A theranostic platform for Joint Repair

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PhD Candidate

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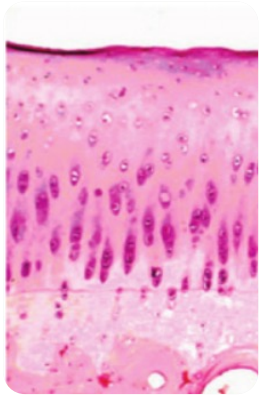
POLITECNICO
MILANO 1863

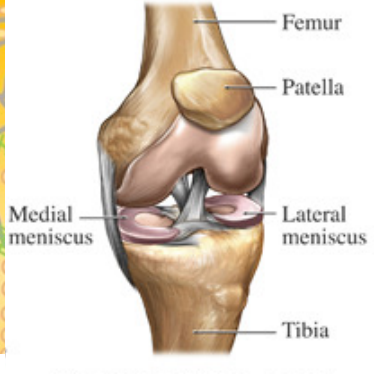
HOUSTON
Methodist®
LEADING MEDICINE

Post-traumatic osteoarthritis (PTOA)

Osteoarthritis (OA): most common degenerative disease of Articular Cartilage (AC) involving disruption of Extracellular Matrix (ECM) resulting in loss of cartilage and lack of effective replacement.

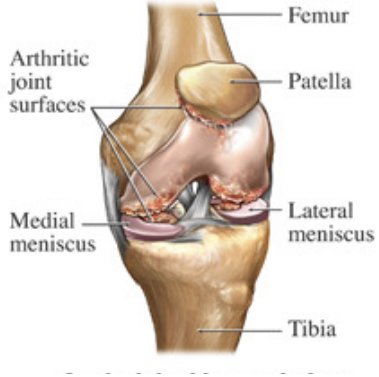
Healthy cartilage

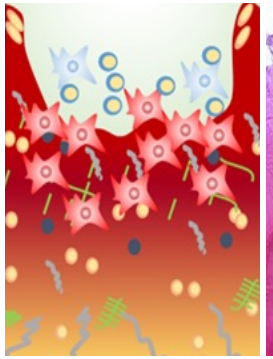


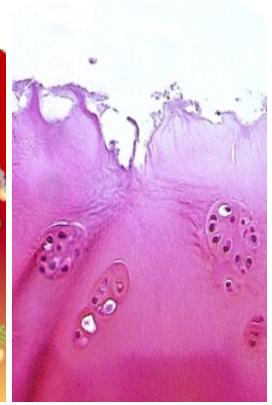


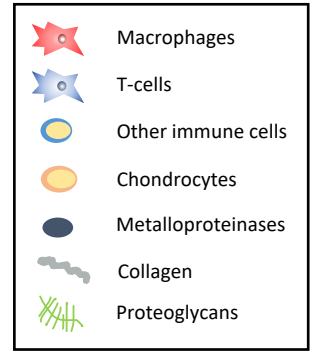
TRAUMA

Damaged cartilage





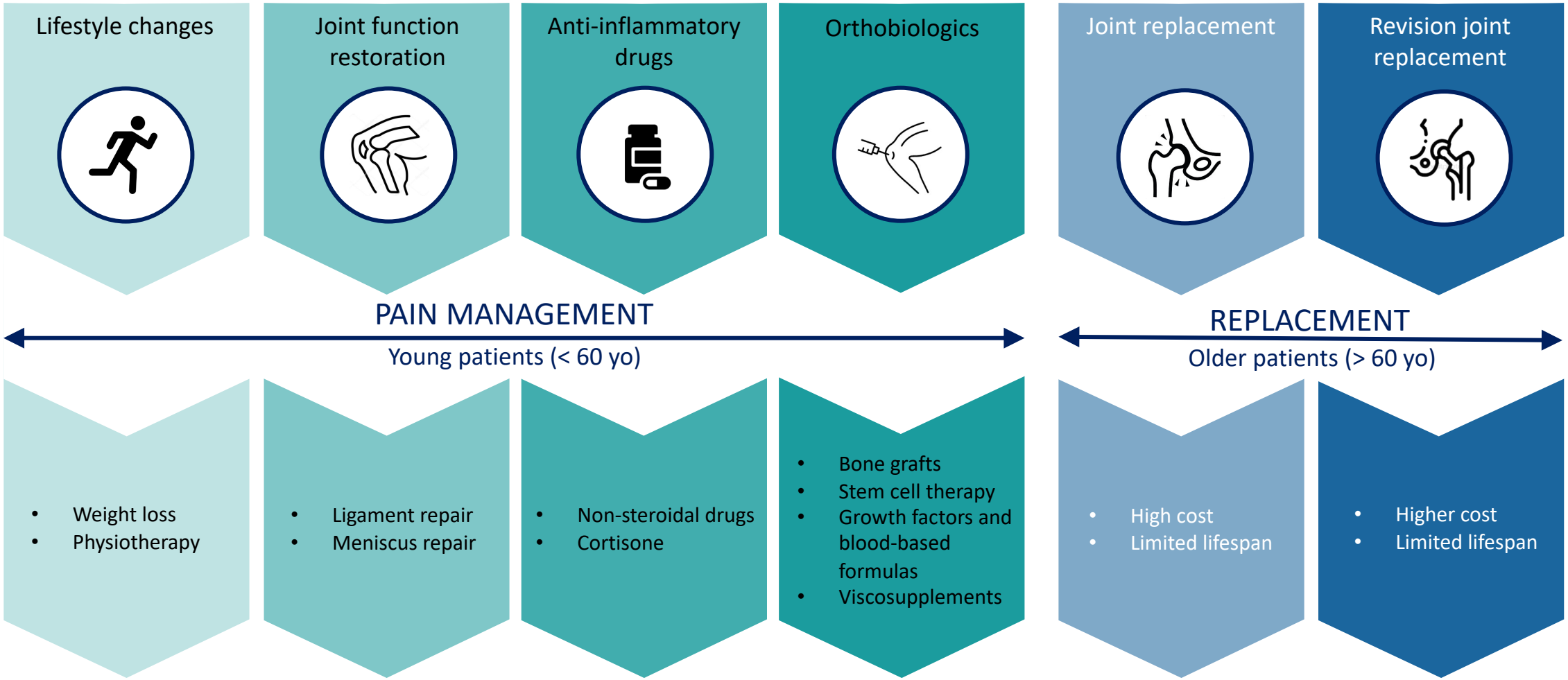




Worldwide estimates are that **9.6% of men and 18.0% of women** aged over 60 years have symptomatic osteoarthritis

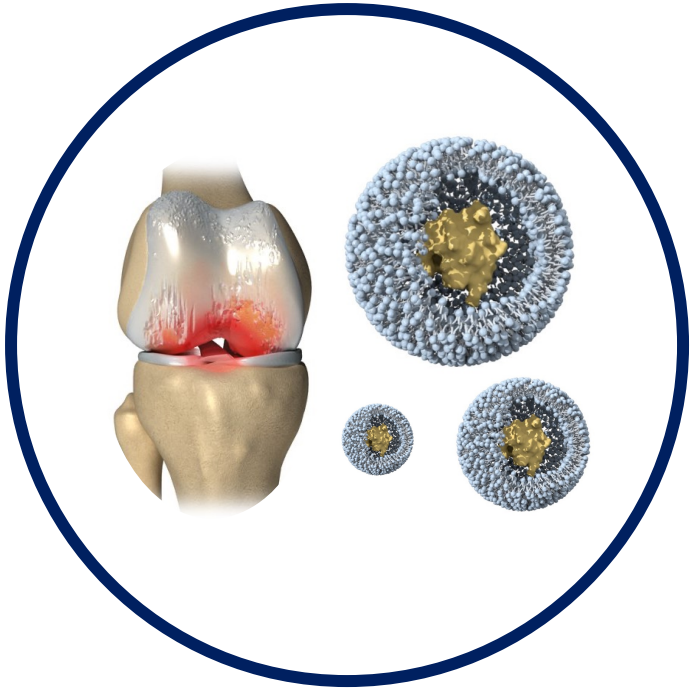
People with **PTOA account for nearly 12% of all cases of symptomatic OA** in the United States

Current therapies & Limitations



Punzi, Leonardo, et al. "Post-traumatic arthritis: overview on pathogenic mechanisms and role of inflammation." *RMD open*2.2 (2016): e000279.

Current therapies & Limitations



Nanoparticles (NPs) encapsulating DISEASE MODIFYING DRUGS (DMDs) ensure:

- ✓ Decreased off-target toxicity
- ✓ Increased drug stability and circulation time within the body
- ✓ Improved cell internalization of the cargo
- ✗ Selective targeting of the affected joint/s:
  - › Intra-articular (IA) administration has shown short retention time
  - ? Systemic administration has not been explored

Uptake by mononuclear phagocyte system (MPS)

Clearance by filtering organs

Selective targeting of inflammation

New targeted therapies delivering molecules aimed at the underlying drivers of PTOA are needed

Hypothesis: theranostic platform

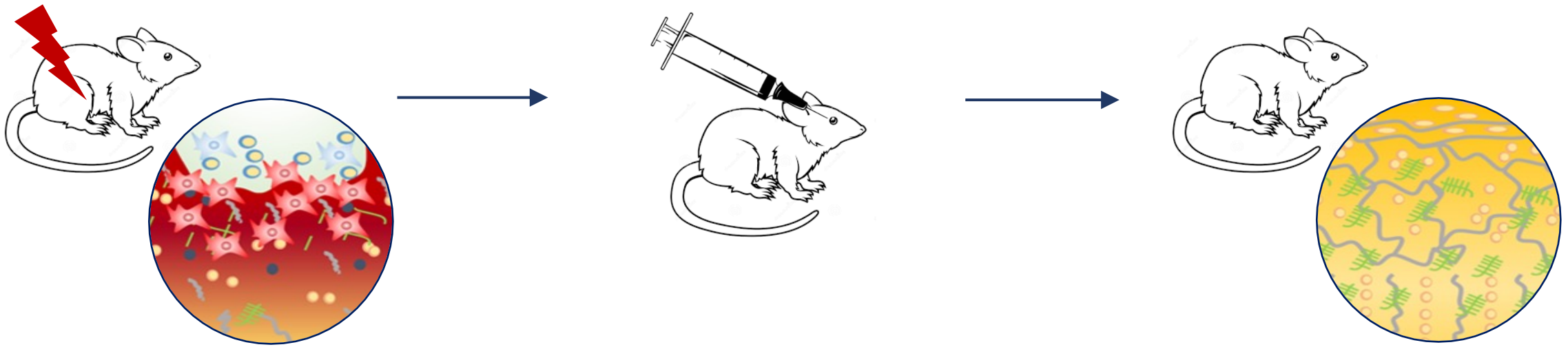
We hypothesize that *intravenous administration of liposome-based nanoparticles* that use macrophage membrane proteins as building blocks - Orthosomes - will allow us to **target** the **inflamed synovium** during the **acute phase of PTOA** and **deliver potent therapeutics**, ultimately leading to recovery of cartilage structure and restoration of the tissue homeostasis

TRANSLATIONAL MEDICINE

PTOA Induction

Systemic NP administration

Cartilage recovery



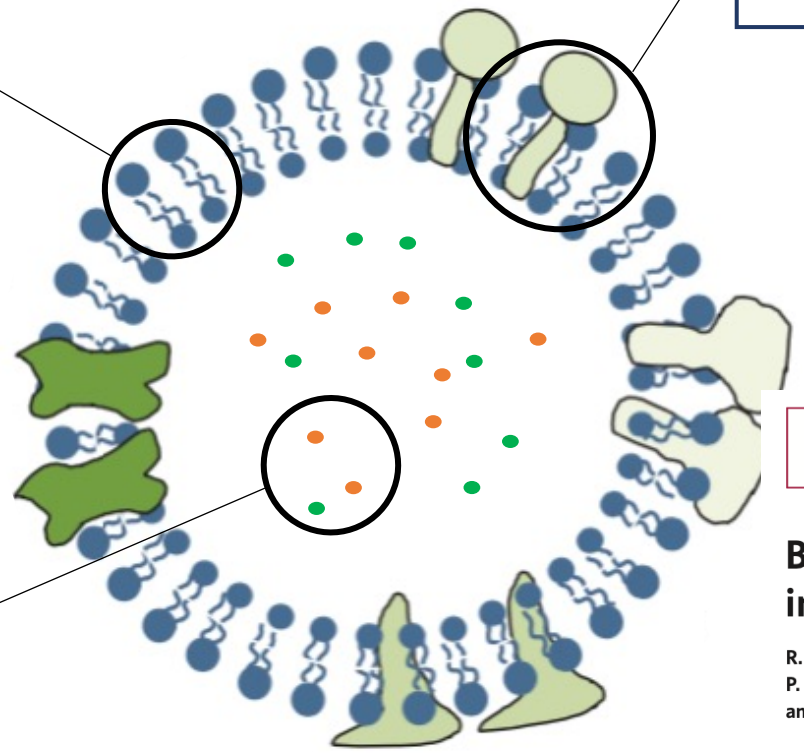
Orthosomes, an overview

Liposome:
- Advantages of nanotechnology
- Phospholipid bilayer: biomimicry

DPPC
DSPC
CHOLESTEROL
DOPG

Macrophage membrane proteins:
- Mediate biological behavior to target sites of inflammation
- Biomimicry

Transport proteins (ex. ATP-binding cassette)
Adhesion proteins (ex. Integrins)
Immunity
Signaling/Receptors



Drug
- Delivery
- Sustained release

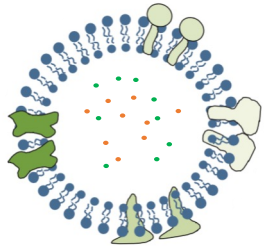
nature materials
ARTICLES
PUBLISHED ONLINE: 23 MAY 2016 | DOI: 10.1038/NMAT4644

Biomimetic proteolipid vesicles for targeting inflamed tissues

R. Molinaro[1], C. Corbo[1,2,3], J. O. Martinez[1], F. Taraballi[1,4], M. Evangelopoulos[1], S. Minardi[1], I. K. Yazdi[1], P. Zhao[5], E. De Rosa[1], M. B. Sherman[6], A. De Vita[7], N. E. Toledano Furman[1], X. Wang[1], A. Parodi[1,3] and E. Tasciotti[1,8]*

A multitude of micro- and nanoparticles have been developed to improve the delivery of systemically administered pharmaceuticals, which are subject to a number of biological barriers that limit their optimal biodistribution. Bioinspired drug-delivery carriers formulated by bottom-up or top-down strategies have emerged as an alternative approach to evade the mononuclear phagocytic system and facilitate transport across the endothelial vessel wall. Here, we describe a method that leverages the advantages of bottom-up and top-down strategies to incorporate proteins derived from the leukocyte plasma membrane into lipid nanoparticles. The resulting proteolipid vesicles—which we refer to as leukosomes—retained the versatility and physicochemical properties typical of liposomal formulations, preferentially targeted inflamed vasculature, enabled the selective and effective delivery of dexamethasone to inflamed tissues, and reduced phlogosis in a localized model of inflammation.

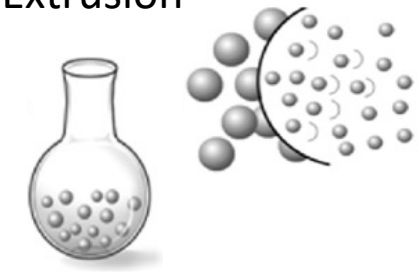
Targeting PTOA with Orthosomes



Protein presence on Orthosomes (ORTHO) mediates biological behavior to target sites of inflammation

1 Synthesis of empty NPs with membrane proteins from murine macrophages

- ✓ Thin layer evaporation (TLE)
- ✓ Extrusion



2 Analysis of NP properties

- ✓ Dynamic light scattering (DLS)
- ✓ Transmission electron microscopy (TEM)
- ✓ SDS page & Western blotting (WB)

3 *In vivo* targeting behavior of the NPs

- ✓ In vivo (Intravital microscopy, IVM) & ex vivo targeting (IVIS)
- ✓ Ex vivo biodistribution (IVIS)
- ✓ Detection of inflammation markers (Imaging mass cytometry, IMC)

Orthosomes characterization

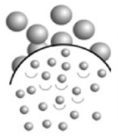
Synthesis of Orthosomes: TLE

1

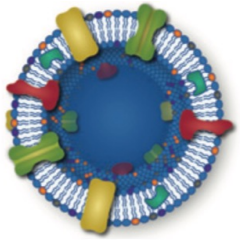
Dried ***lipid film***

- DPPC
- CHOLESTEROL
- DSPC
- DOPG

Aqueous phase w/ J774 murine macrophages membrane proteins

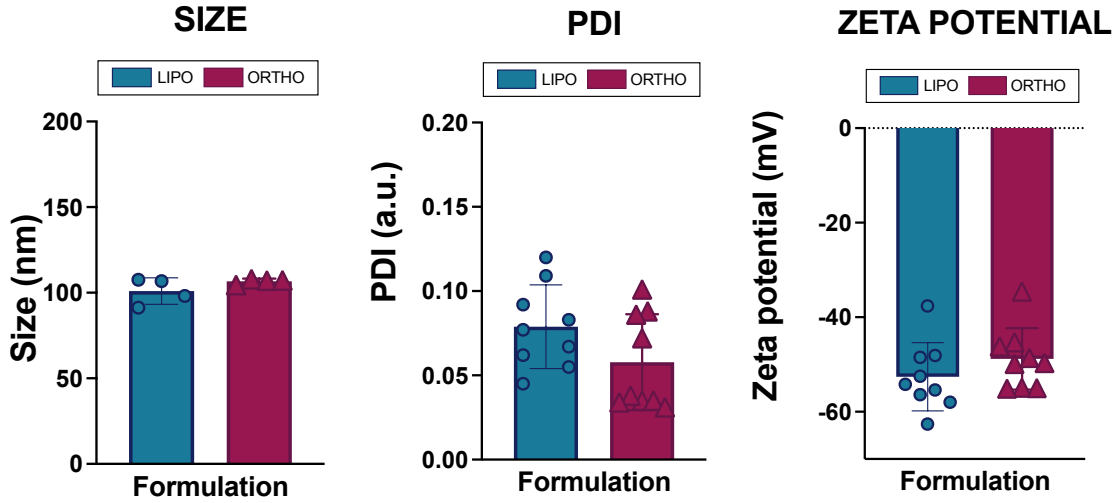


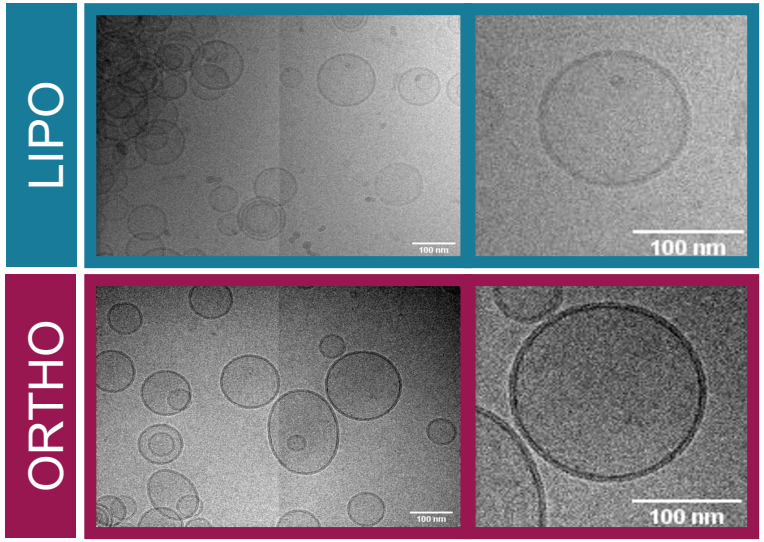
Extrusion

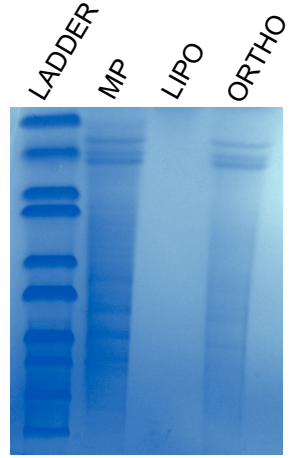


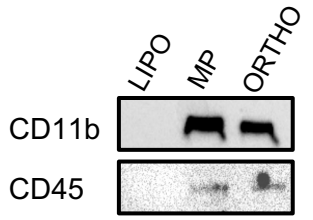
Analysis of NP properties

2







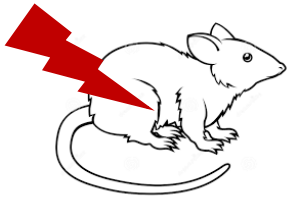


Targeting PTOA

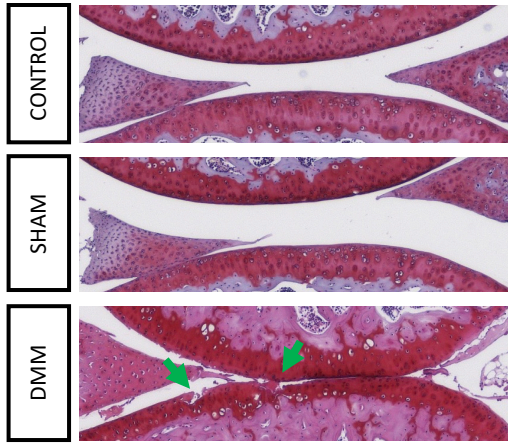
AIM: *identification of the timepoint with highest NP accumulation* in the damaged joint during the acute phase

PTOA Induction

Surgical Disruption of Medial Meniscus (DMM)

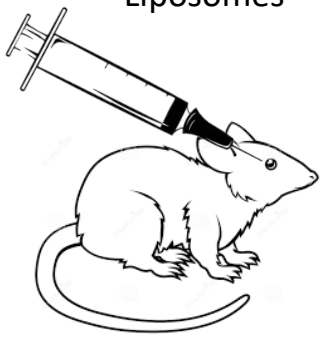


Surgically Induced PTOA mouse model (male & female C57BL/6 mice)



NP administration (IV)

- Orthosomes
- Liposomes



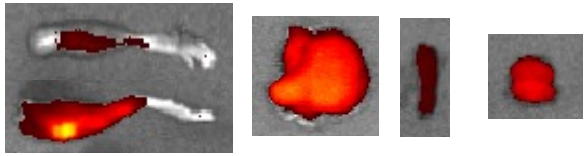
Fluorescently labeled NPs administered at:
24H - 48H -72H - 7D

Imaging

6h post injection

IVIS

Quantification of fluorescence signal from NPs accumulation in legs and organs (liver, spleen, kidneys)



IVM

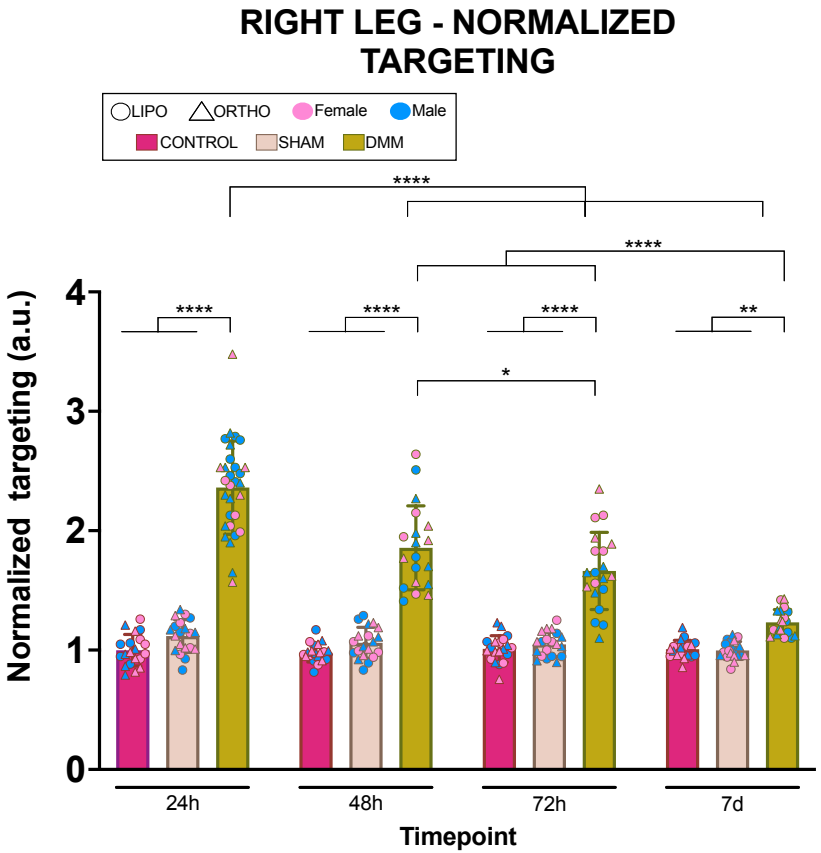
Qualitative evaluation of NPs accumulation in the synovial area

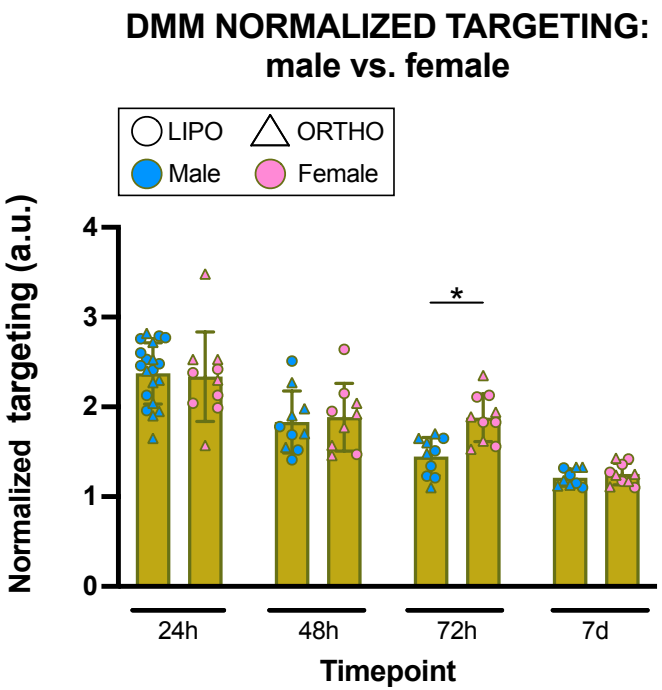
CyTOF

Quantitative evaluation of inflammation markers on harvested and sectioned tissue slides

TOTAL N° OF MICE: n=240

Targeting PTOA: IVIS *ex vivo*



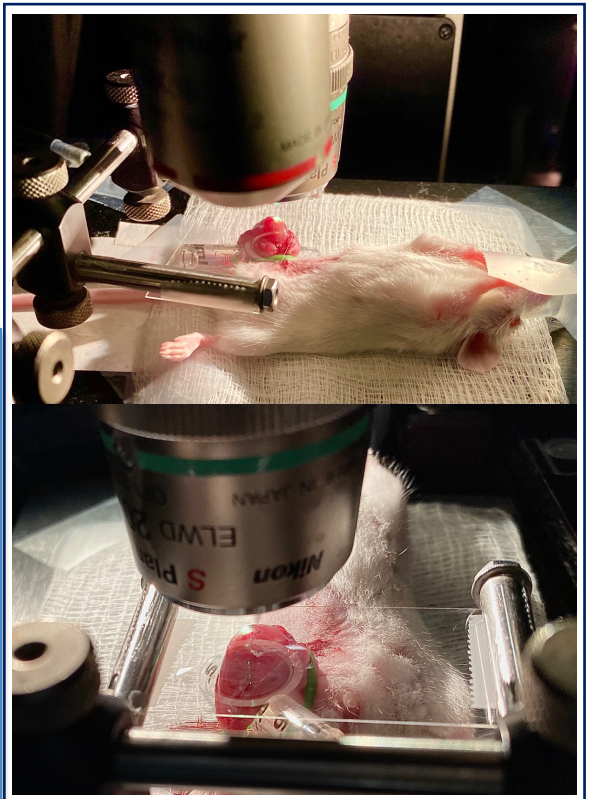


Successful targeting of the right leg in the DMM group

- Leaky vasculature → significantly higher NP accumulation
  - NP accumulation decreases at later timepoints
    - No difference between Lipo & Ortho → *IMC*
      - No difference between male & female

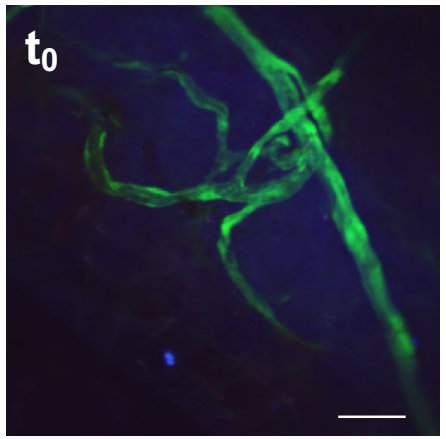
Targeting PTOA: IVM *in vivo*

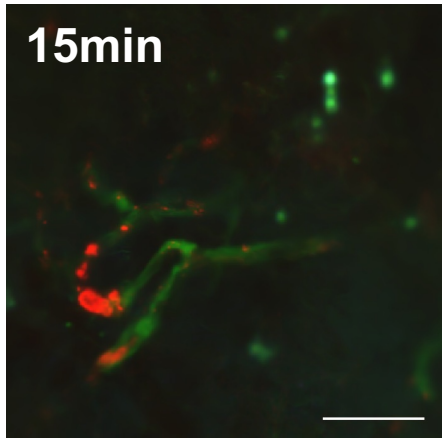
Animal positioning

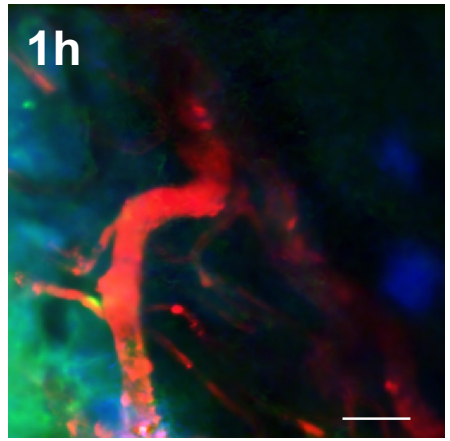


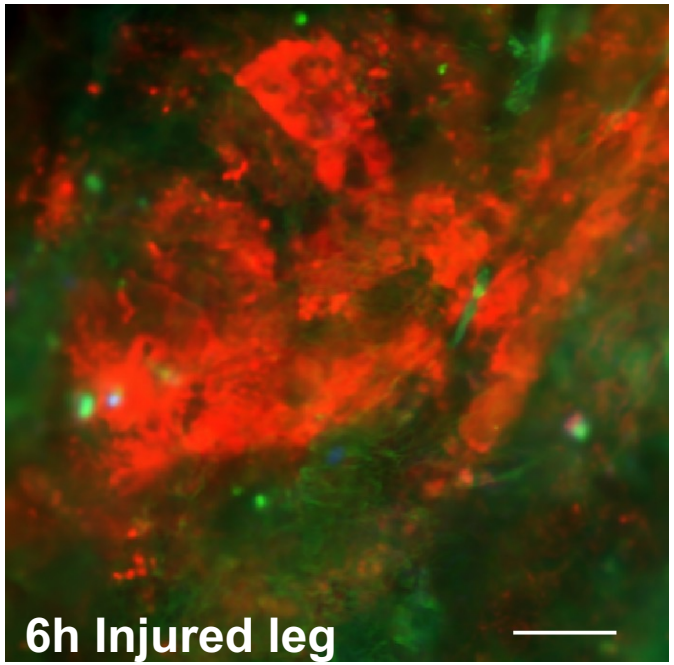
Female mice, 24h timepoint

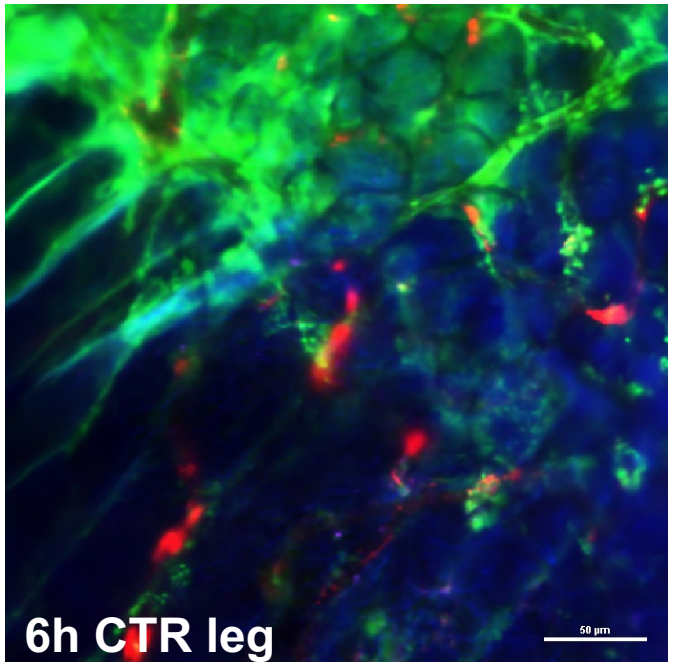
6 h post injection (lipo)







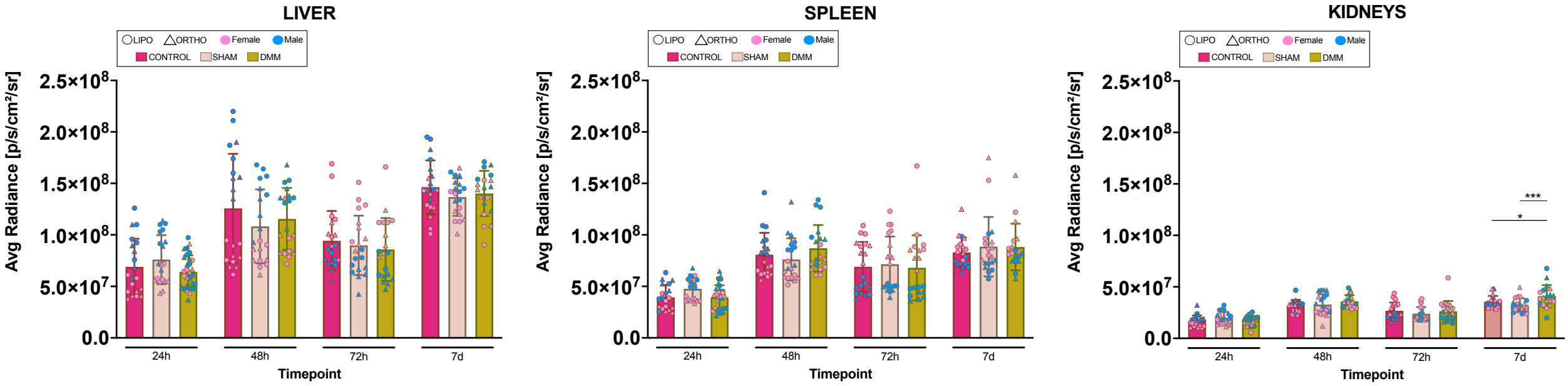




* Scalebars 50 µm

Biodistribution: IVIS *ex vivo*

When NPs are loaded, the drug's side effect will be more evident in the organs with highest NP accumulation

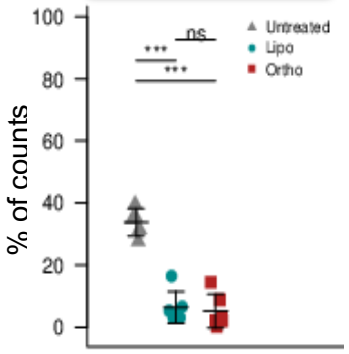


As expected, LIPO and ORTHO accumulated in filtering organs with a similar profile

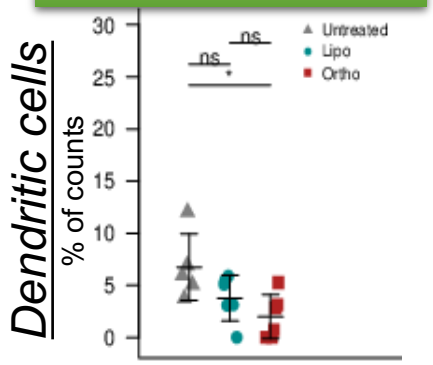
Imaging Mass Cytometry (IMC)

MYELOID COMPARTMENT

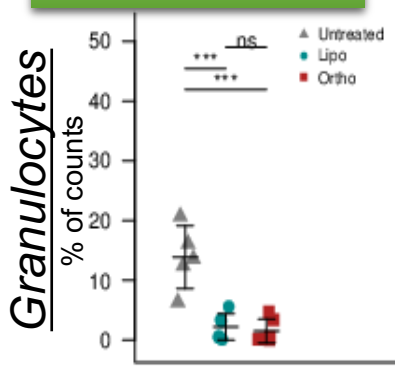
CD11b⁺ vs total



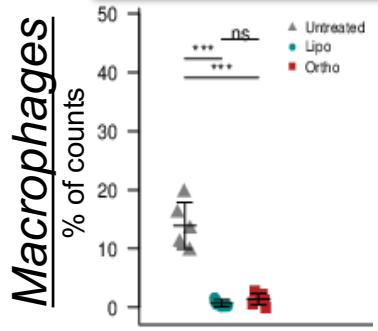
CD11b⁺/CD11c⁺ vs CD11b⁺



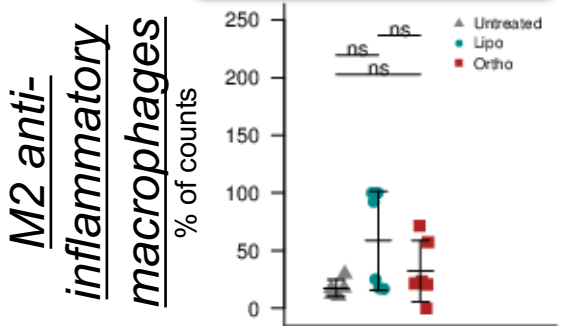
CD11b⁺/Ly6G⁺ vs total



CD11b⁺/F480⁺ vs total

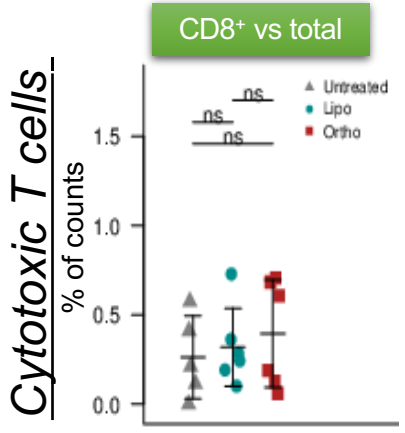


F480⁺/CD206⁺ vs F480⁺

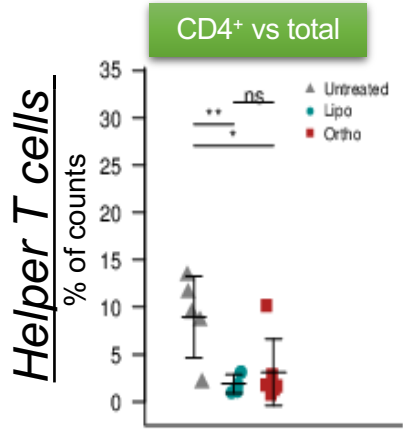


LYMPHOID COMPARTMENT

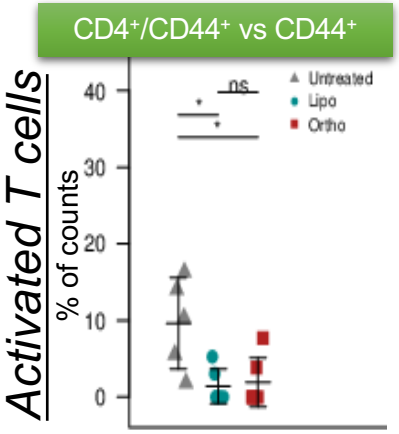
CD8⁺ vs total



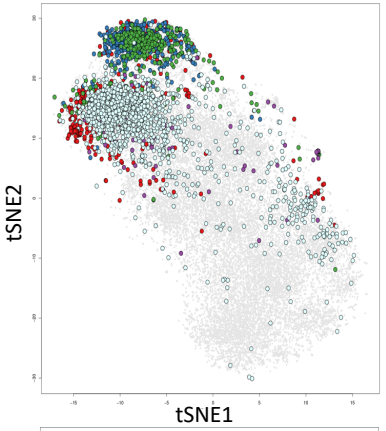
CD4⁺ vs total



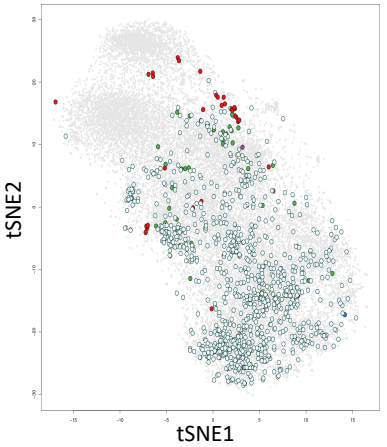
CD4⁺/CD44⁺ vs CD44⁺



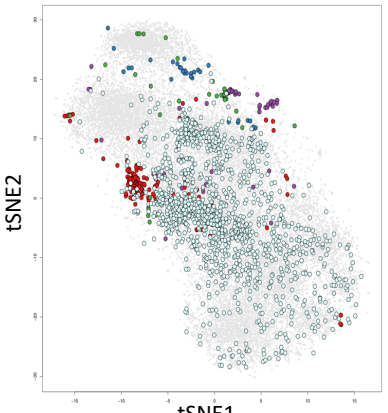
UNTREATED



LIPO



ORTHO

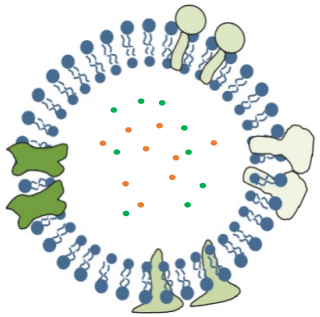


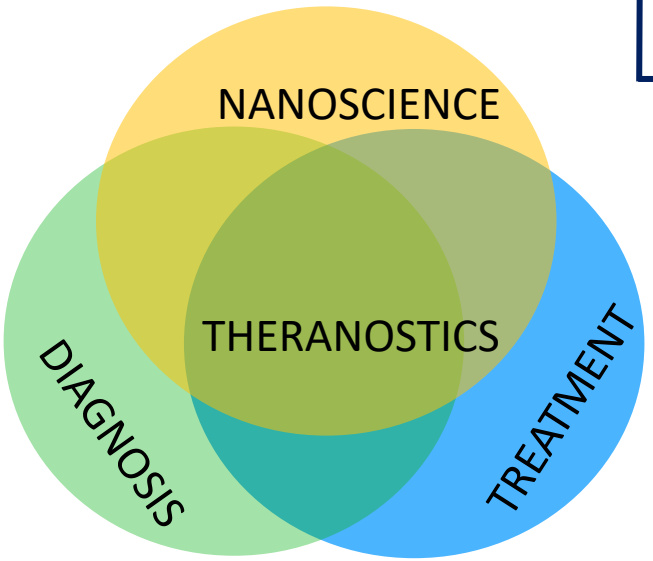
Summary

Challenges in PTOA treatment:

1. Targeting inflammation
2. Delivery of new therapeutics

Engineering Orthosomes for PTOA





A

Targeting PTOA with Orthosomes

- ORTHO preferentially accumulate within the injured leg for up to 7 days after injury in a murine PTOA model

B

Tuning inflammatory environment with Orthosomes

- ORTHO treatment caused drastic reduction of cell infiltrates from the myeloid compartment, and an increasing trend of anti-inflammatory macrophage infiltrates

ACKNOWLEGMENTS

Francesca Taraballi, PhD

Anna Pasto, PhD

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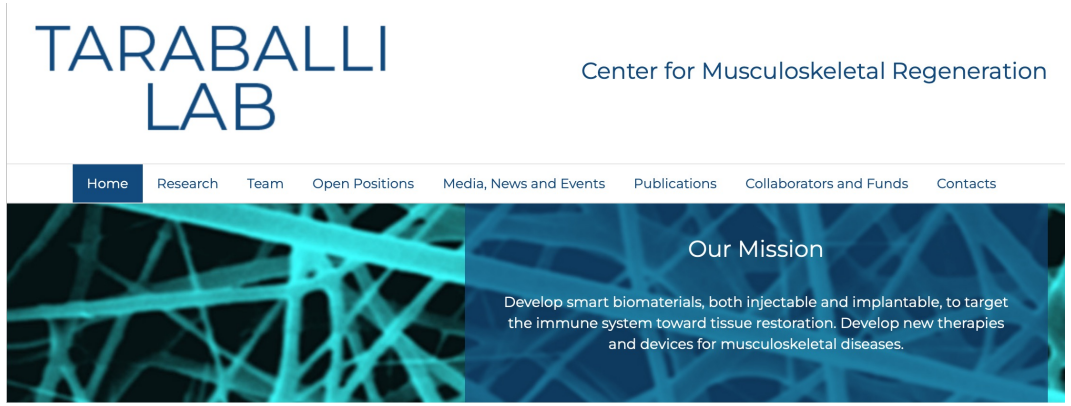


CPRIT

Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation

SCAN ME

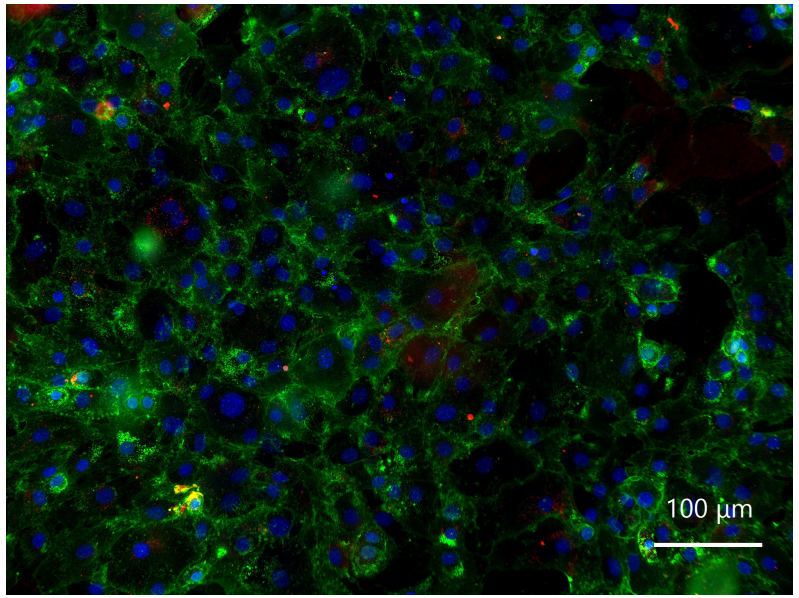
THANK YOU

ANY QUESTIONS?

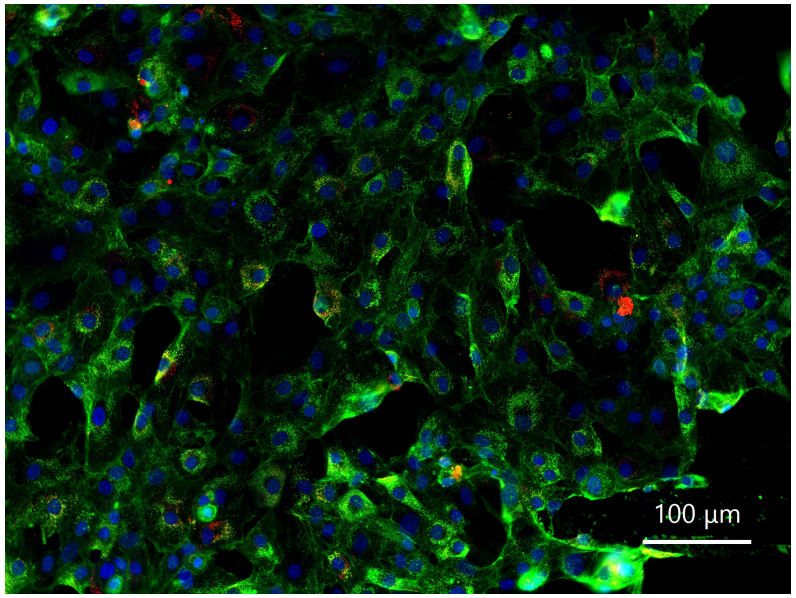
Liposome uptake - Pilot

- Primary Murine Articular Chondrocytes
- Empty Liposomes (Rhodamine-labeled) - 100 µM
- Timepoints: 6 h -24 h

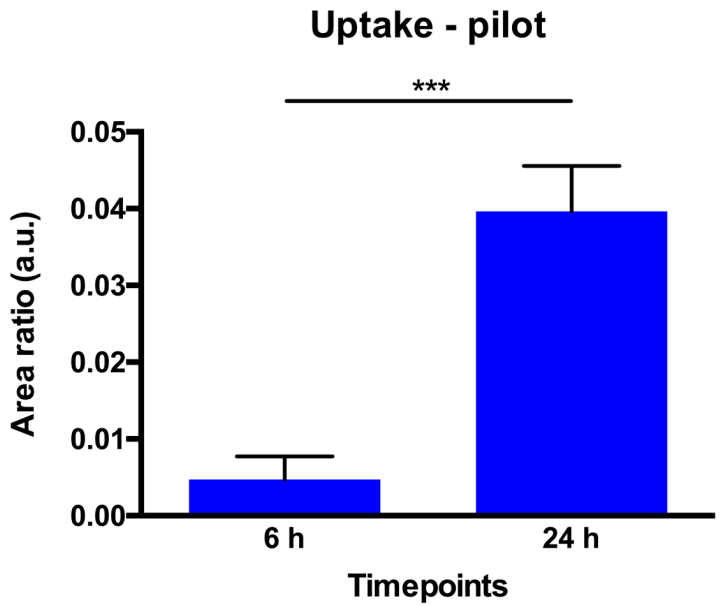
6 hours



24 hours



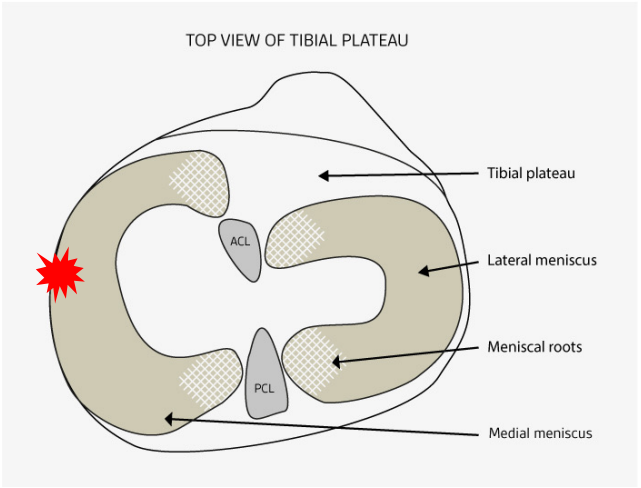
WGA - Green Nuclei - Blue Liposomes - Red



8-FOLD INCREASE

Surgically induced model

Surgical Disruption of Medial Meniscus (DMM)



- Medial meniscus bears more load than the lateral in mice
- Joint instability
- Moderate OA after 4 weeks

Pros

- High incidence
- Mirrors the progression of the disease in humans
- Causes joint instability

Cons

- Time consuming (30 min/mouse)
- Slower progression
- Challenging technique
- Need for skilled surgeon
- Risk of infection

Bapat, Santul, et al. "Pros and cons of mouse models for studying osteoarthritis." *Clinical and translational medicine* 7.1 (2018): 36.
Blaker, Carina L., Elizabeth C. Clarke, and Christopher B. Little. "Using mouse models to investigate the pathophysiology, treatment, and prevention of post-traumatic osteoarthritis." *Journal of orthopaedic research* 35.3 (2017): 424-439.