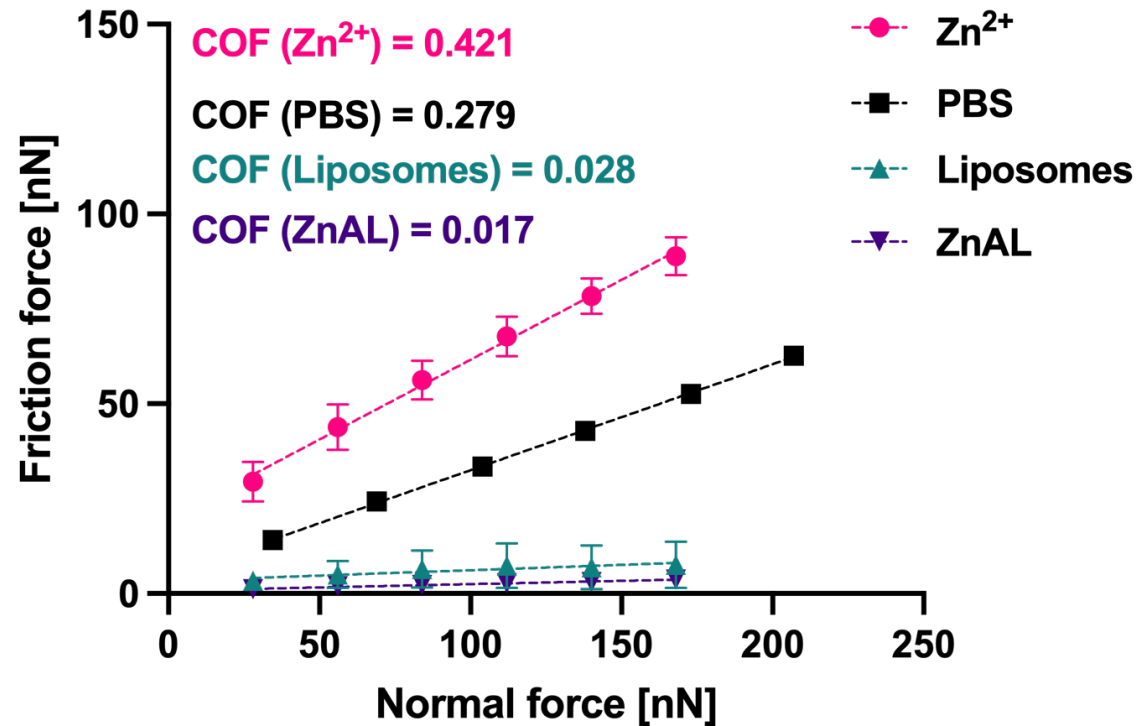


Bordon et al. *J Colloids Interface Sci* (2023) 650:1659-1670

Lubrication properties

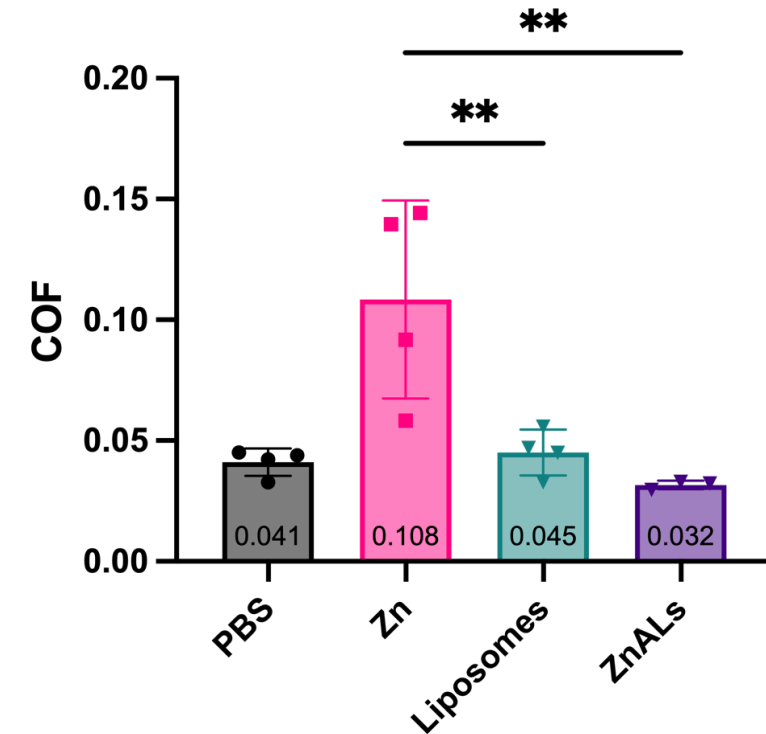
Nanotribology

Colloidal probe lateral force microscopy



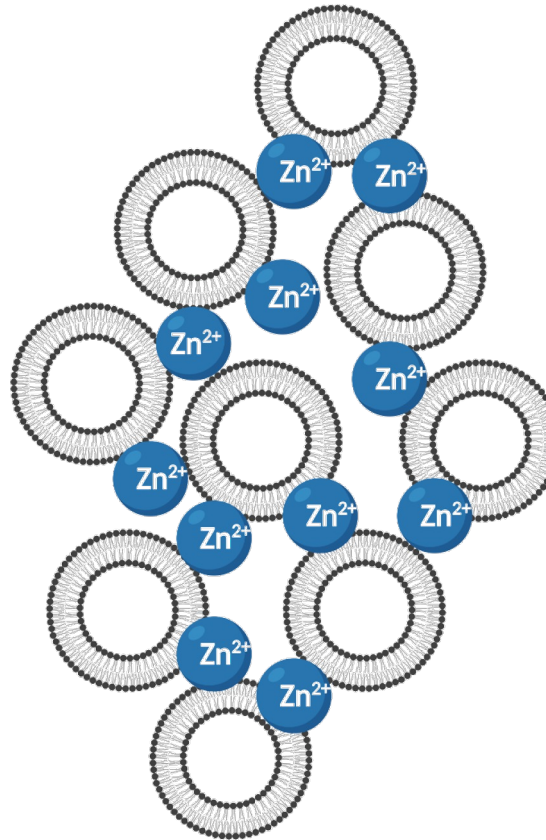
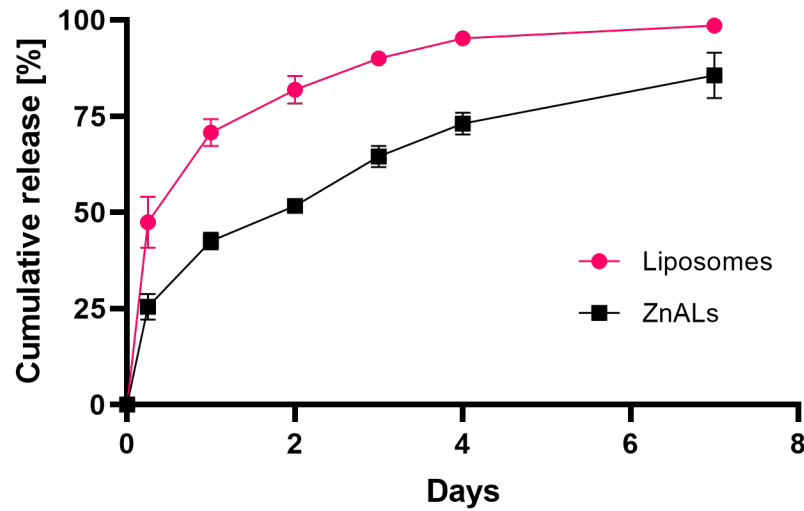
Macrotribology

Tribometer with ex vivo cartilage



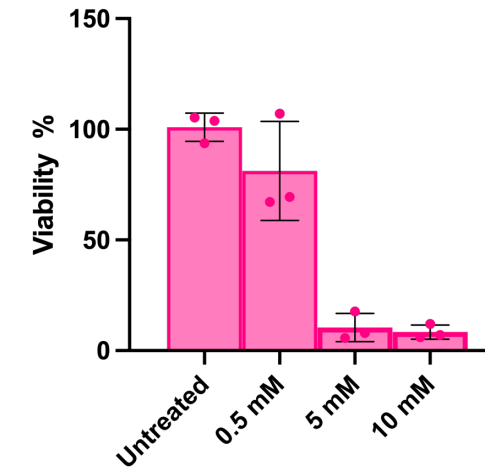
Rapamycin-loaded aggregated liposomes

Release from Zn ALs

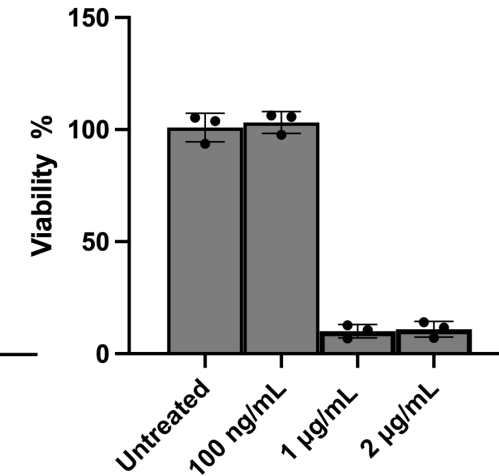


Toxicity study on human synovial fibroblasts from patients at USZ

Zn^{2+} toxicity

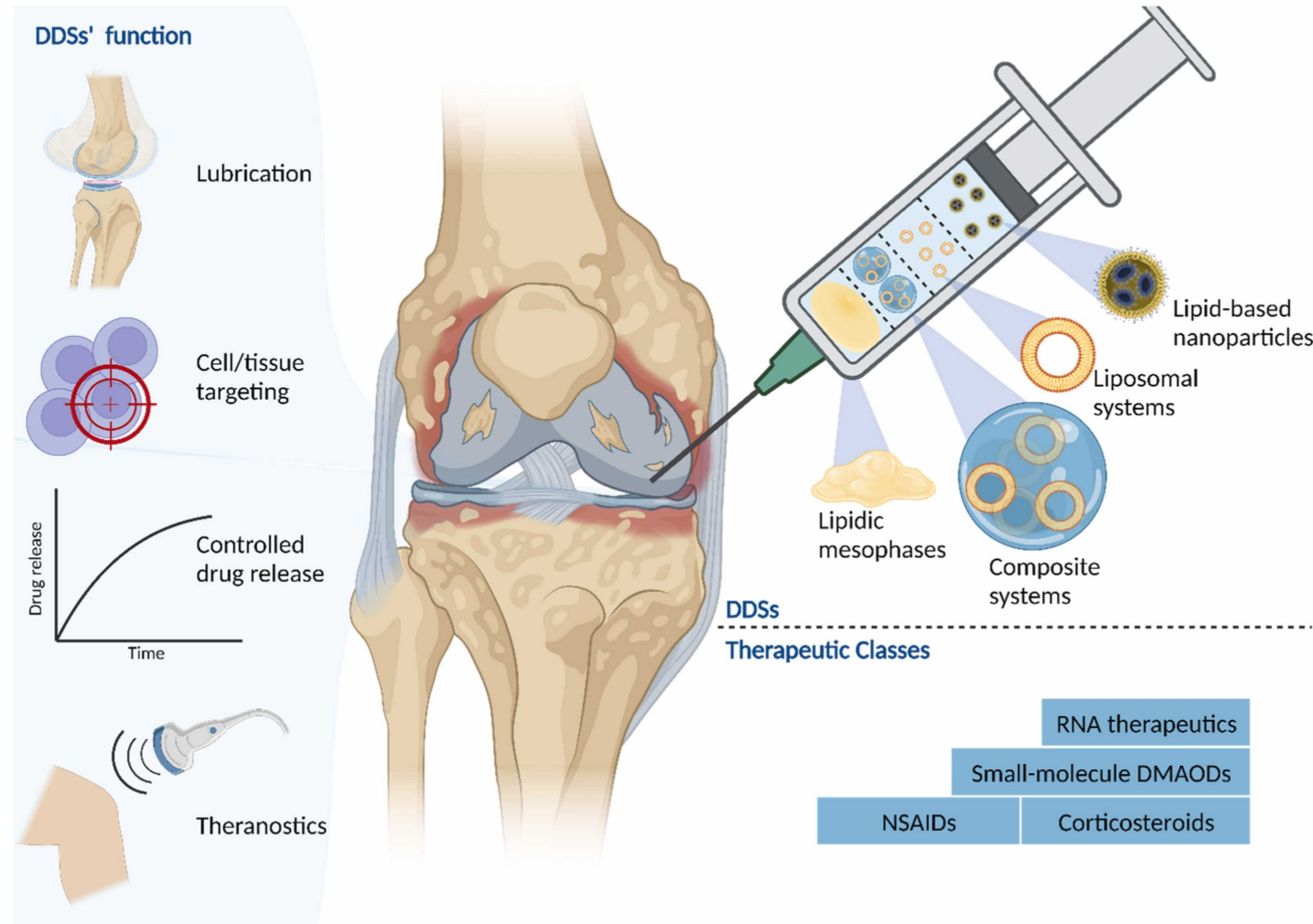


ZnAL toxicity



RAPA content in purified ZnALs with 0.5 mM Zn^{2+}

ZnALs for intraarticular administration



Liposomal CBD (Innocan Pharma)

The Solution

Innocan Solutions: Patent Pending Injectable Cannabinoids Delivery Platform to Treat Pain

A unique CBD-loaded Liposome Technology (LPT) facilitating **exact dosing, prolonged** and **controlled** release of CBD into the bloodstream

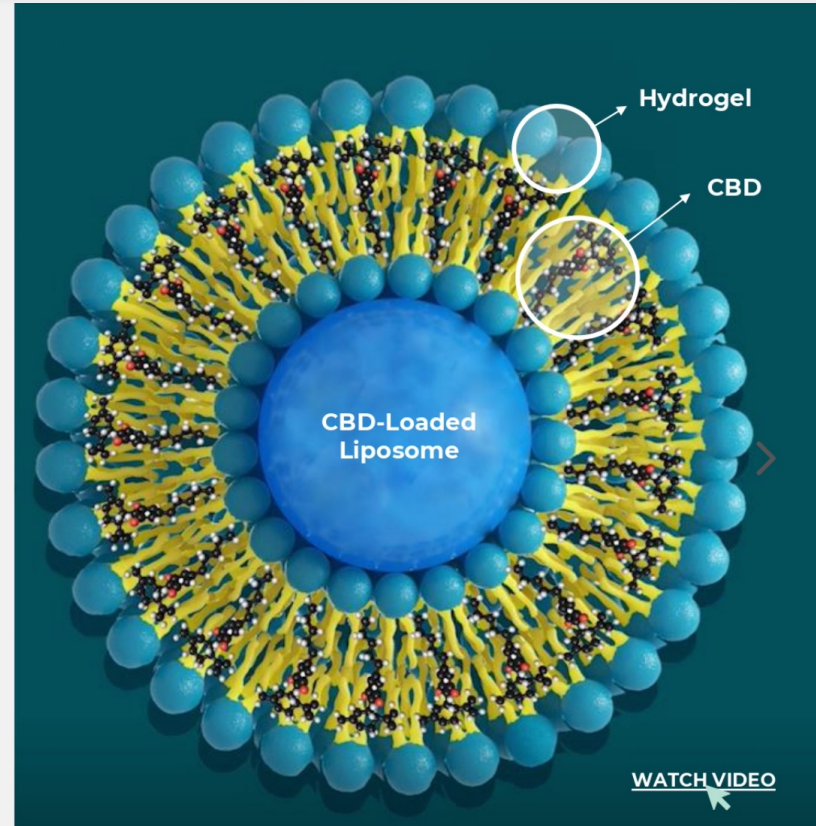
Liposomes are spherical vehicles composed of a few layers of lipids that can carry drugs through the human vascular system.

~100%

CBD Bioavailability
in a Dog Clinical
Study Platform

+42 days

Prolonged &
controlled release
of CBD in
bloodstream



LPT: Liposomal CBD

50 mg/mL CBD into hydrogenated soy phosphatidylcholine (HSPC) liposomes

Dogs: subcutaneously at a dose of 5 mg/kg (0.1 mL/kg) using a 21-gauge 1-inch needle

Liposomal-CBD administered subcutaneously produced detectable CBD plasma concentrations for 6 weeks with minimal side effects

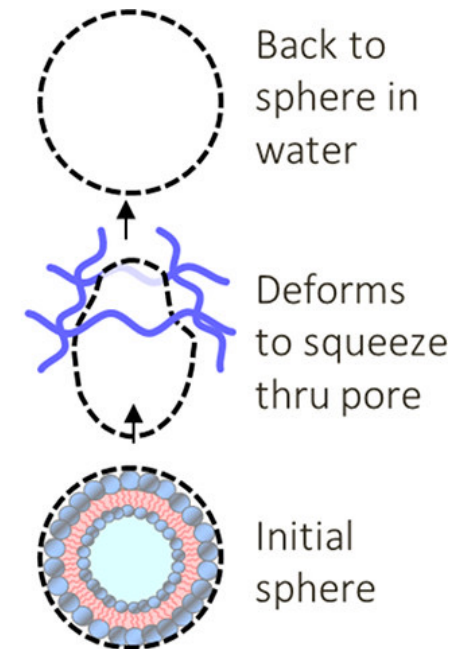
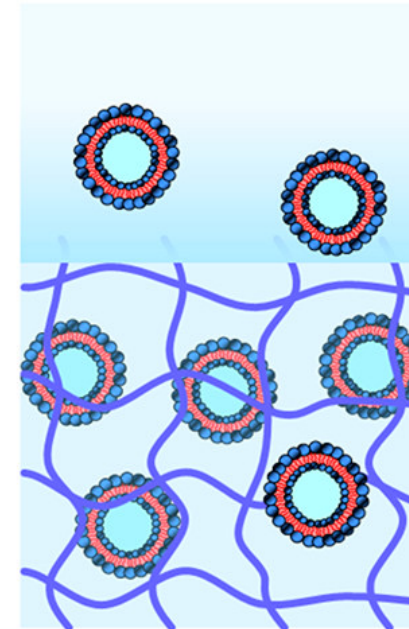
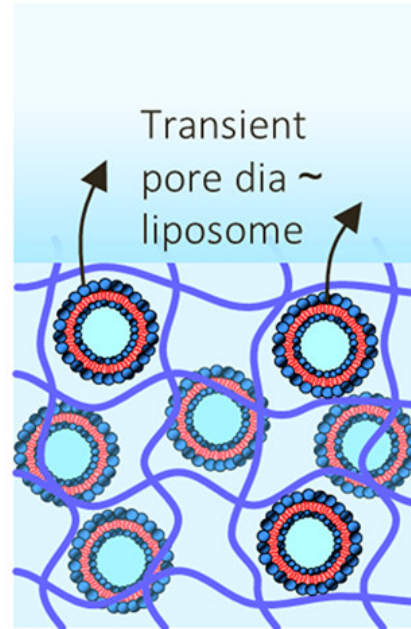
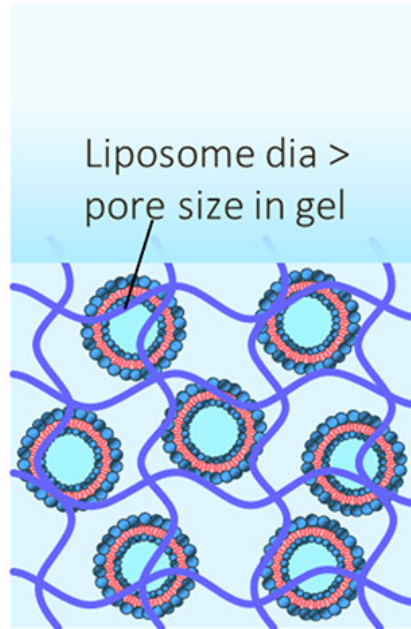
<https://innocanpharma.com>

PCT/IL2020/05168: Presence of a dispersing agent of a cannabinoid

Shilo-Benjamini et al. *Front Vet Sci* (2023) 10:1224452

Composite liposomal depots

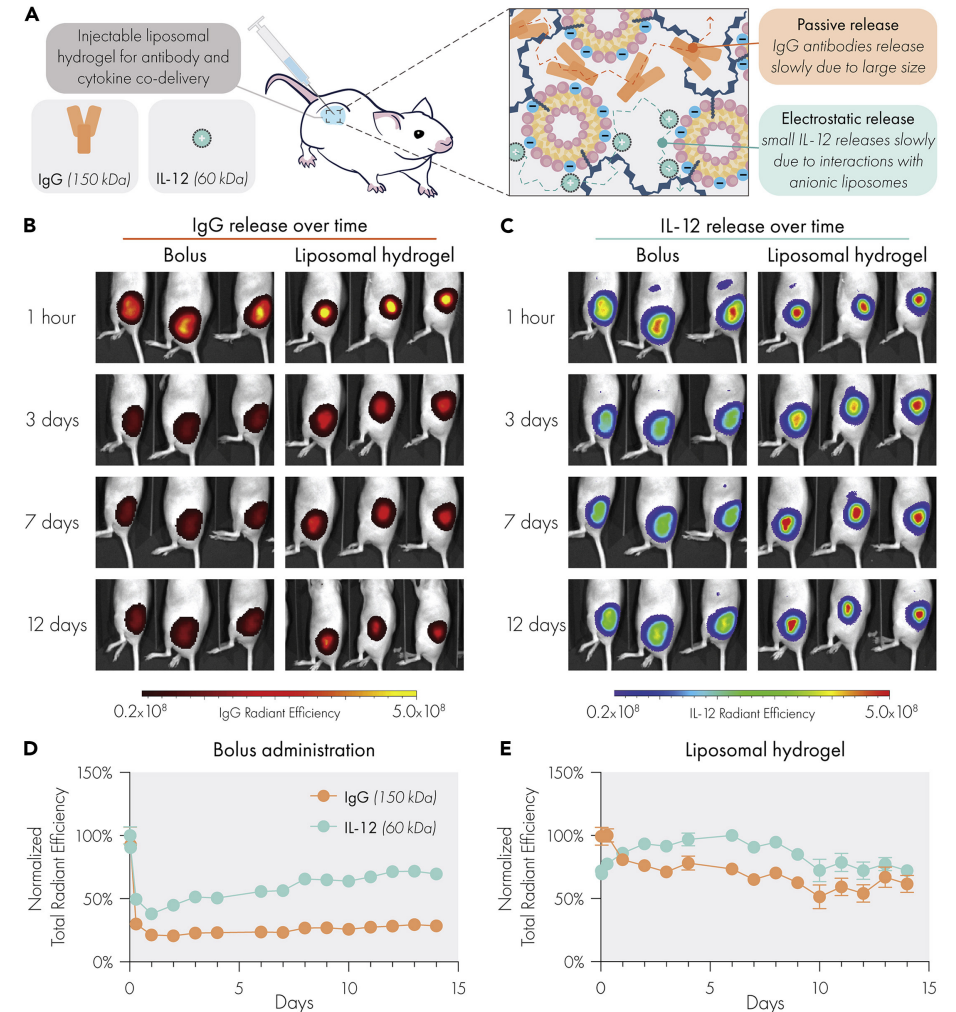
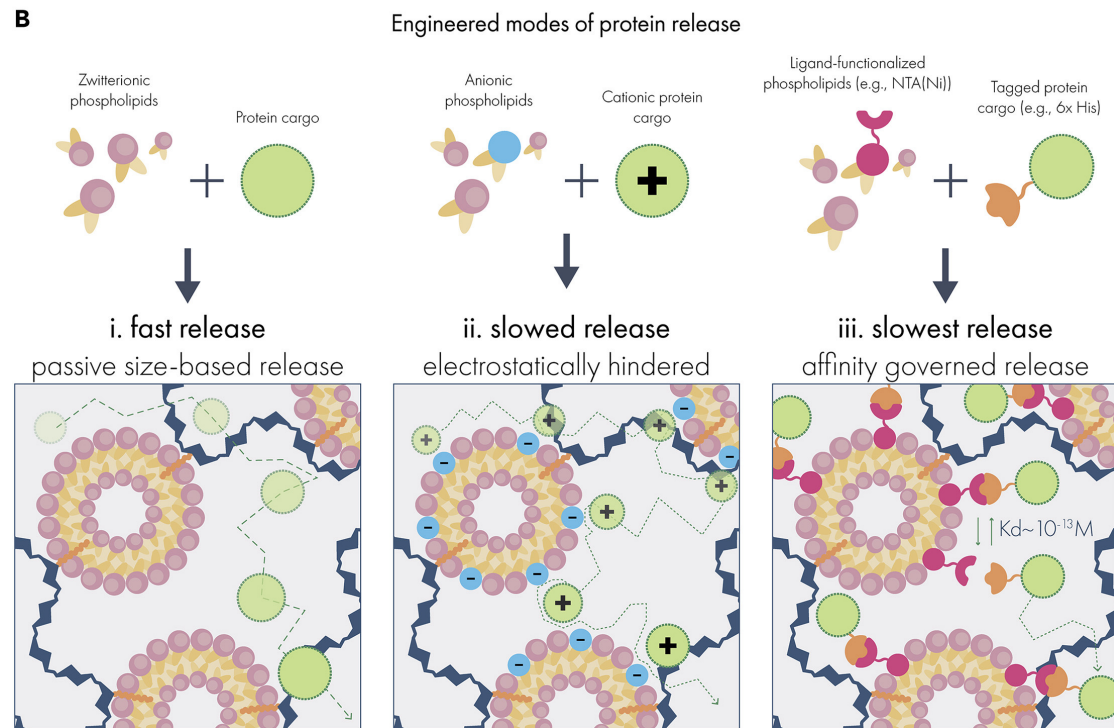
- Liposomes can be embedded in hydrogels
- Release rates and rheological behaviour can be tuned



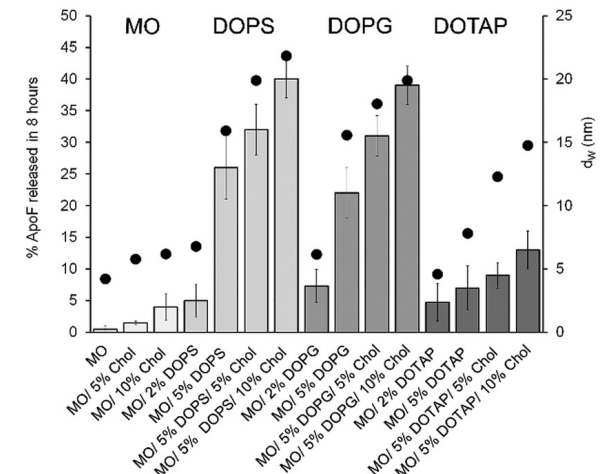
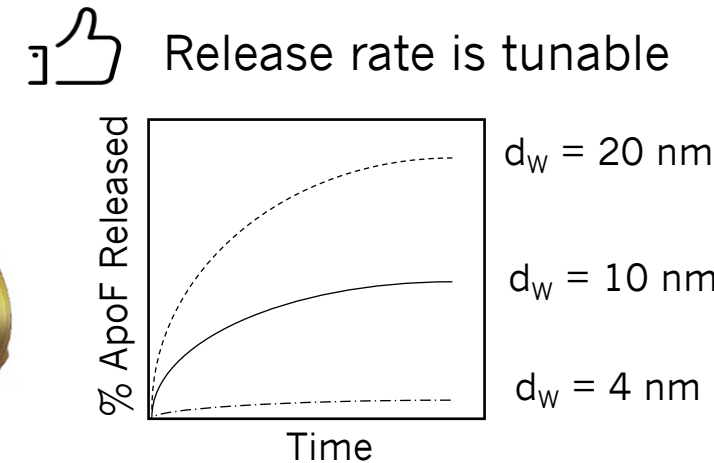
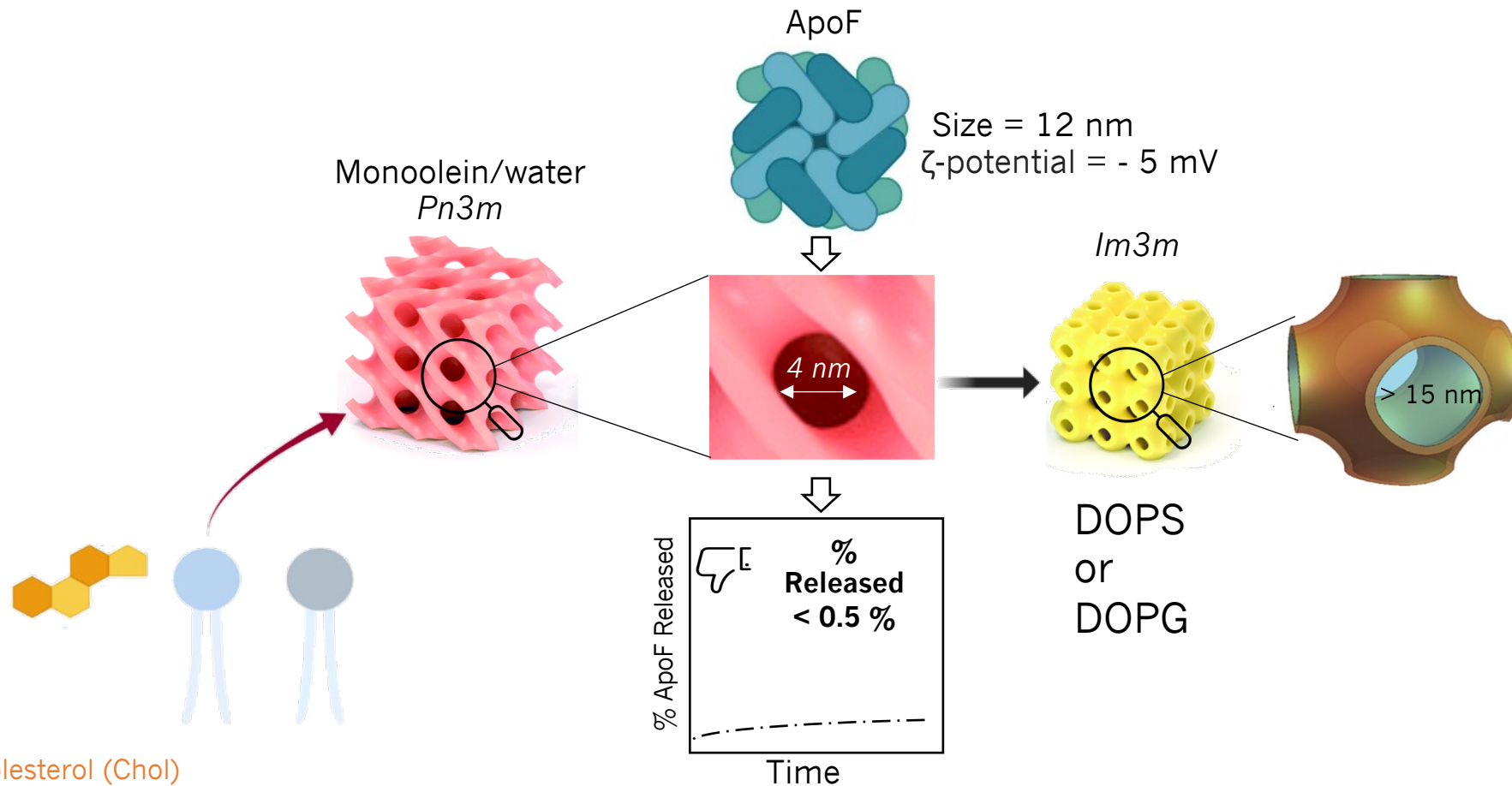
Adapted from Thompson et al. *Langmuir* (2020) 36:7268–7276

Liposome-based supramolecular hydrogels

Injectable nanocomposite hydrogels obtained by mixing liposomes and modified cellulose polymers acting as depot for biotherapeutics



Phospholipid-doped ultra-swollen LMPs

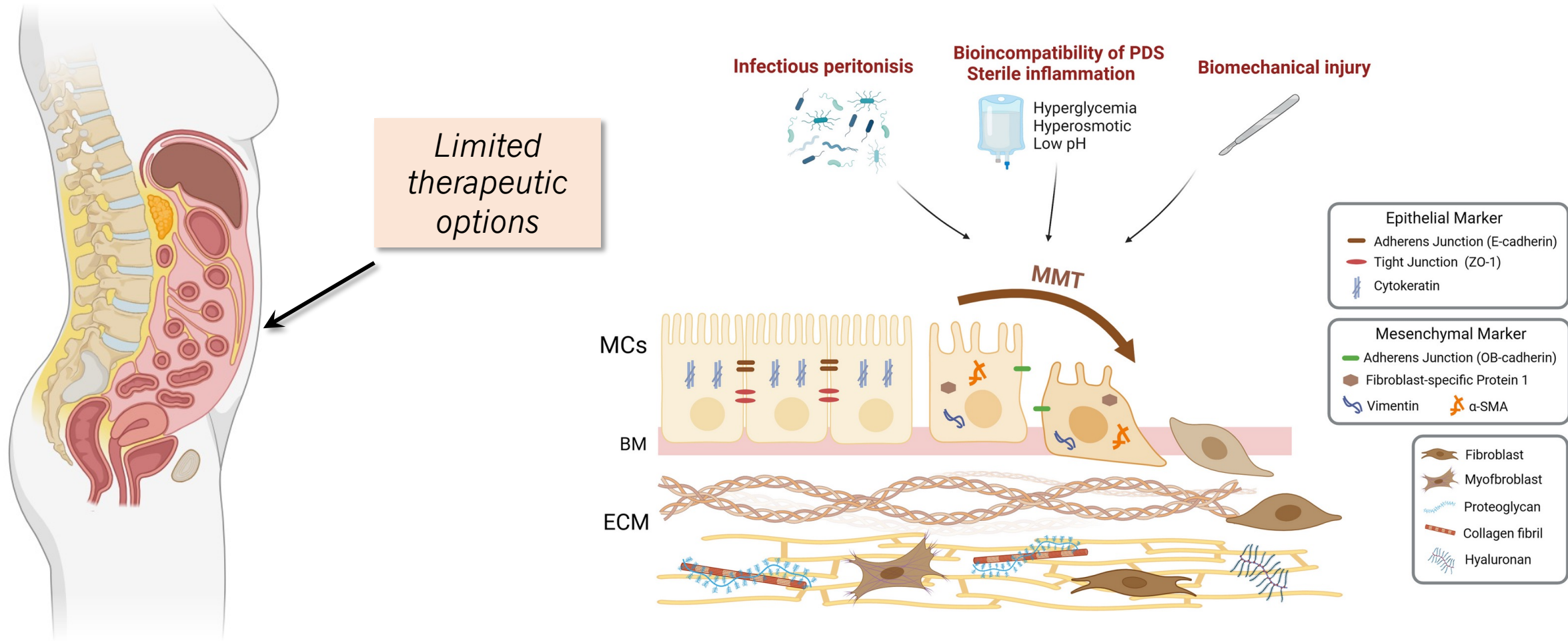


Cholesterol (Chol)
1, 2-dioleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (DOPG)
1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS)
2-dioleoyl-3-trimethylammonium propane (DOTAP)

Elzenaty et al, *J. Mater. Chem. B* (2022) 10:3876-3885

Broadening the therapeutic possibilities for peritoneal diseases u^b

^b
UNIVERSITÄT
BERN

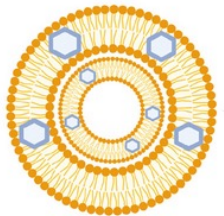


Huang et al. *Stem Cell Res Ther* (2023) 14: 293

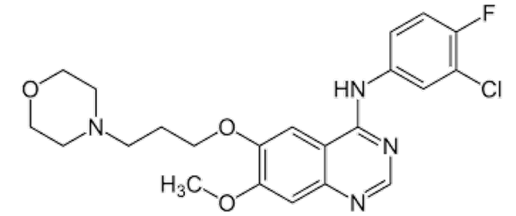
Broadening the therapeutic possibilities u^b for peritoneal conditions

^b
UNIVERSITÄT
BERN

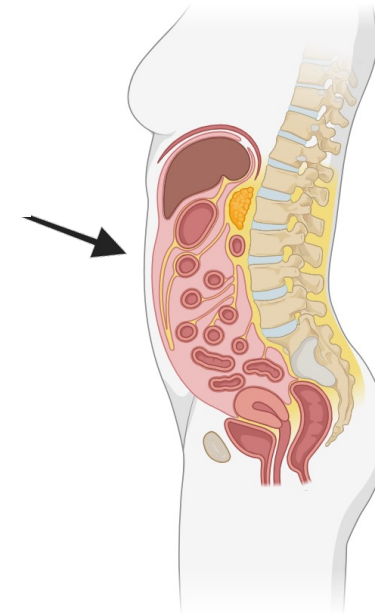
Alginate Hydrogel



Liposome + GEF

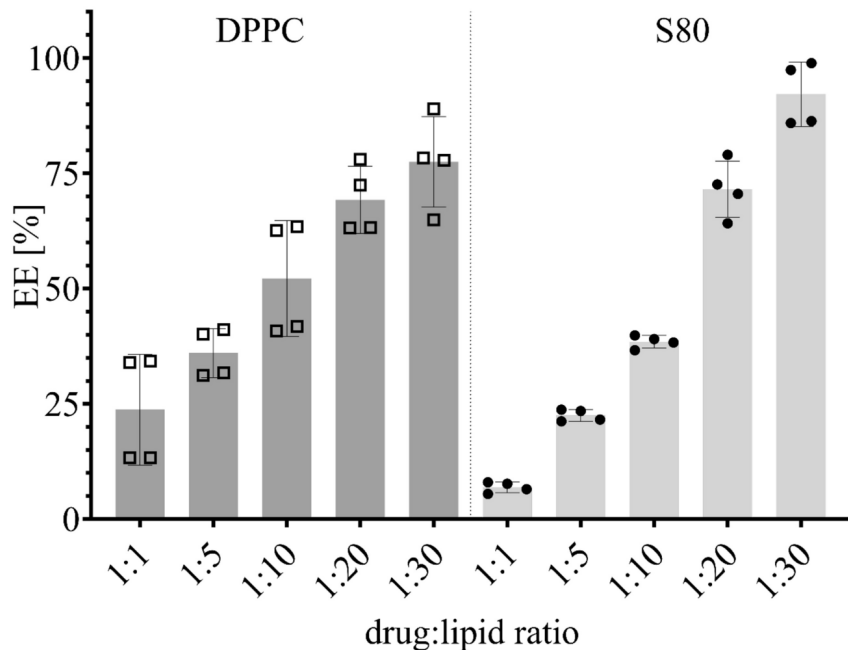


Gefitinib, GEF:
epidermal growth
factor receptor
(EGFR) inhibitor

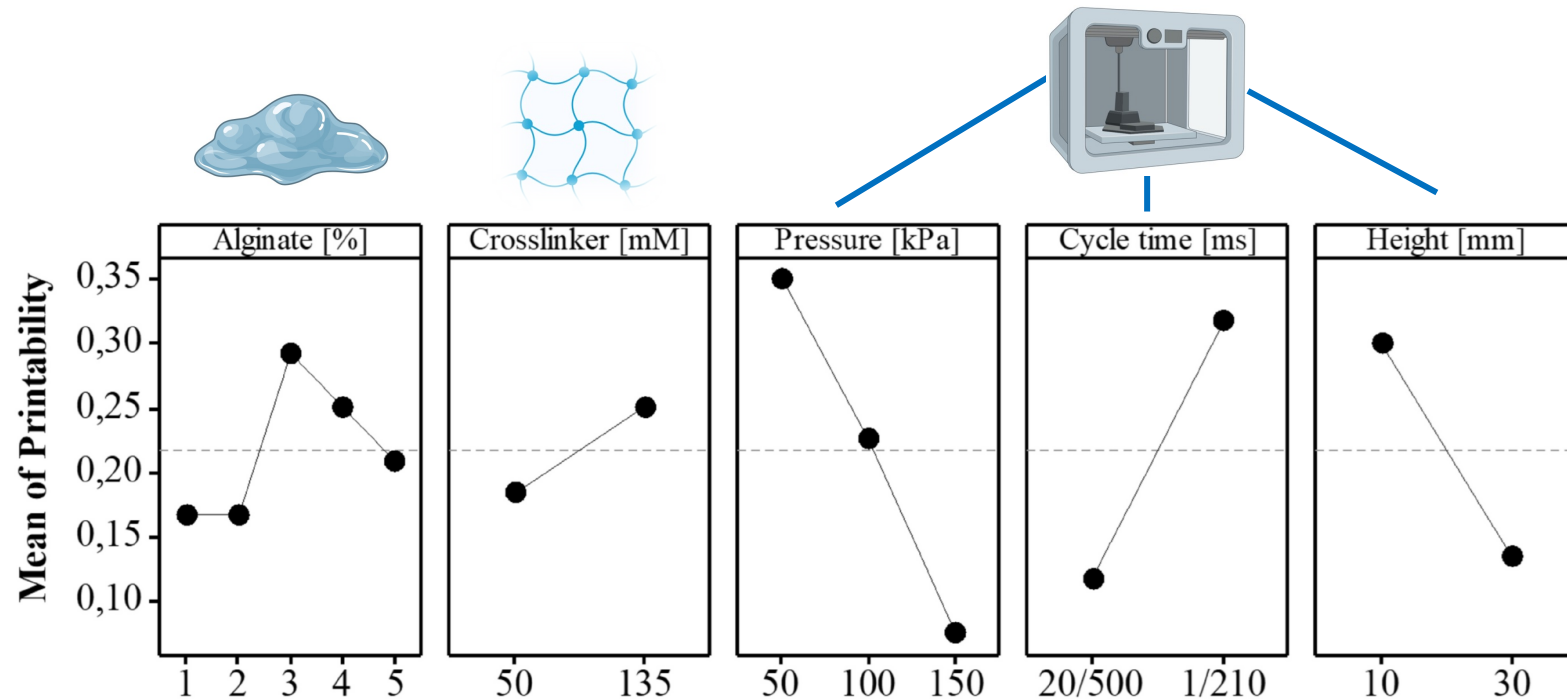


Liposomal ink: some properties

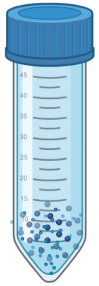
GEF encapsulation in MLVs



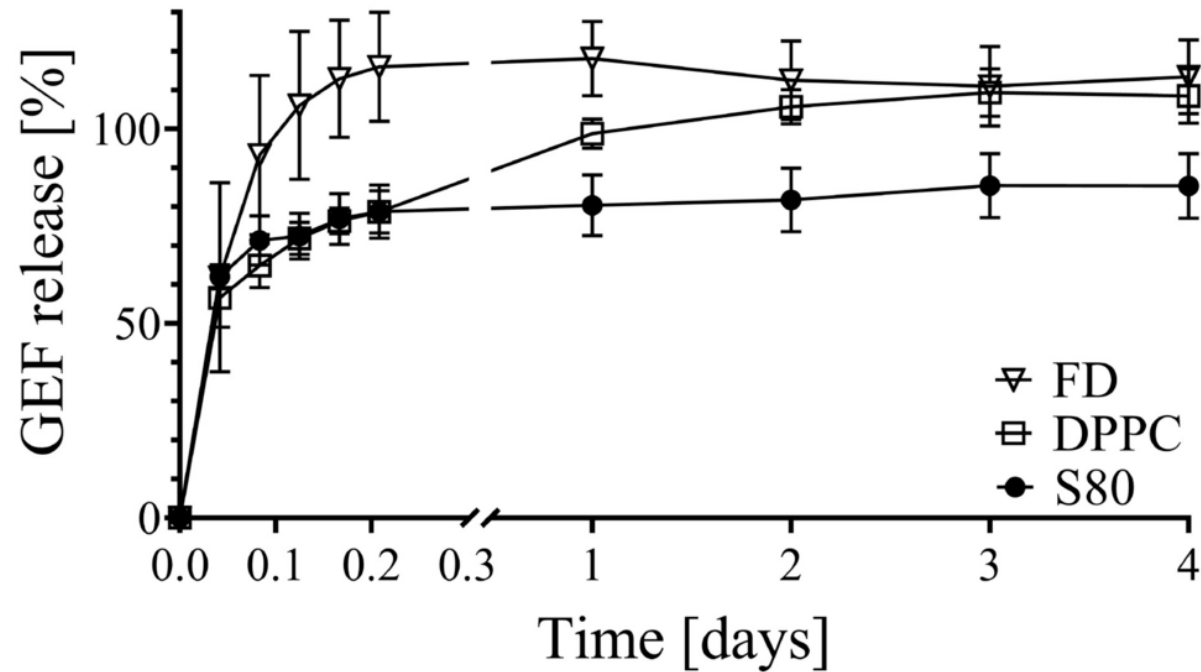
Main effects plot for printability
in a multifactorial experimental design



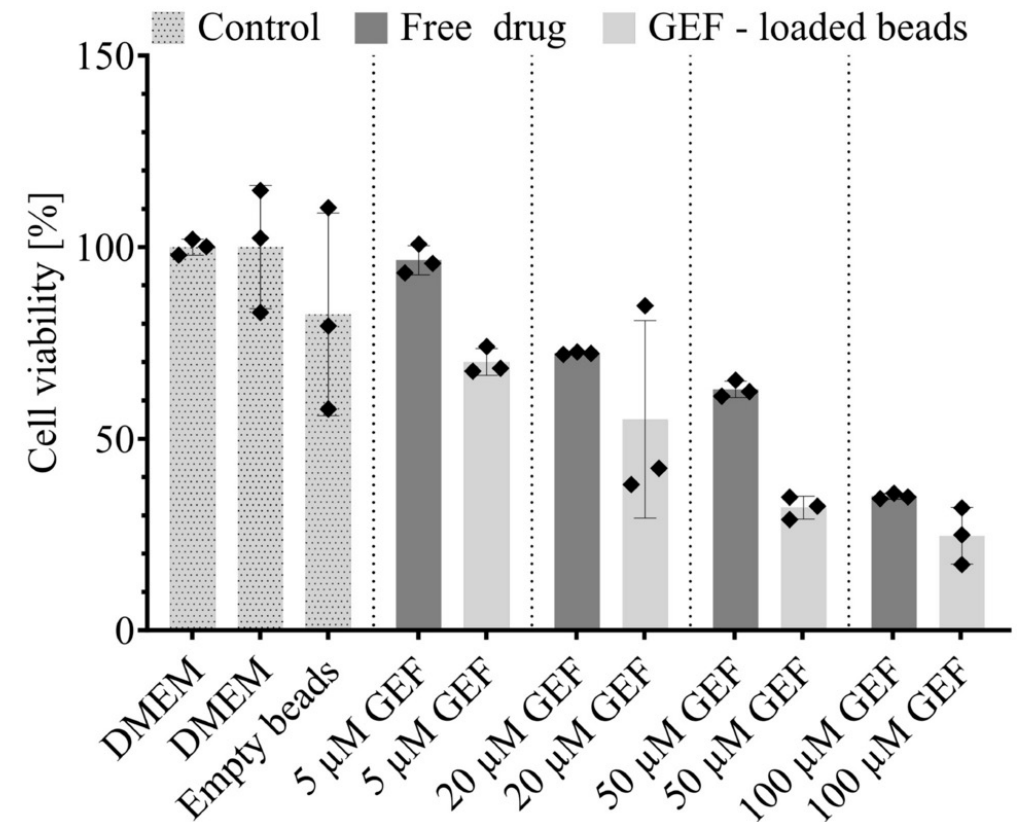
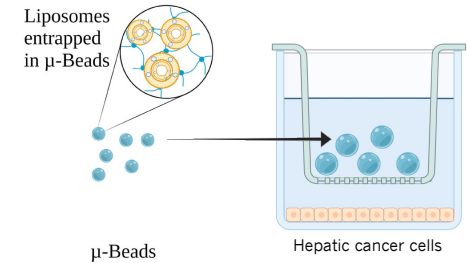
Gefitinib release from liposomal beads



Microbeads suspended in peritoneal simulated fluid



Microbeads tested on Huh7 cell line using inserts



What next?

Open issues and possibilities to explore

- Manufacturing/Sterility
- Standardization of the release methods
- Investigating the tissue reaction
- Exploring new hybrid materials

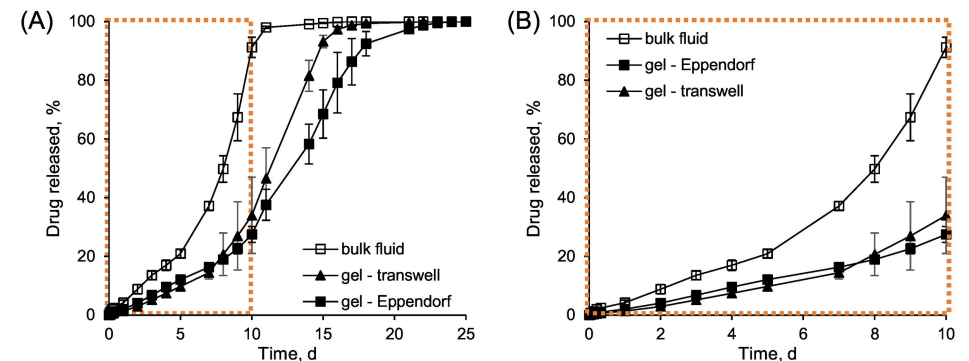
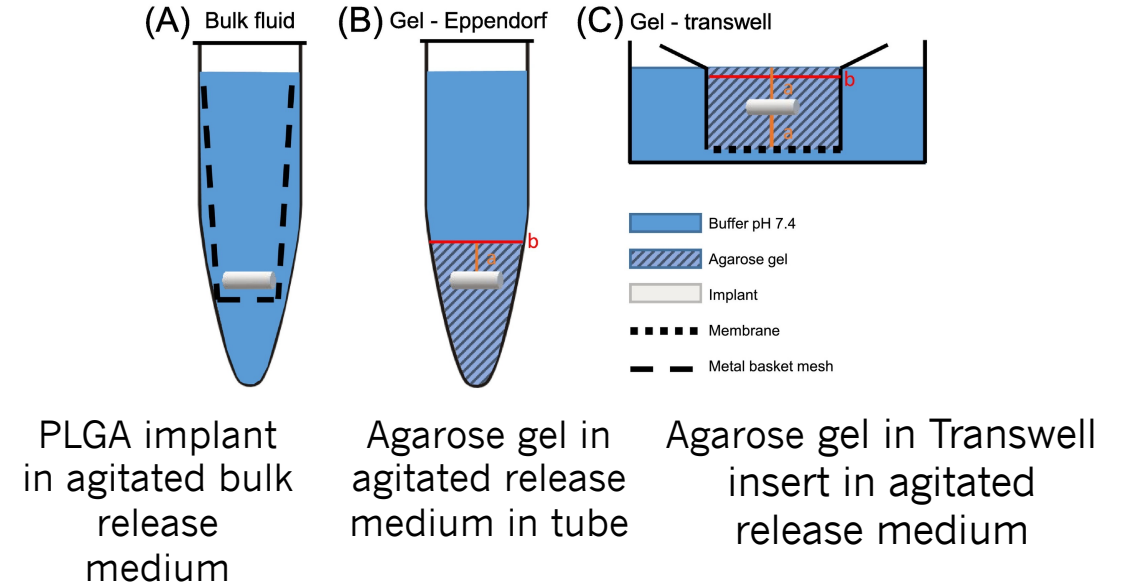
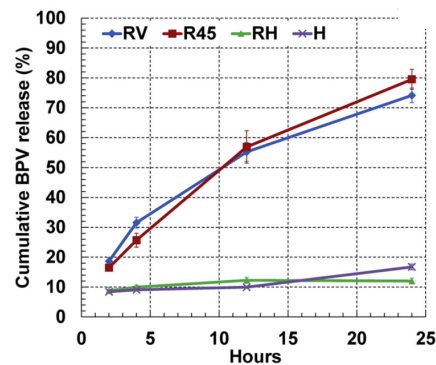
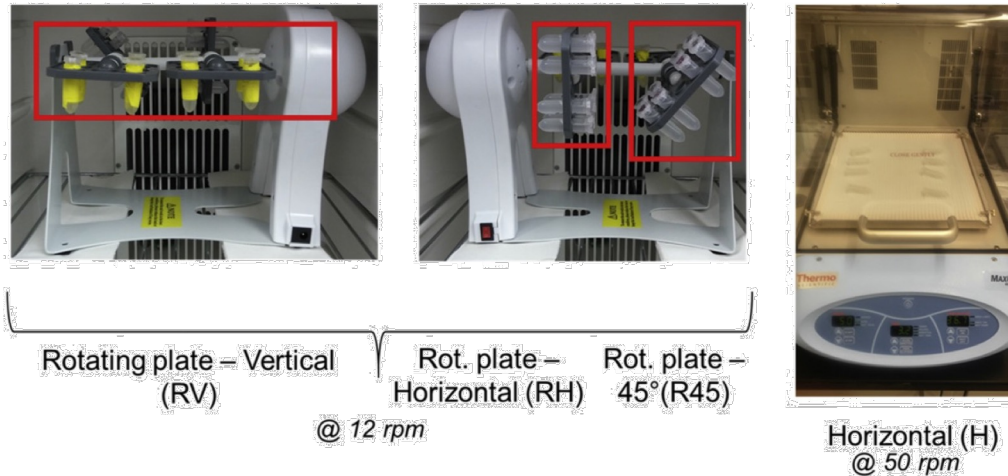
The quest for the ideal *in vitro* release test

There are currently **no standard methods** for *in vitro* release testing of controlled release parenterals

- Burst release
- Drug stability
- Sink conditions
- Extent of *in vitro* release
- *In vivo* relevance
- Accelerated release
- Dissolution vessel selection
- *Reaction at the injection site*

Importance of the experimental release setup

Sample-and-separate method For bupivacaine release from Exparel



SCISSOR

Subcutaneous Injection Site Simulator:

- Investigates the release performance of subcutaneous formulations under simulated physiological conditions
- “The only commercially available solution that bridges the gap between standard dissolution and solubility results and *in vivo* Pharmacokinetics (PK) studies”



<https://pion-inc.com/>



Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Evaluation of In Vitro Tools to Predict the In Vivo Absorption of Biopharmaceuticals Following Subcutaneous Administration



Christian Bender^{a,*}, Sabine Eichling^b, Lutz Franzen^c, Viktoria Herzog^b,
Ludger M. Ickenstein^d, Dhananjay Jere^e, Lara Nonis^e, Gregoire Schwach^f,
Philipp Stoll^d, Marta Venczel^g, Shalin Zenk^g

^a Bayer AG, Pharmaceuticals, Research & Development, Building 0520, 42096 Wuppertal, Germany

^b AbbVie Deutschland GmbH & Co. KG, NBE Advanced Formulation Development, Building 11, 67061 Ludwigshafen, Germany

^c Bayer AG, Pharmaceuticals, Research & Development, Building 0226, 23096, 42096 Wuppertal, Germany

^d Boehringer Ingelheim Pharma GmbH & Co. KG, Pharmaceutical Development Biologicals, Building G55, Birkendorfer Straße 65, 88397 Biberach an der Riss, Germany

^e Drug Product Services, Lonza AG, Hochbergerstrasse 60G, CH-4057 Basel, Switzerland

^f F.Hoffmann-La Roche Ltd., Pharmaceutical Development and Supplies Europe Biologics, Grenzacherstrasse 124, 4070 Basel, Switzerland

^g Sanofi-Aventis Deutschland, Global CMC Development, Industriepark Hoechst, Building H770, 65926 Frankfurt am Main, Germany

Methods: We examined seven commercially available biopharmaceuticals using three instruments, i.e., the Subcutaneous Injection Site Simulator (Scissor), the Osmomat 050, and a dialysis system using three artificial extracellular matrices, two dissolution apparatuses, i.e., the USP4 and the USP7, and two evaluation tools, i.e., the affinity-capture self-interaction nanoparticle spectroscopy (AC-SINS) and the Developability Index (DI). Results were evaluated for their usefulness to predict the bioavailability and other pharmacokinetic parameters in humans using the Pearson correlation.

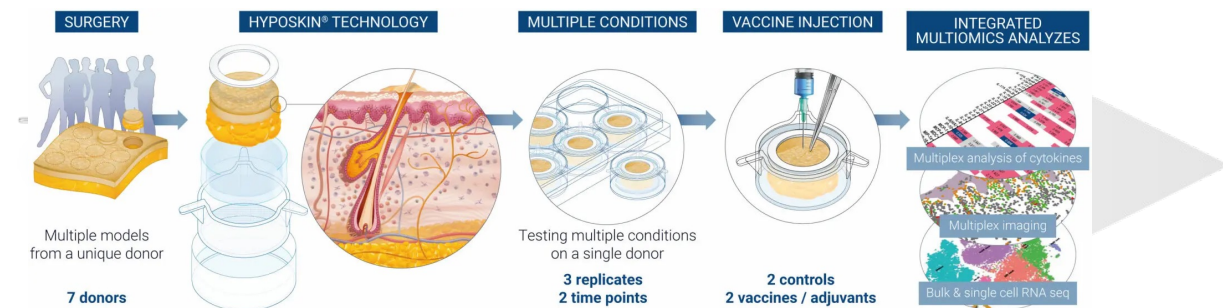
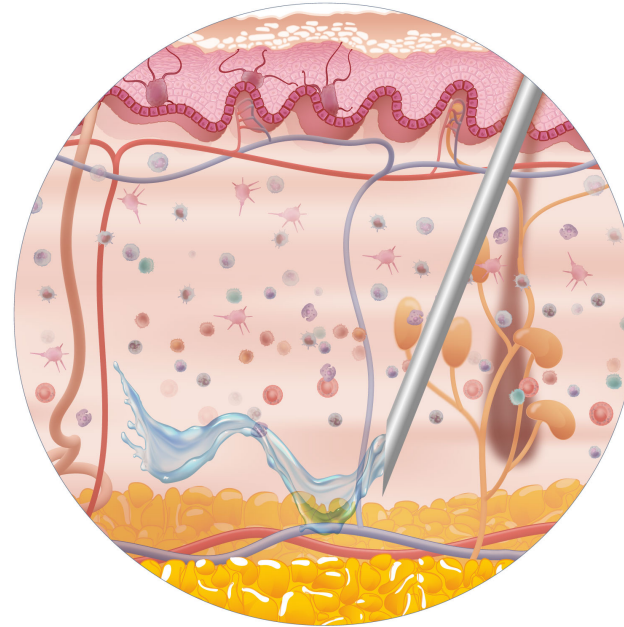
Results: None of the tested instruments and methods could reliably approximate bioavailability. Only pressure values derived with the Osmomat 050 instrument correlated with C_{max} with a Pearson correlation coefficient greater than 0.8.

Conclusion: No single in vitro method confidently predicted the bioavailability in humans. We only found a correlation to maximum plasma concentration values for one of the tested approaches. However, a more focused evaluation would be necessary to confirm our findings and test combinations of orthogonal methods that may improve the confidence of such a prediction

Bender et al, *J Pharm Sci* (2022) 111:2514-2524

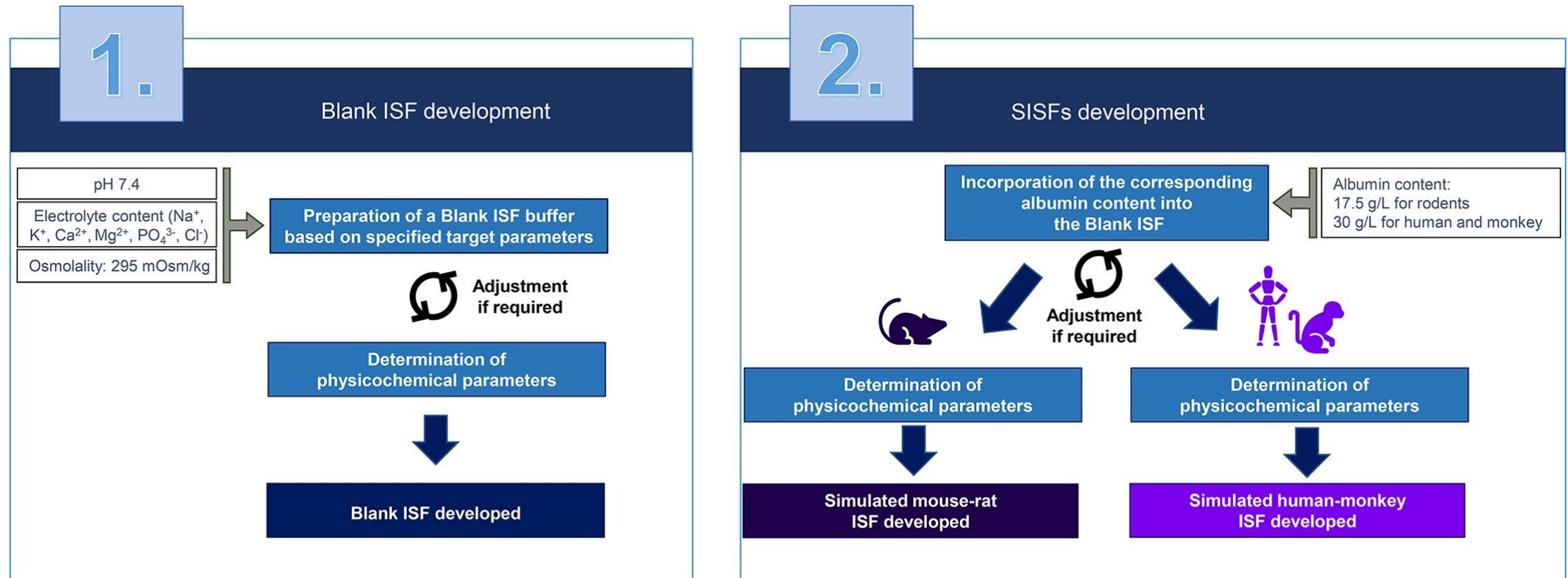
Commercial alternatives to in vivo tests

HypoSkin from GenoSkin contains the three layers of the skin: epidermis, dermis and hypodermis

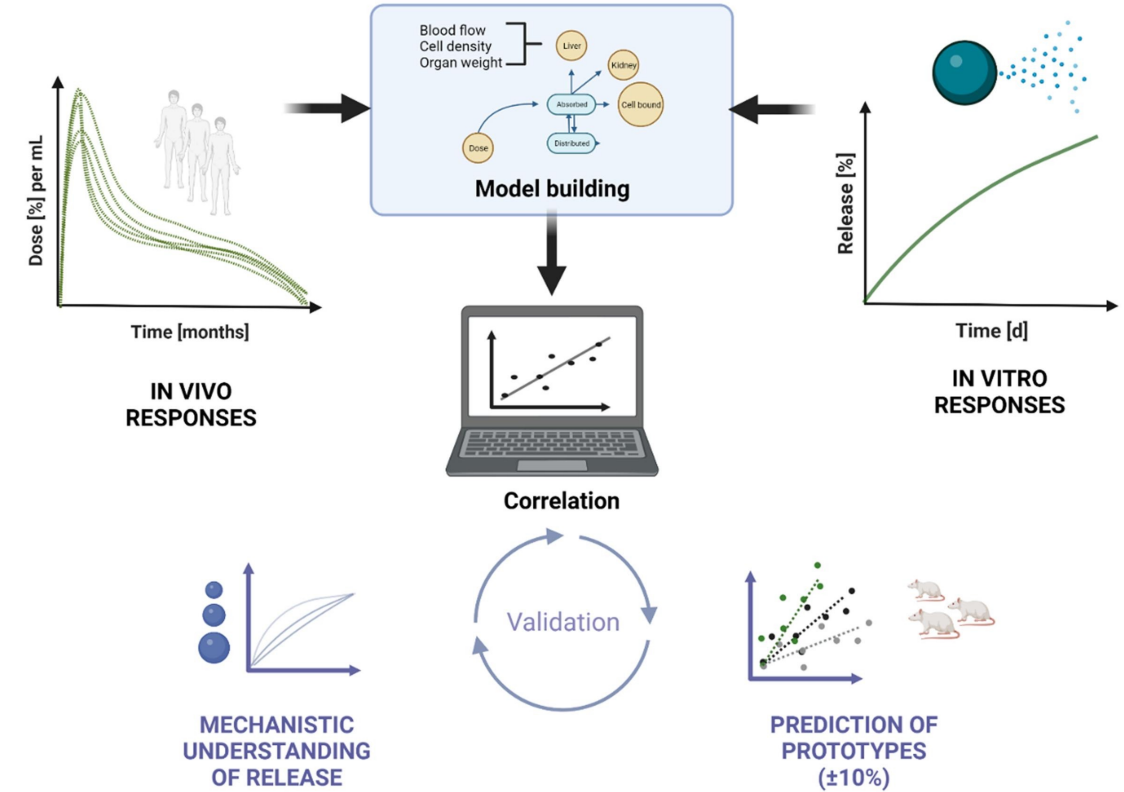
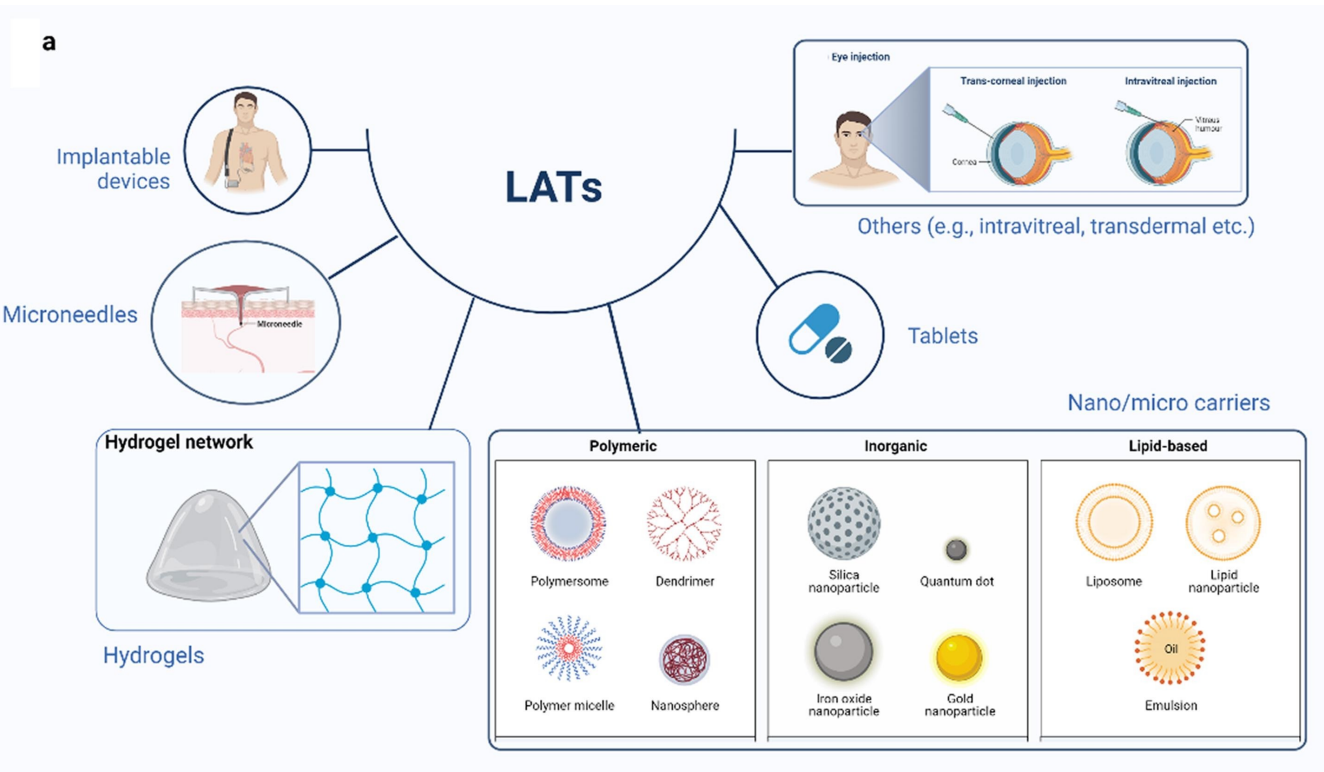


Simulated interstitial fluid media for predictive subcutaneous in vitro assays

Simulated mouse-rat interstitial fluid (ISF) and a simulated human-monkey ISF



In vitro to *in vivo* correlation development for long-acting therapeutics



Acknowledgements



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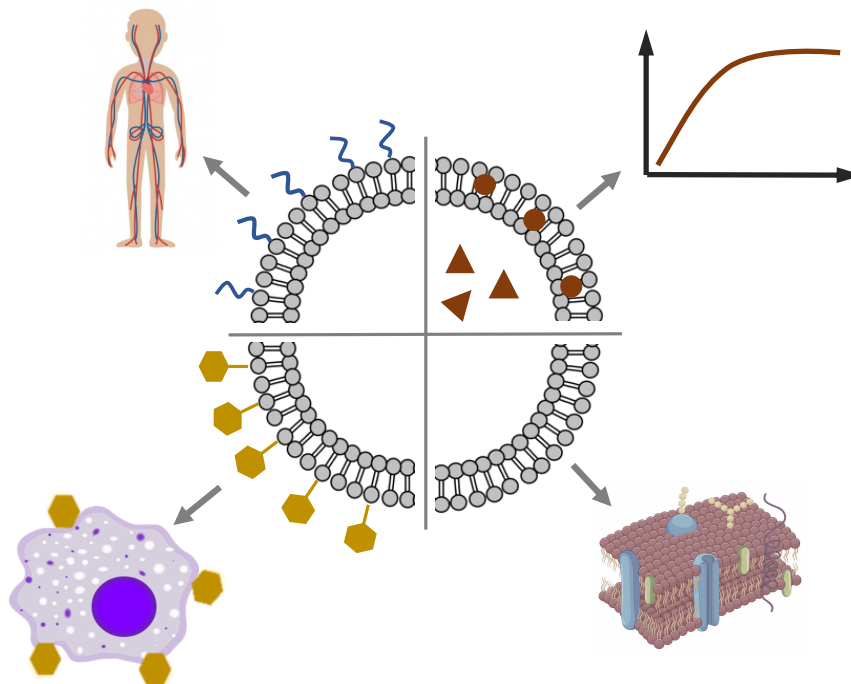


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