CRS 2025 ANNUAL MEETING & EXPOSITION
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Syracuse University
College of Engineering & Computer Science

Nanoparticles in Microparticles(NiM) as Macrophage Targeting Immunomodulatory Drug Delivery System for Osteoarthritis Treatment

Paul Sagoe, Kaixiang Zhang, and Era Jain, PhD

PhD student
Biomedical and Chemical Engineering Department
Bioinspired Institute, Syracuse University

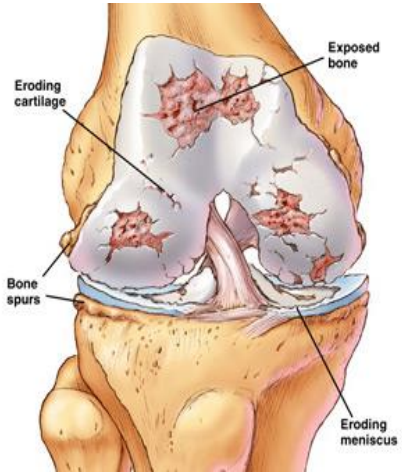
07/16/2025

Osteoarthritis (OA)

OA is a **painful** degenerative joint disease with **no known cure**

- Articular cartilage degradation
- Synovial inflammation

Arthritic knee joint



Pain, Stiffness, Swelling, Loss of Mobility

↓

Joint dysfunction and Disability

1. Bowles RD, LA Setton et al., *Arthritis Rheumatology*, 2014
2. March, Lyn, et al. "Osteoarthritis: A Serious Disease: Submitted to the US Food and Drug Administration.", 2016

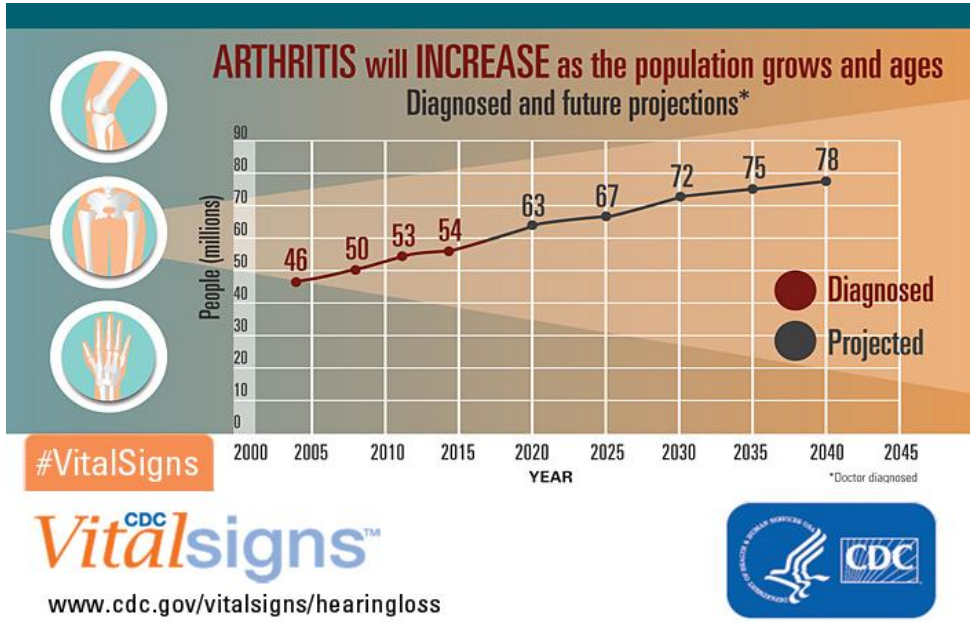
Clinical need for osteoarthritis (OA)

Socioeconomic burden [1]

- Cost approaches $200 billion annually in the US

Increasing Prevalence [2,3]

- OA will impact ~70M people in the US by 2030



Science

HEALTH >

The unstoppable rise of osteoarthritis: One billion to be affected by 2050

Published August 21, 2023

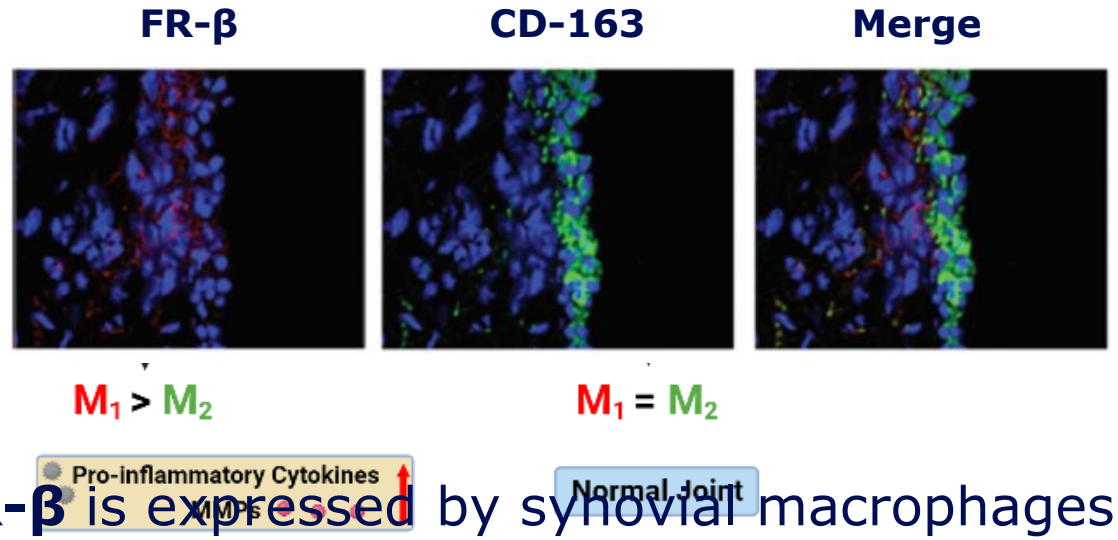
The Lancet: New study reveals the most common form of arthritis, osteoarthritis, affects 15% of the global population over the age of 30

Designing curative intervention is necessary

1. Zhao, Xiaohui, et al, Osteoarthritis and cartilage, 2019
2. Steinmetz JD, et al, Lancet Rheumatol, 2023
3. Hootman JM, et al, Arthritis Rheumatol. 2016.

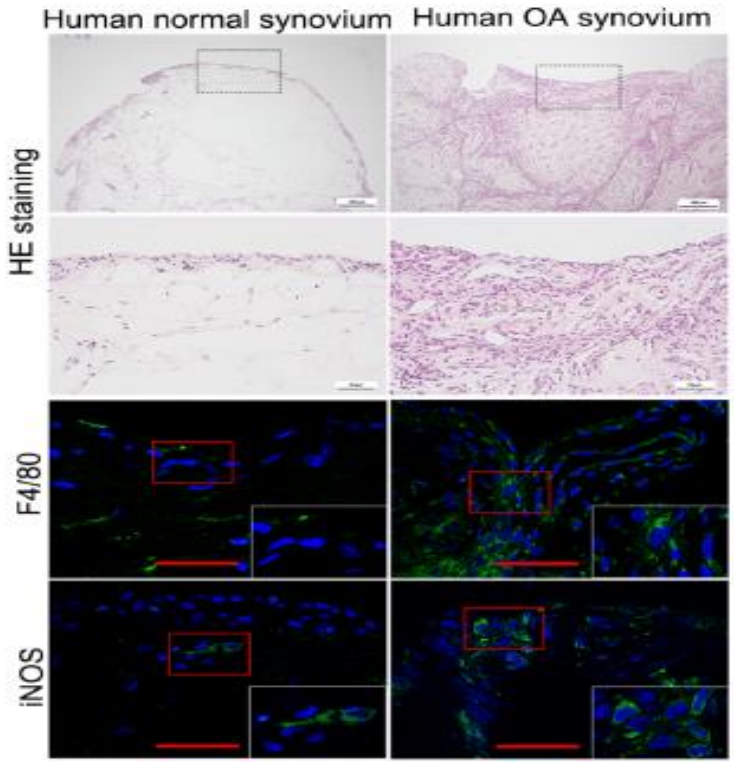
Macrophage targeting for modulating inflammation

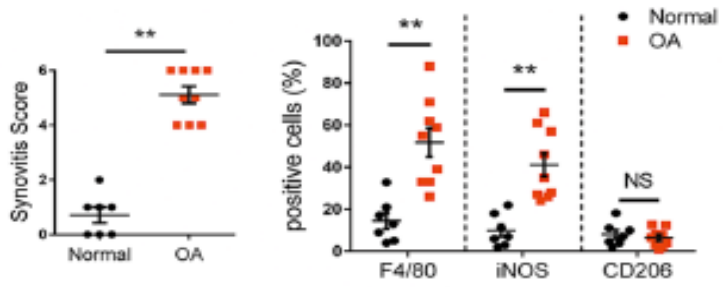
Synovial macrophages contributes significantly to the inflammatory cascade that drives OA pain and progression [1]



FR-β is expressed by synovial macrophages in OA [2]

- Enable specific target and delivery of therapeutics to macrophages
  - Modulate joint inflammation and disease outcome

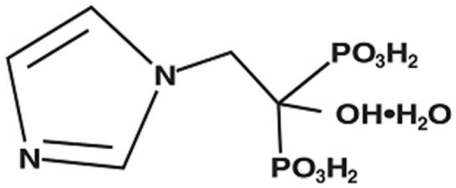




M1 Marker **(iNOS);** M2 Marker **(CD206);** Pan Marker **(F4/80)**.

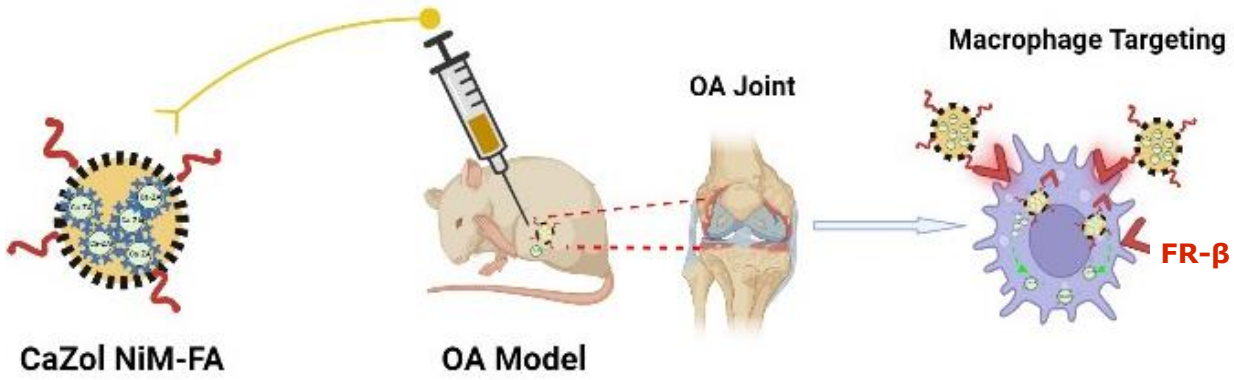
1. Zhang, H. Bai X., et.al., Annals of rheumatic diseases, 2018
2. Tsuneyoshi Y.,et.al., Scan J Rheumatol, 2012

Macrophage targeting intra-articular delivery system for osteoarthritis therapy



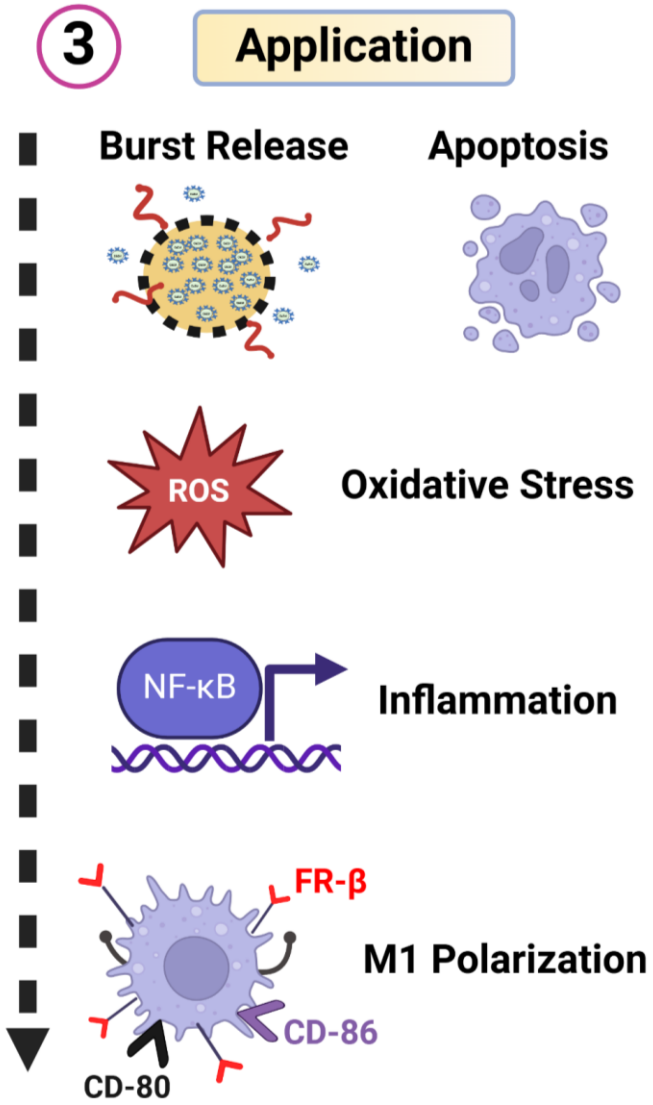
Zoledronate (Zol)

- ✓ Strong affinity for macrophage
- ✓ Chondroprotective benefit
- ✓ Anti-inflammatory potential
- ✗ Rapid joint clearance
- ✗ Toxic at elevated dose



Modulate

Suppress



Figures created in Biorender.com

1. Rogers, T. L., & Holen, I., (J. Transl. Med). 2011
2. Evans, C. H., Kraus, V. B., & Setton, L. A., (Nat Rev Rheumatol). 2014
3. Gao, J., Xia, Z., Mary, H. B., Joseph, J., Luo, J. N., & Joshi, N., (TIPS). 2022

Synthesis of Nanoparticles in Microparticles(NiM) by coaxial flow-phase separation technique

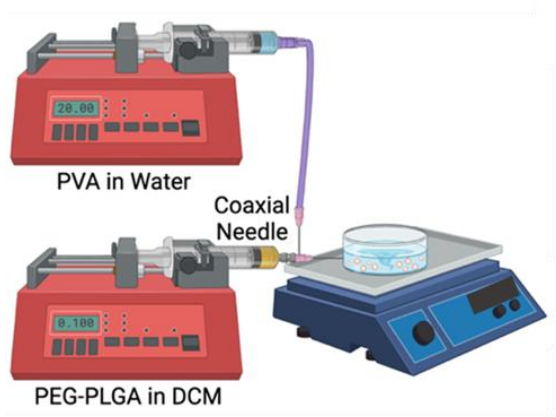
Drug Delivery and Translational Research
https://doi.org/10.1007/s13346-025-01889-7

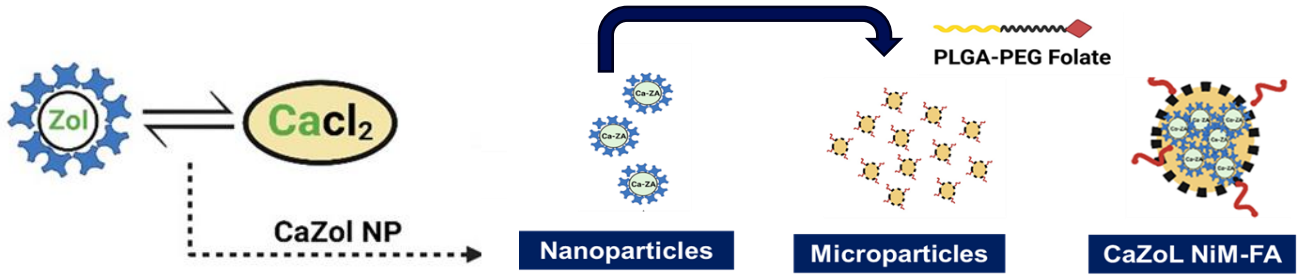
ORIGINAL ARTICLE

Check for updates

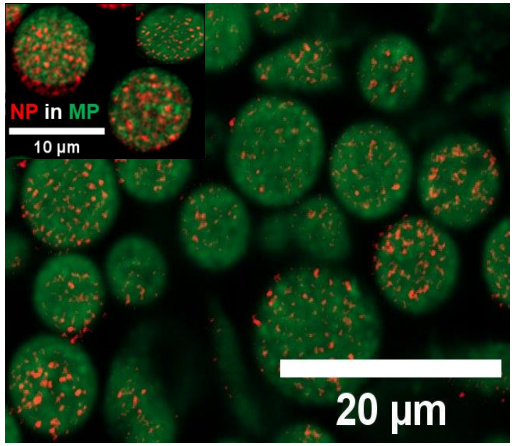
Bisphosphonates loaded nanoparticles in microparticles: a potential macrophage targeting and repolarizing drug delivery system

Paul N. K. Sagoe[1,2] · Benjamin Zink[3] · Era Jain[1,2]

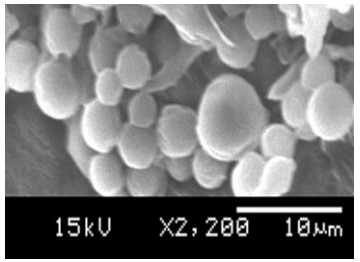




CaZol NiM – NP in MP



6.9 ± 2.1 µm



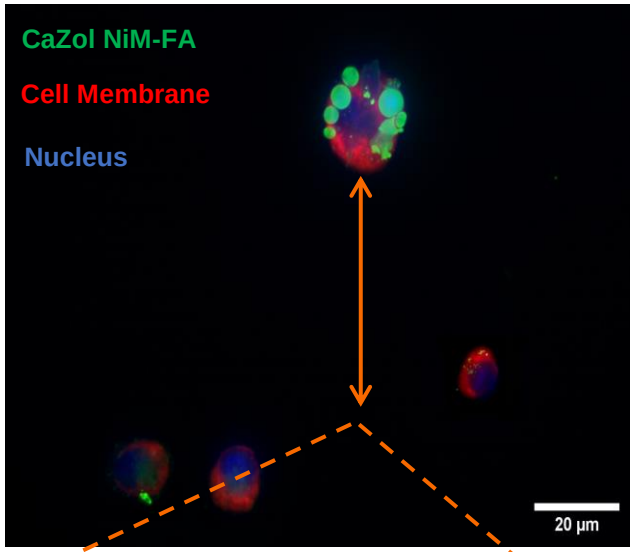
Encapsulation Efficiency (EE%)	55.8 ± 9.0
Drug Loading (DL%)	3.9 ± 0.1
#NP per MP	32 ± 12

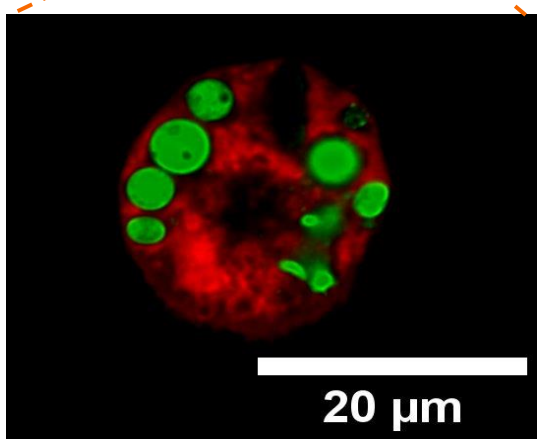
CaZol: Calcium-Zoledronate; NiM: Nanoparticles(NP) in Microparticles(MP); FA: Folic Acid

Sagoe, P. Jain, E., et. al., DDTR, 2025
Sagoe, P. Jain, E., et. al., Molecules, 2023

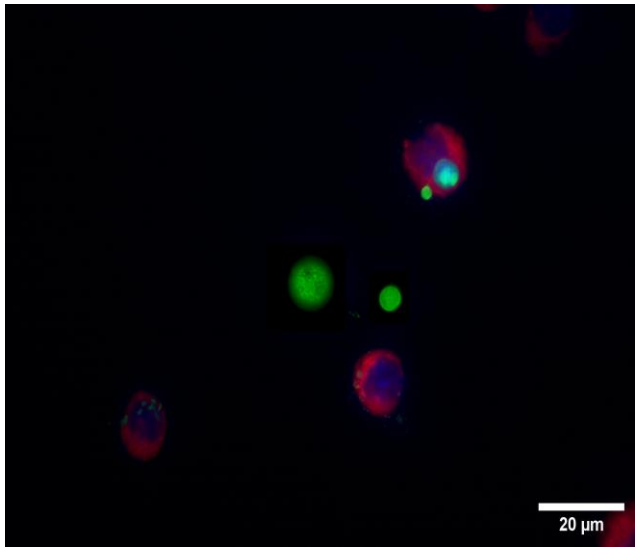
Folic Acid(FA) conjugation mediates targeted uptake of NiM by macrophages

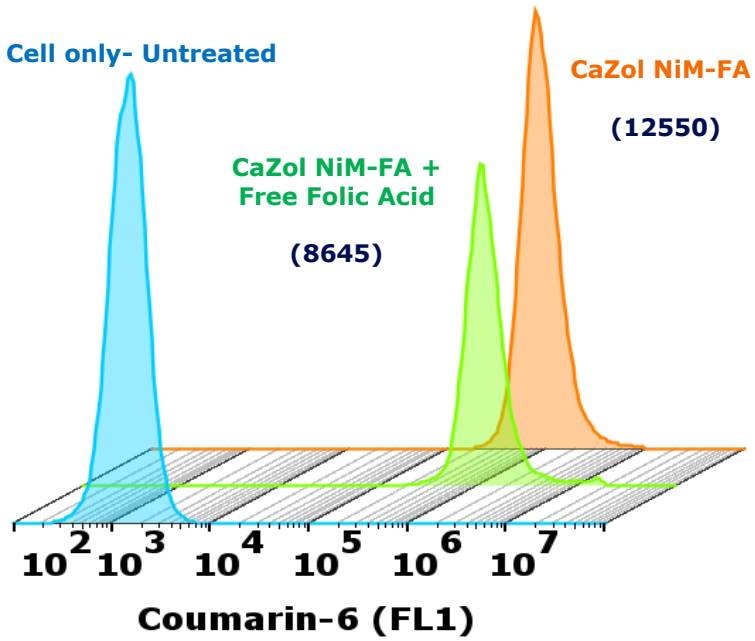
NiM-FA treated cells





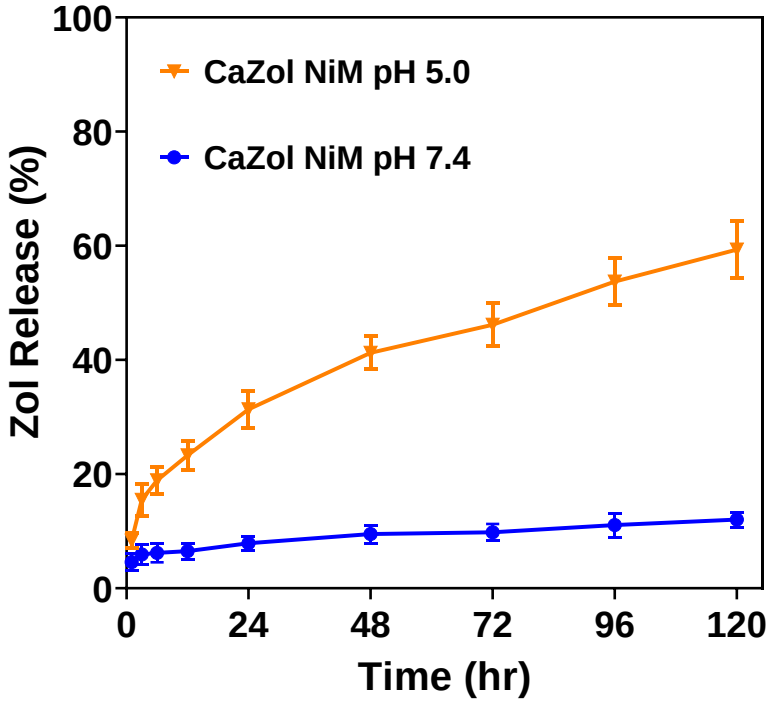
NiM-FA + free folic acid treat

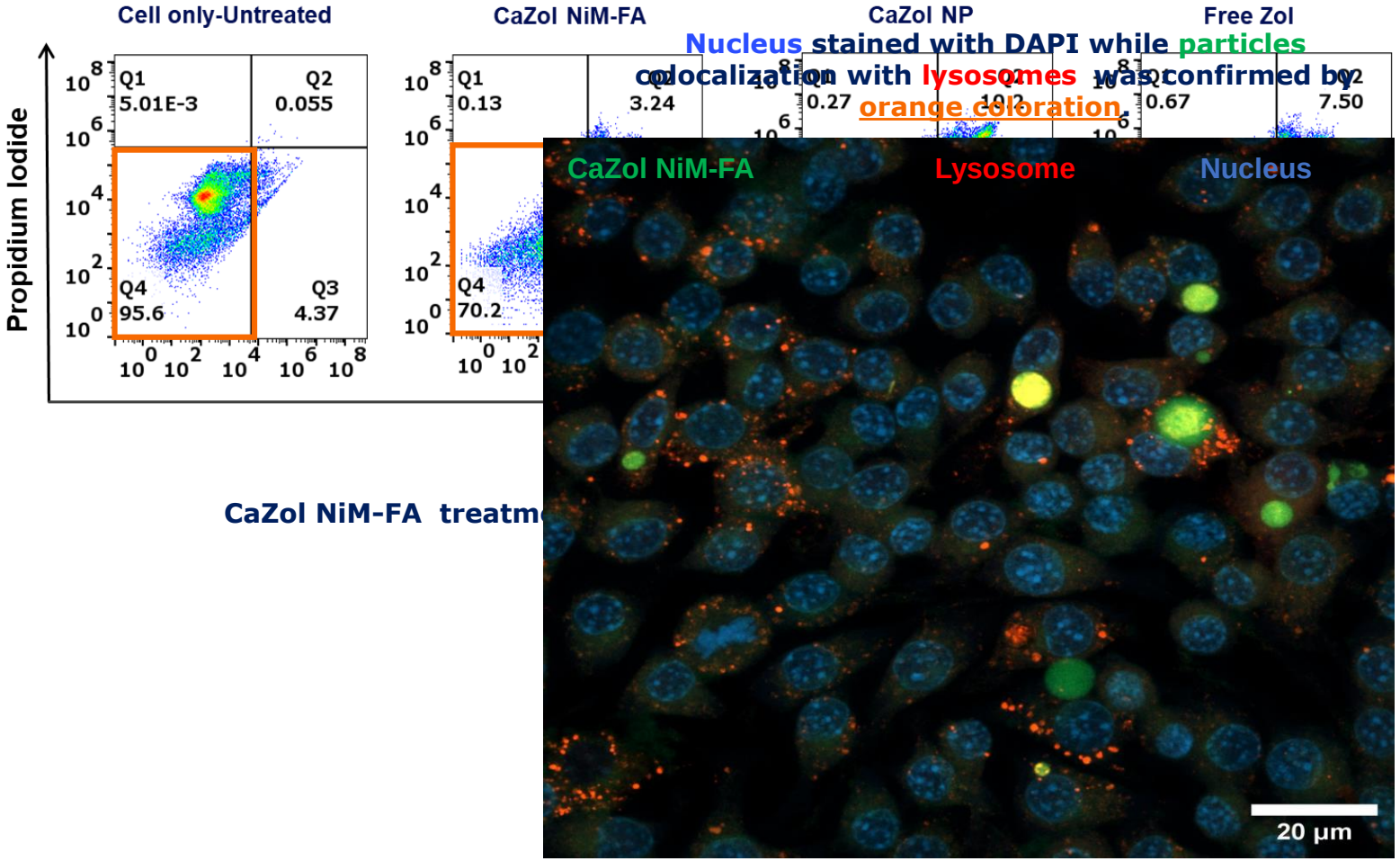




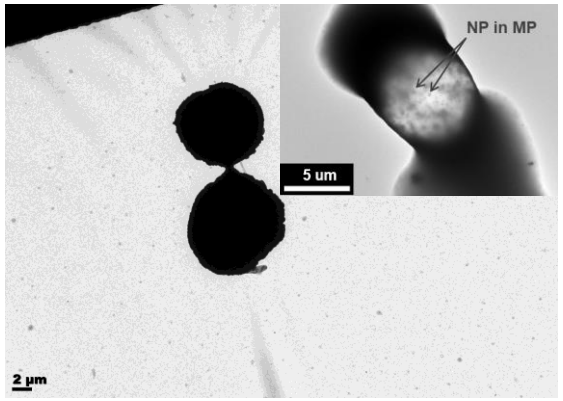
Sagoe, P. Jain, E., et. al., DDTR, 2025

NiM-FA sustainably releases Zol allowing to minimize apoptosis



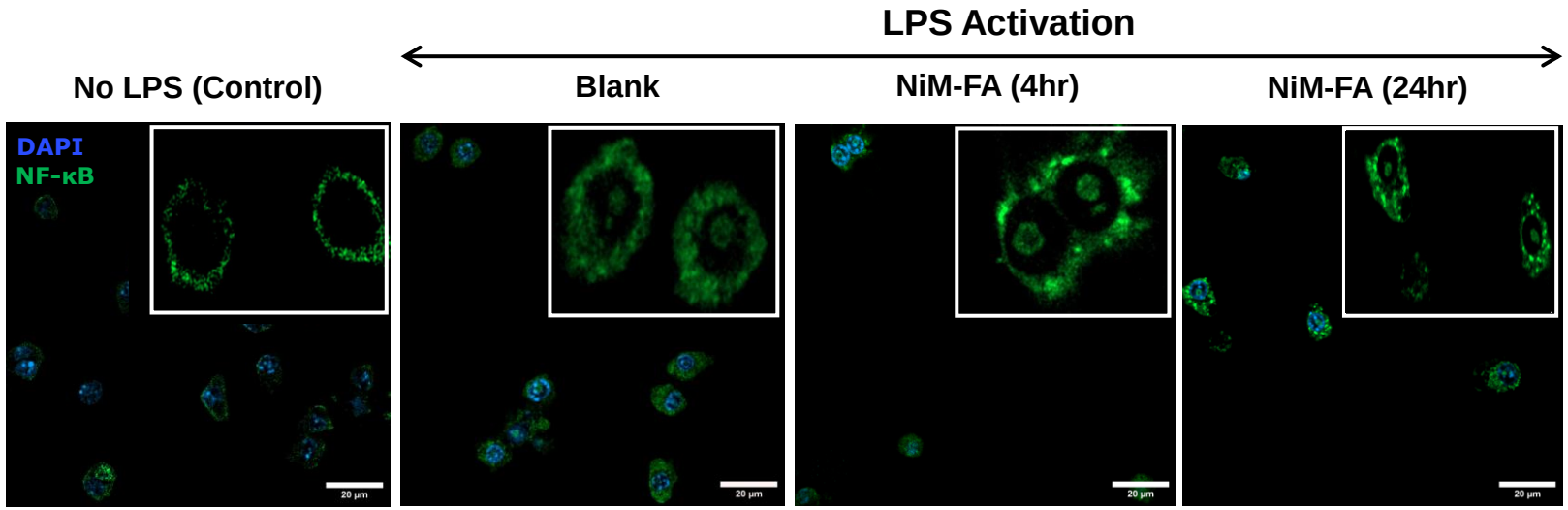


CaZol NiM-FA treatm

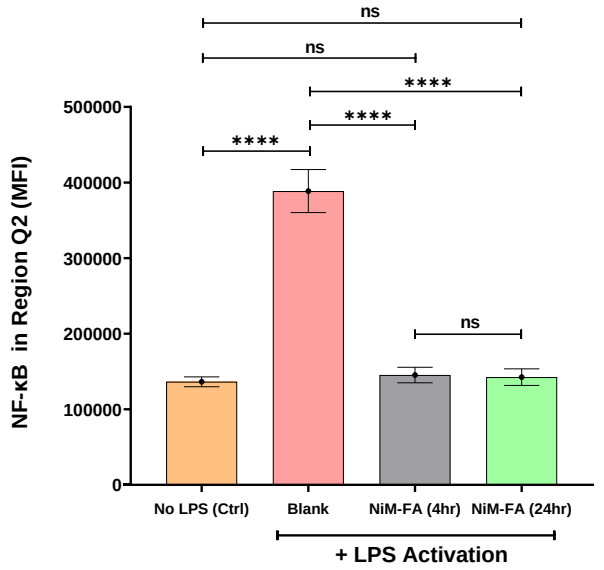


TEM Image showing the control release of NP from NiM

NiM-FA can modulate inflammation in macrophages by suppressing NF-κB

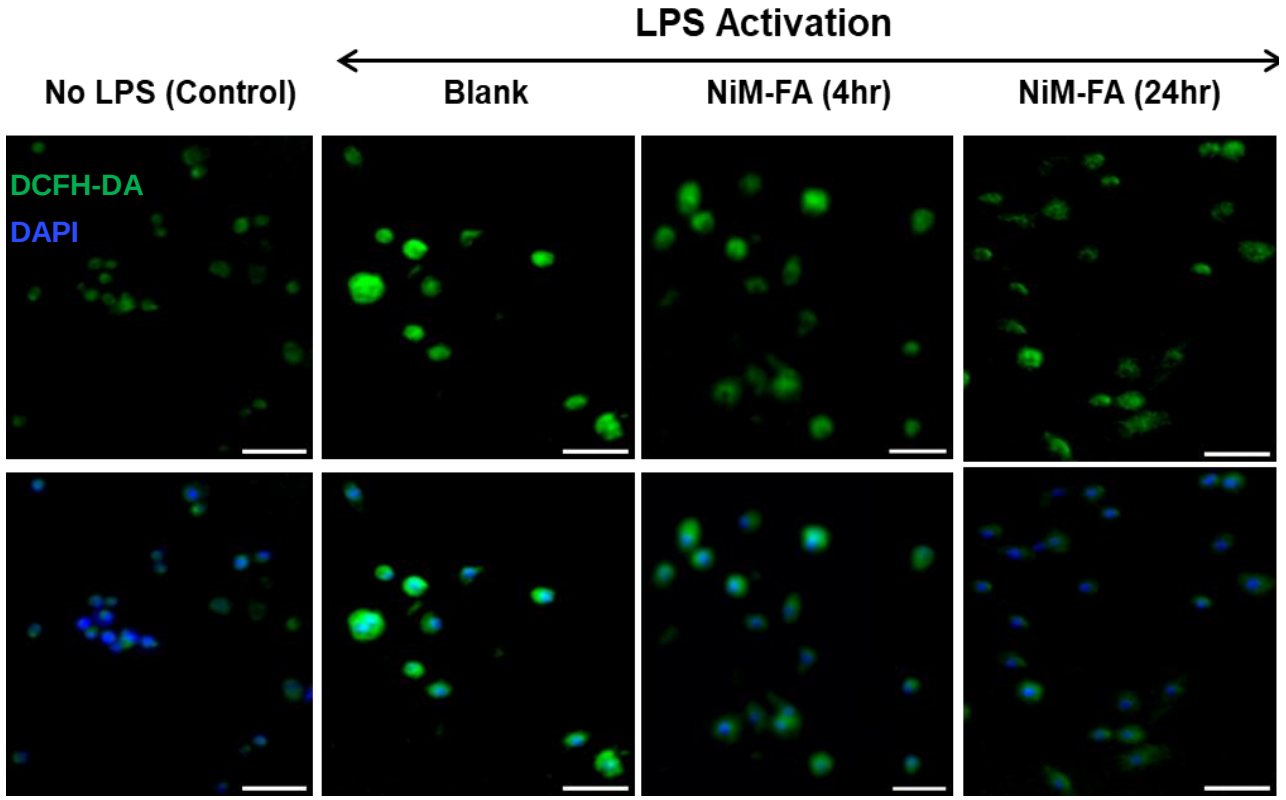


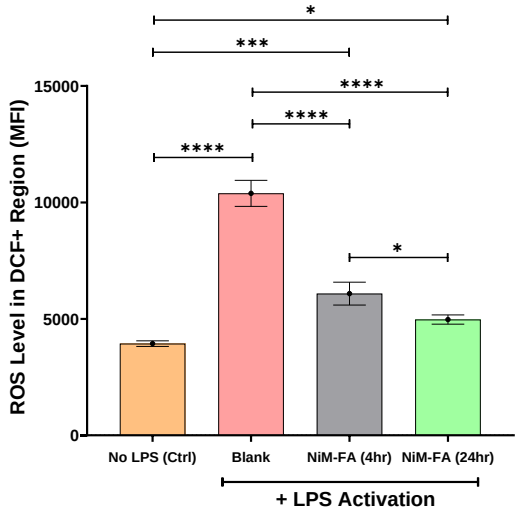
- **NF-κB** is a master regulator of inflammation
- **NF-κB** was activated by LPS stimulation and confirmed by nuclear translocation.

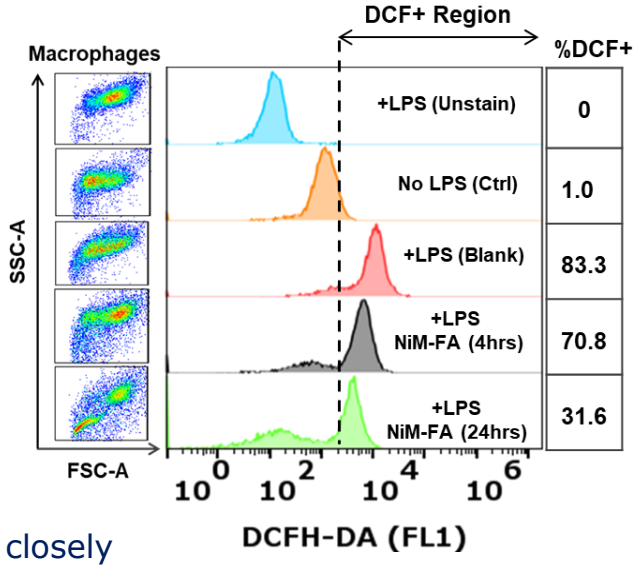


- NiM-FA suppressed **NF-κB,** closely matching the MFI of non-LPS control

NiM-FA shows antioxidant effect by scavenging ROS produced by inflammatory macrophages



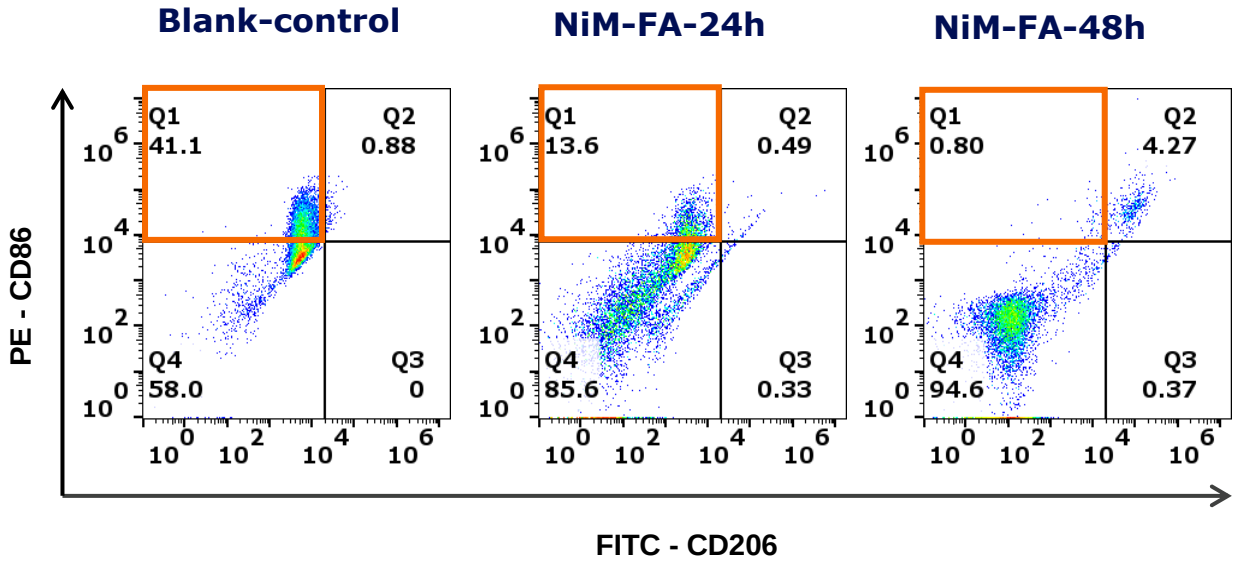


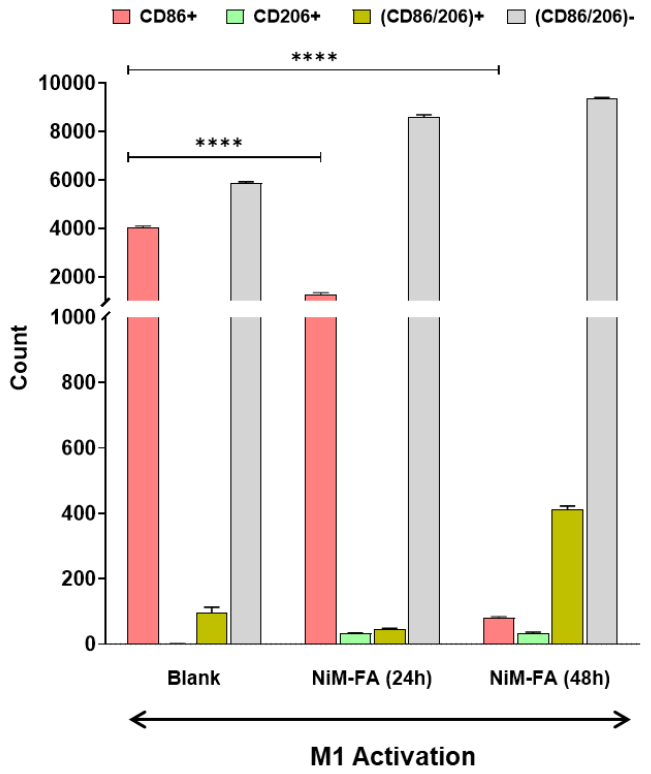


- **ROS** was induced by LPS stimulation and confirmed by DCFH-DA fluorescent probe.
- NiM-FA suppressed **ROS,** closely matching the MFI of non-LPS control

Sagoe, P. Jain, E., et. al., DDTR, 2025

NiM-FA shows anti-inflammatory effect by reprogramming macrophages from inflammatory state





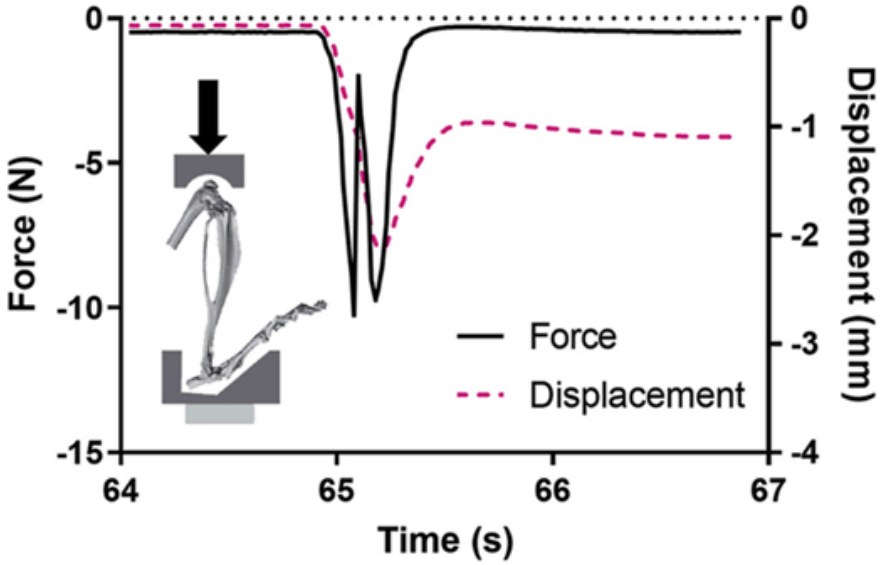
- Cells were polarized to M1 phenotype and further treated with NiM-FA for 24h and 48h.
- **M1:** LPS(100ng/ml) and IFN-γ(10ng/ml)

M1 marker: PE CD86

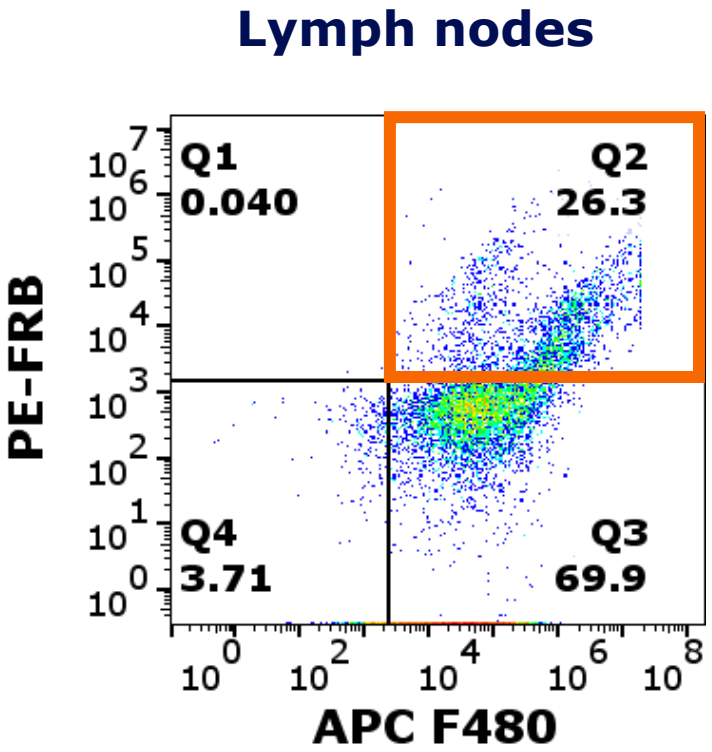
M2 marker: FITC CD206

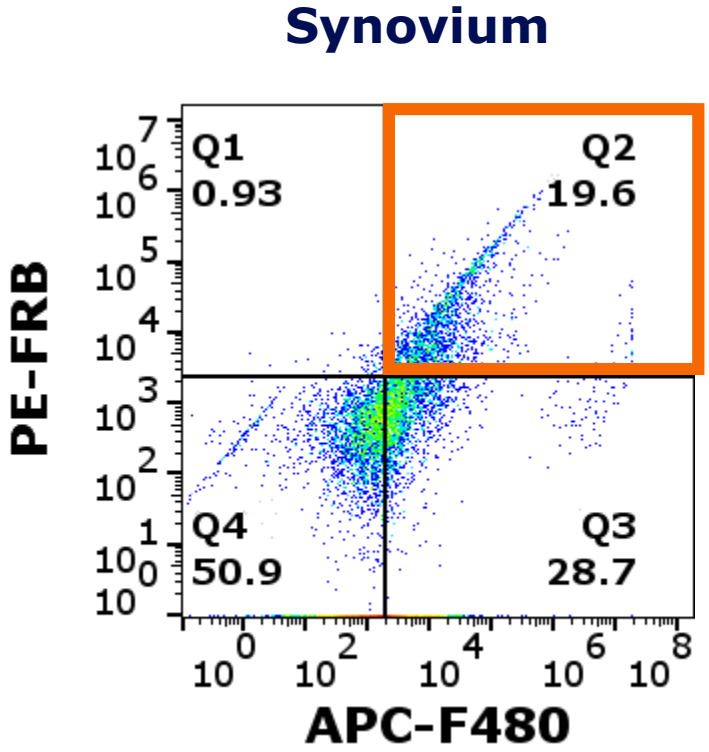
Sagoe, P. Jain, E., et. al., DDTR, 2025

FR-β positive macrophages are present in the synovium and lymph nodes of non-surgical mouse model of OA



Berke, I. M., Jain, E., et al, Osteoarthritis and cartilage, 2021

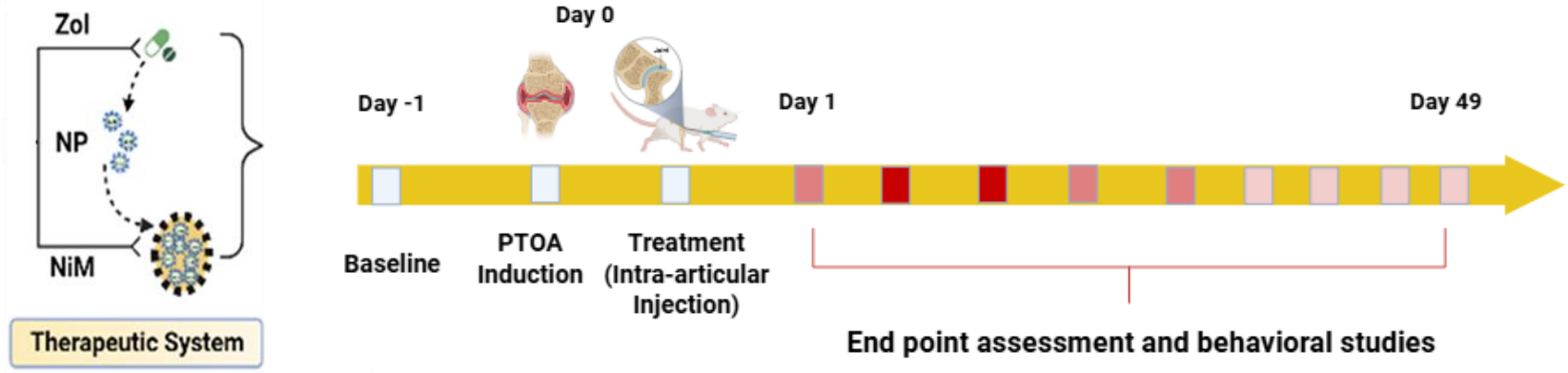




- Knee joints were subjected to 60 cycles of 12 N axial compressive load, leading to:
- ACL rupture
- Osteophyte formation
- Early synovial inflammation

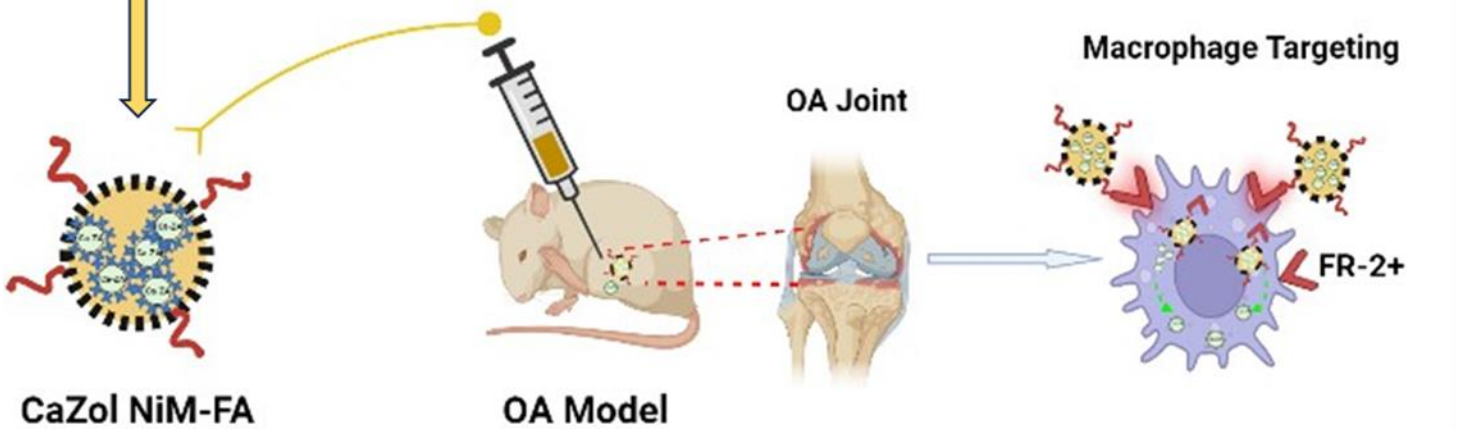
Sagoe, P. Jain, E., et. al., Unpublished data

Targeted delivery of CaZol NiM-FA to FR-β positive macrophages in non-surgical mouse model of OA



End-point Studies

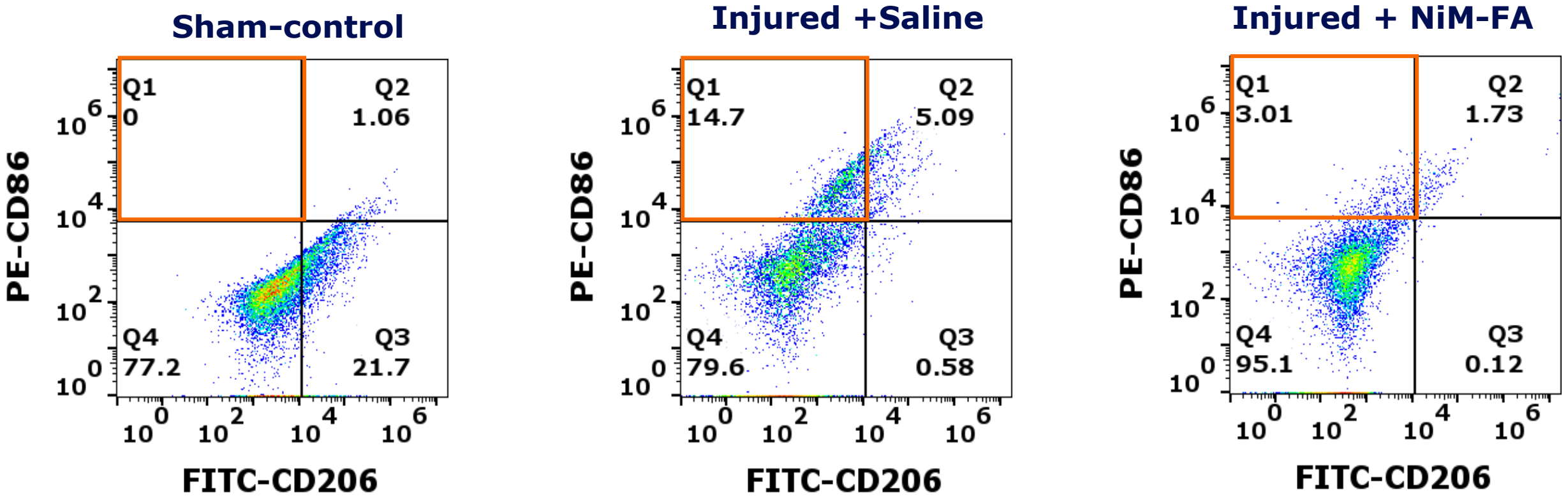
- Inflammation
- Pain
- Gait



Modeling the residence time of Zol when delivered by NiM shows a joint residence time of 49 days.
Courtesy Dr. Lori Setton
Washington University in St. Louis

Sagoe, P. Jain, E., et. al., Unpublished data

NiM-FA shows anti-inflammatory effect by reprogramming macrophages in the synovium of PTOA mice from inflammatory state

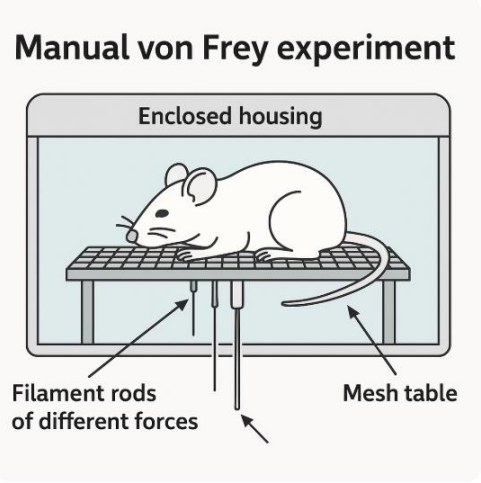


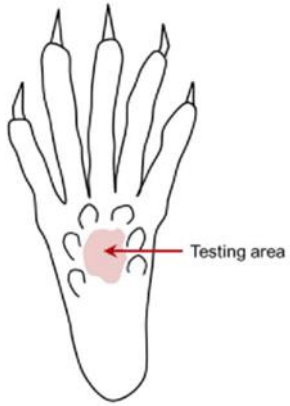
M1 marker: PE CD86

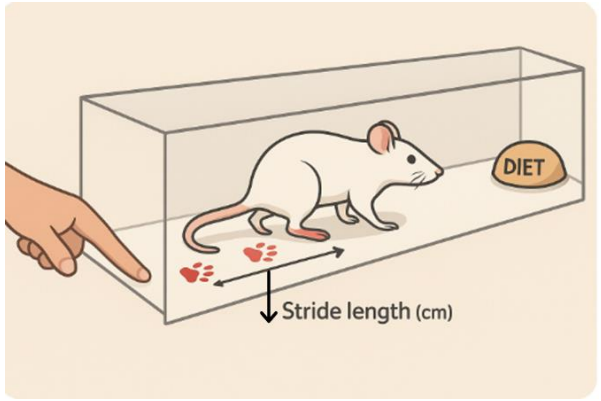
M2 marker: FITC CD206

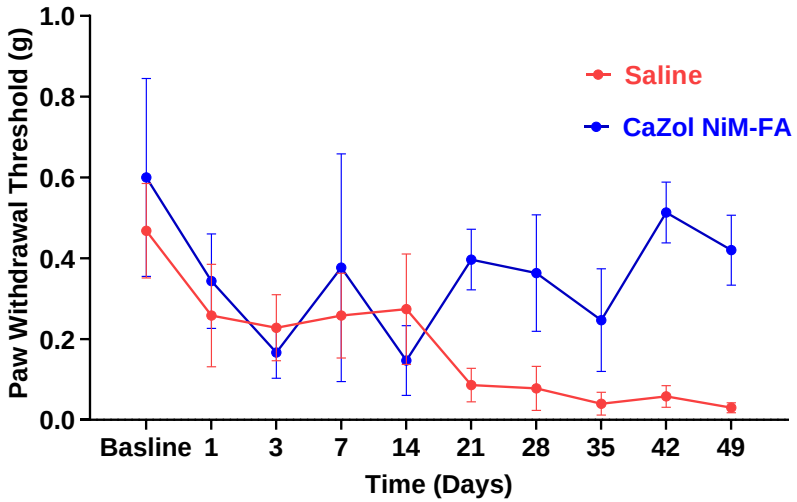
Sagoe, P. Jain, E., et. al., Unpublished data

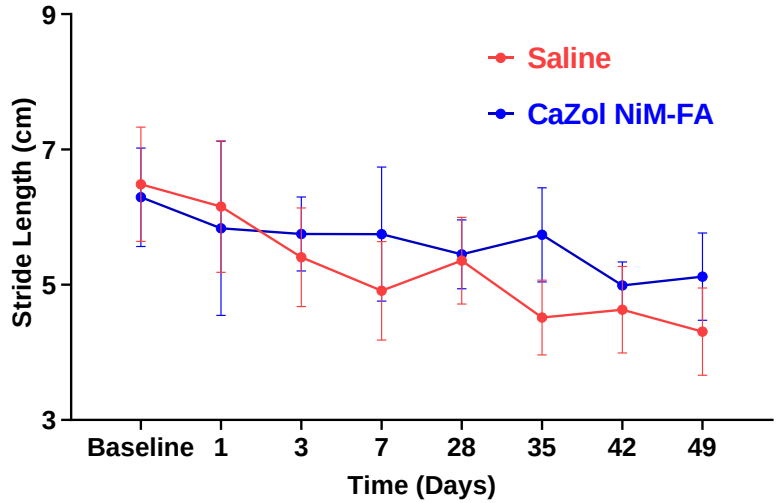
Targeted delivery of NiM in a non-surgical mouse model of OA alleviates pain and improves gait











Sagoe, P. Jain, E., et. al., Unpublished data

Conclusions

- NiM system fosters sustained delivery of Zol and macrophage targeting ability.
- CaZol NiM shows anti-inflammatory and immunomodulatory characteristics.
- CaZol NiM-FA allowed for continual improvement in gait and pain in mice model of osteoarthritis.
- Sustained release of Zol can eliminate the need for repeated injection.
- CaZol NiM-FA can be used to repurpose Zoledronate an FDA approved drug for osteoarthritis treatment.

Ongoing/Future Studies

- Investigate the therapeutic efficacy of NiM-FA in modifying disease outcome:
  - In larger animal model of PTOA.
  - Upon delivering Zol simultaneously or at a later timepoint after ACL rupture.

Acknowledgement

Current Members

Paul Sagoe (Ph.D.)

Michael Seitz (Ph.D.)

Kaixiang Zhang (Ph.D.)

Jason Bae (MS)

Eric Schaffer (UG)

Bailey Snead (UG)

Maddie Olson (UG)

Emily Gao (UG)

Salatiela Alfredo (UG)

Alumni

Yohely Espiritusanto (MS)

Zining Yang (MS)

Tessa DeCicco (UG)

Amelia Gilbert (UG)

Meghan Elder (UG)

Rohail Alvi (UG)

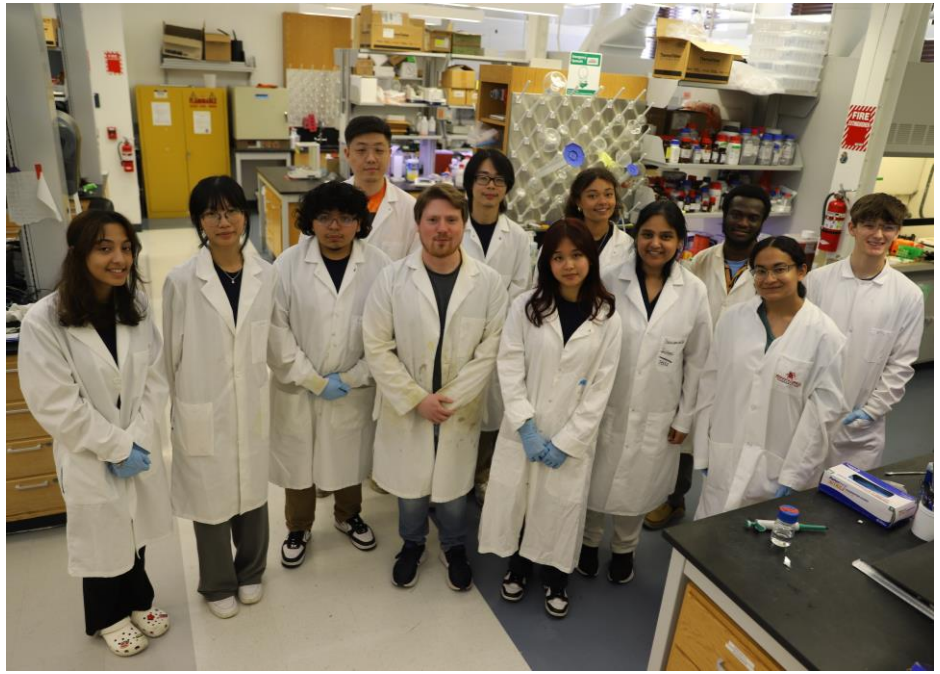
Eduardo Machado (UG)

Isabel Mittal (UG)

Joanna Perera (UG)

Evan Gohil (High school)

Malachi Barksdale (High school)



Jain Lab Members

Dr. Era Jain (PI)

Institute for Veterans & Military Families

JPMorgan Chase & Co., Founding Partner

Syracuse University BioInspired Institute

Supplementary Slides

Synthesis of calcium zoledronate nanoparticle(CaZol NP) by reverse emulsion technique

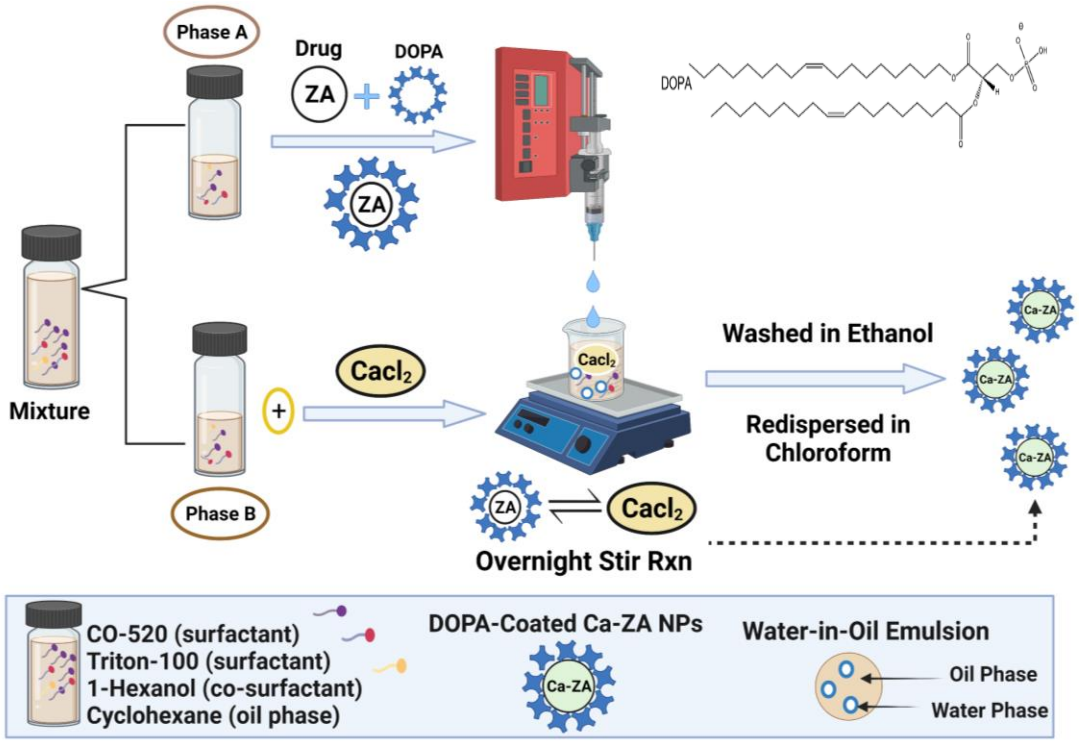
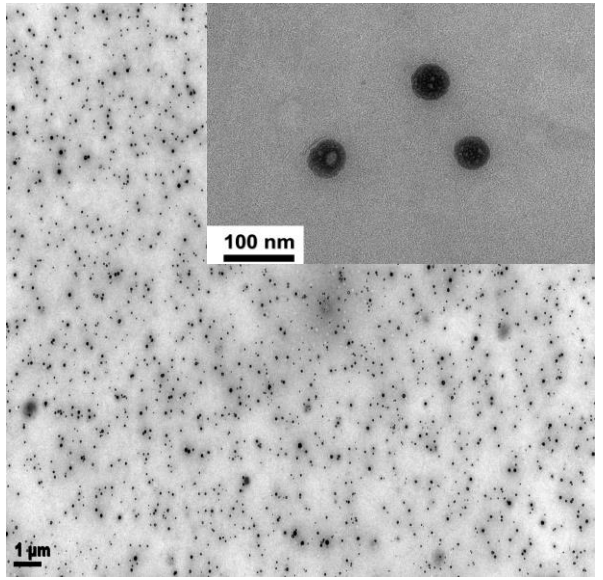
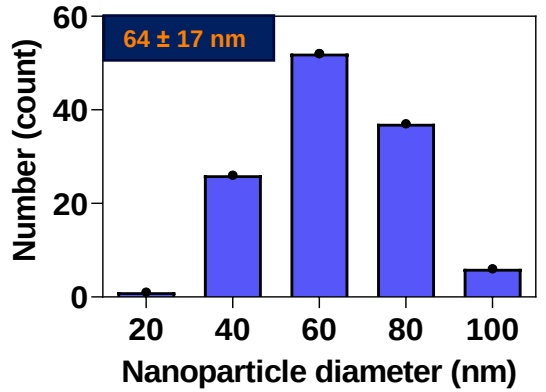


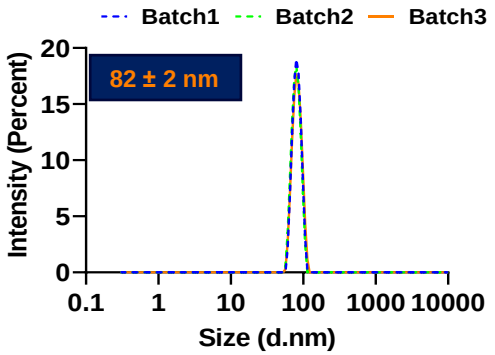
Figure created in Biorender.com



TEM

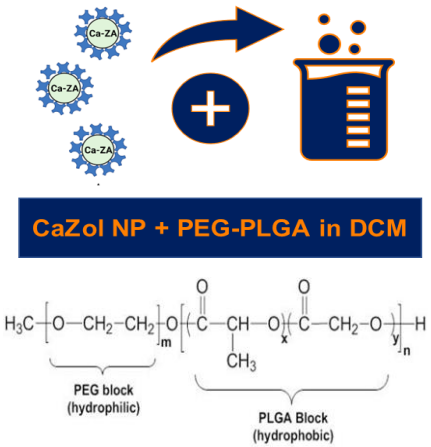


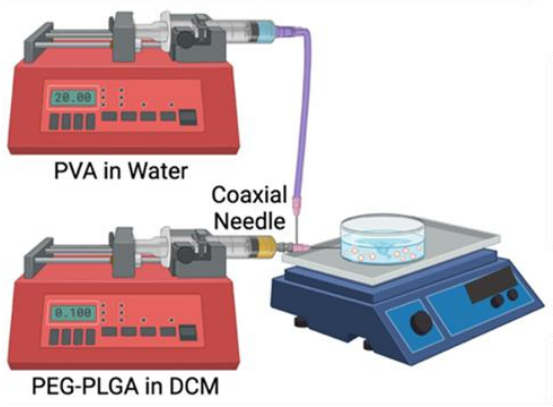
DLS



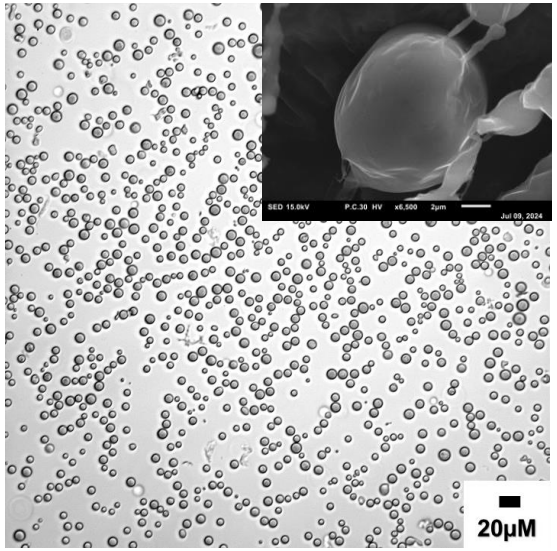
Drug Loading (DL%)	Encapsulation Efficiency (EE%)
4.9 ± 0.1	45.1 ± 0.4

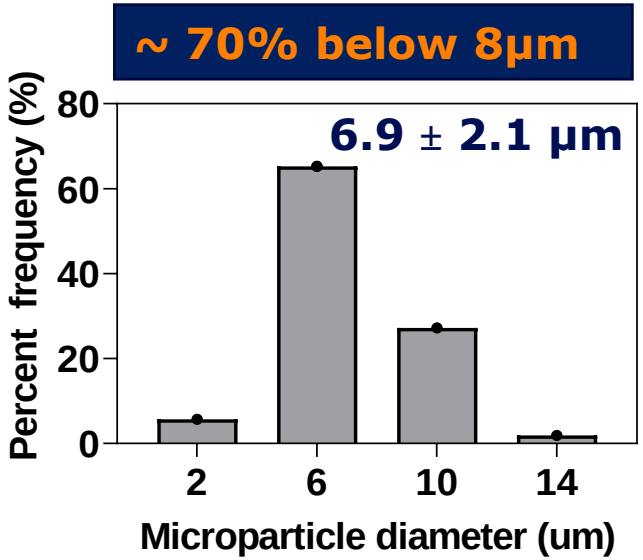
Synthesis of Nanoparticles in Microparticles(NiM) by coaxial flow-phase separation technique



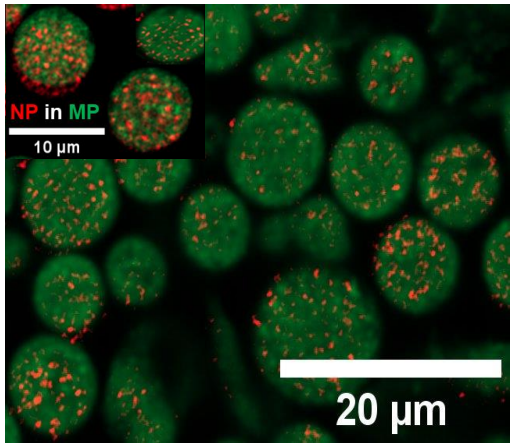


Sagoe, P. Jain, E., et. al., Molecules, 2023

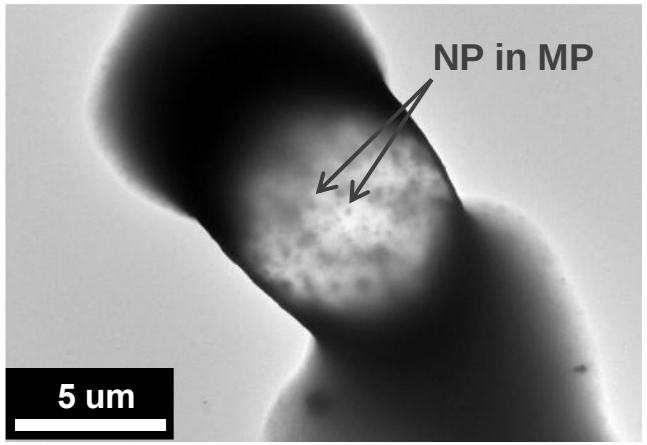




CaZol NiM – NP in MP



TEM Image of CaZol NiM

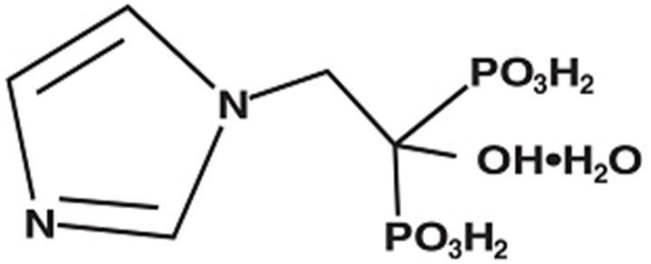


Encapsulation Efficiency (EE%)	55.8 ± 9.0
Drug Loading (DL%)	3.9 ± 0.1
#NP per MP	32 ± 12

Zoledronate (Zol) for macrophage targeting in OA

Zol is a potent 3rd Gen Bisphosphonate (BP) [1]

- High affinity for macrophage

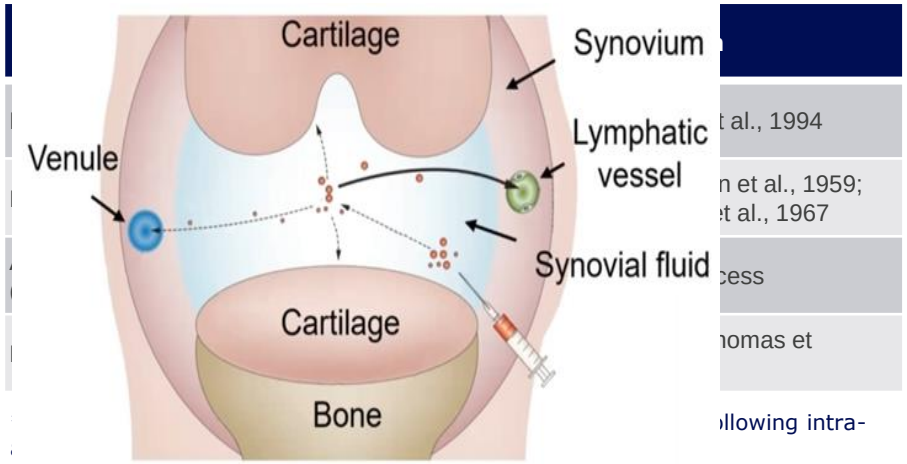


Zoledronate (Zol)

Low bioavailability and short synovial half-life

- Fast clearance from joint space
  - Therapeutic efficacy requires high and repeated dose
    - causes severe adverse effects: **BRONJ**

Kinetics of drug transport in the joint [2]

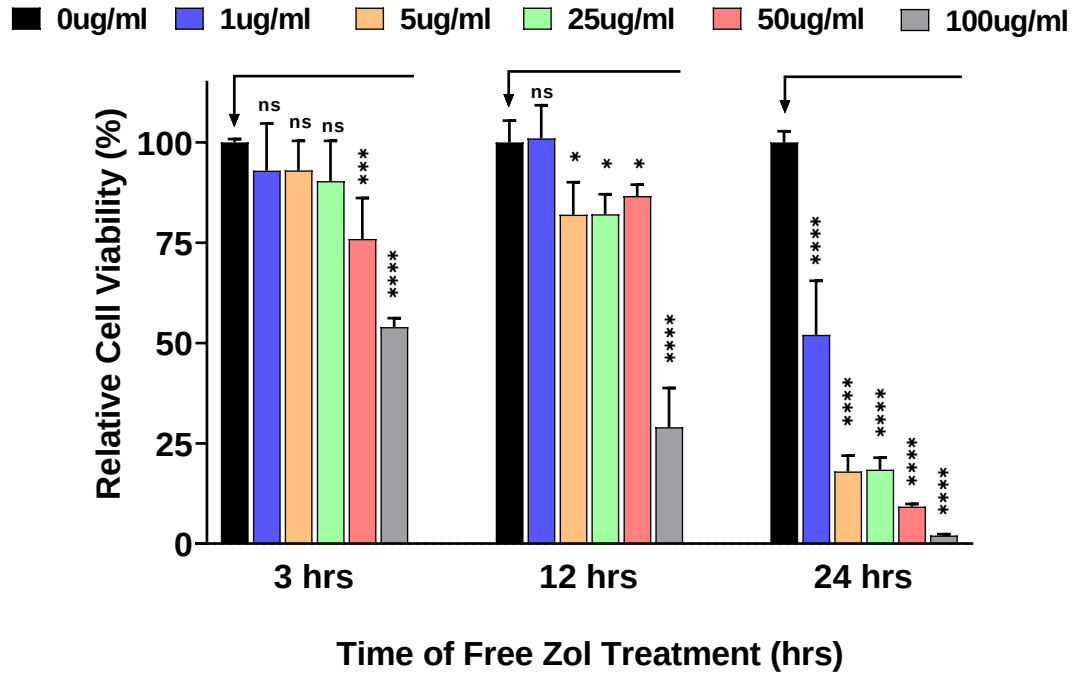


Entry and exit of molecules from joint is size dependent [2,3]

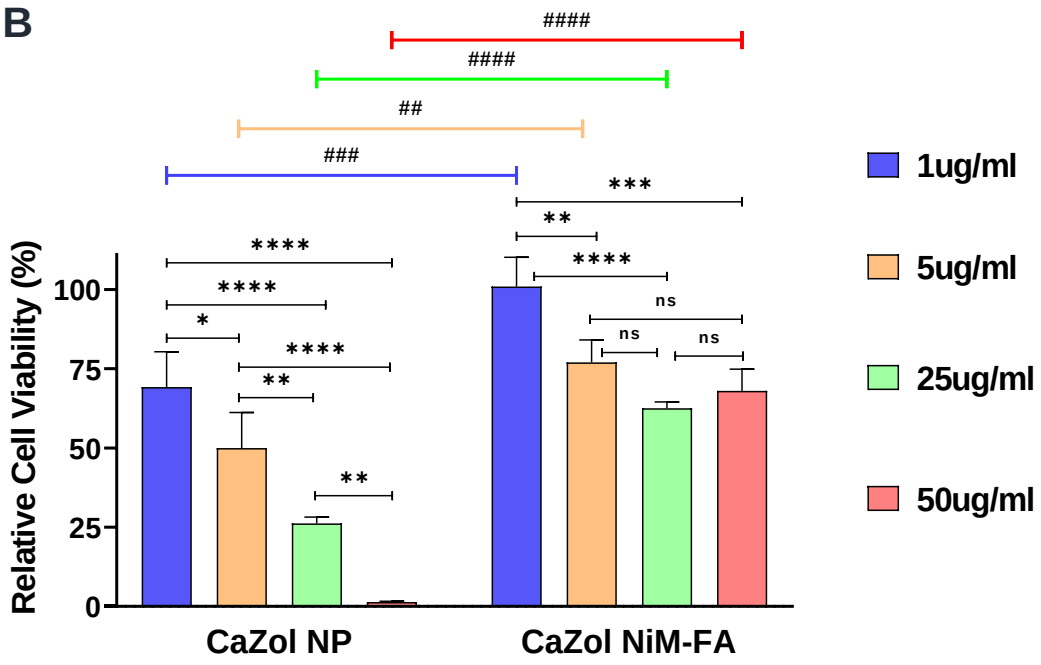
Small molecule drugs like free Zol are cleared faster than biomolecules or drug delivery systems of big size

1. Rogers, T. L., & Holen, I., (J. Transl. Med). 2011
2. Evans, C. H., Kraus, V. B., & Setton, L. A., (Nat Rev Rheumatol). 2014
3. Gao, J., Xia, Z., Mary, H. B., Joseph, J., Luo, J. N., & Joshi, N., (TIPS). 2022

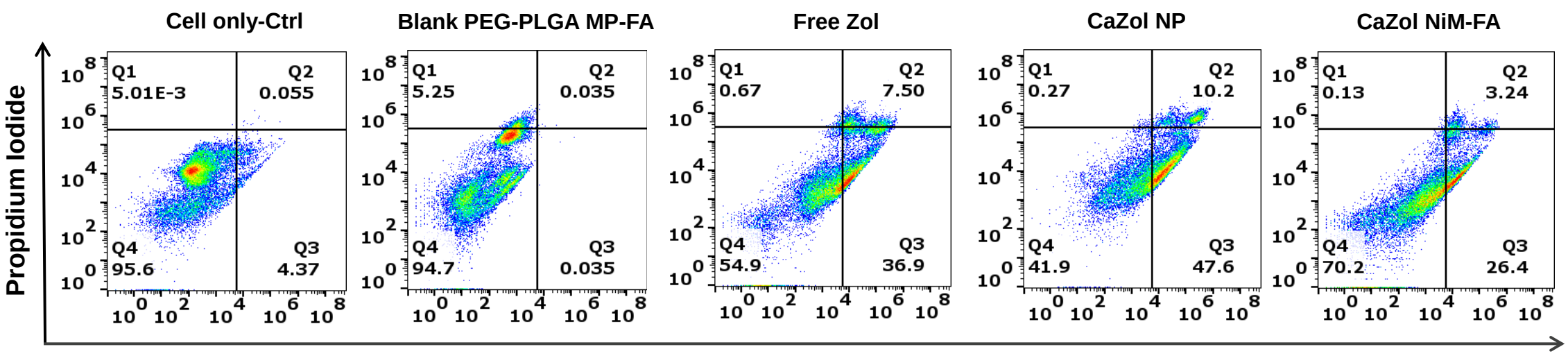
A


0ug/ml
1ug/ml
5ug/ml
25ug/ml
50ug/ml
100ug/ml
Relative Cell Viability (%)
Time of Free Zol Treatment (hrs)
3 hrs
12 hrs
24 hrs

B

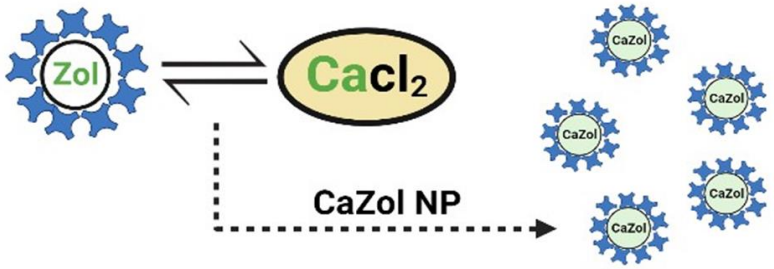

1ug/ml
5ug/ml
25ug/ml
50ug/ml
Relative Cell Viability (%)
CaZol NP
CaZol NiM-FA

C


Cell only-Ctrl
Blank PEG-PLGA MP-FA
Free Zol
CaZol NP
CaZol NiM-FA
Propidium Iodide
FITC - Annexin-V
Q1 5.01E-3
Q2 0.055
Q4 95.6
Q3 4.37
Q1 5.25
Q2 0.035
Q4 94.7
Q3 0.035
Q1 0.67
Q2 7.50
Q4 54.9
Q3 36.9
Q1 0.27
Q2 10.2
Q4 41.9
Q3 47.6
Q1 0.13
Q2 3.24
Q4 70.2
Q3 26.4

Macrophage targeting intra-articular delivery system for osteoarthritis therapy

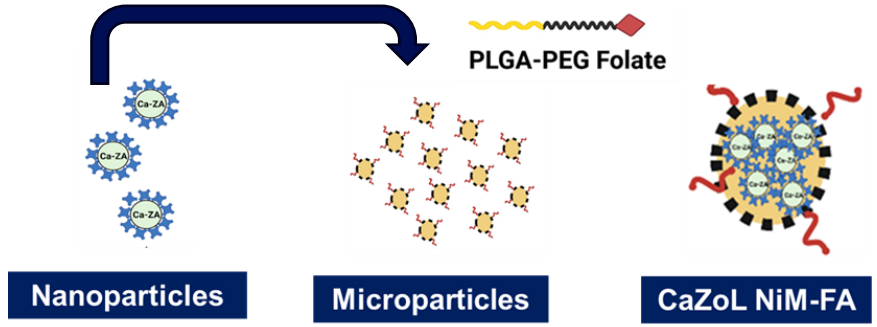
CaZol NP improves Zol bioavailability

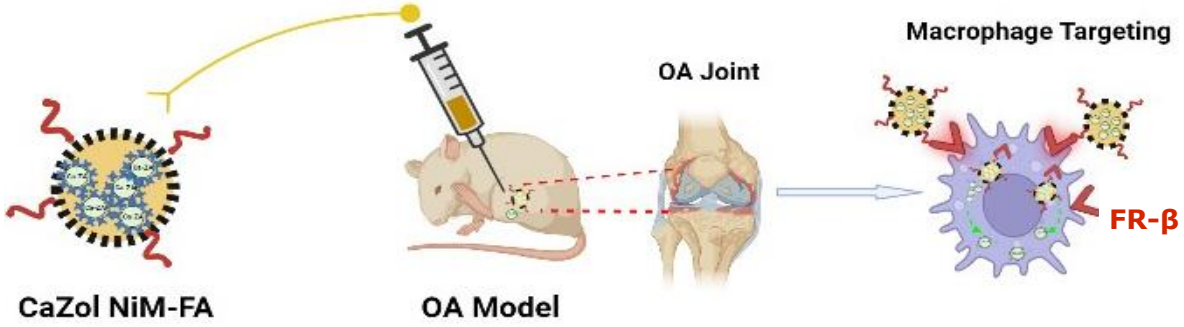


NP in MP

NiM-System

PEG-PLGA-Folate is FDA approved, biocompatible, and biodegradable





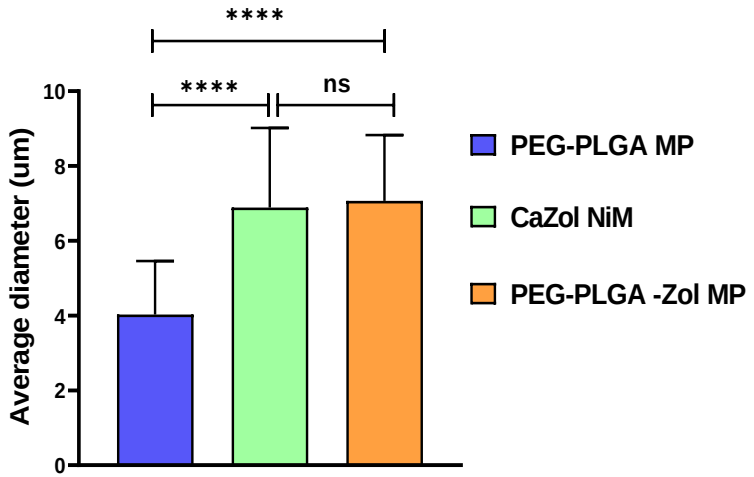
CaZol NiM-FA can:

- Limit non-specific targeting of Zol to bone
- Sufficiently survive synovial fluid clearance
- Prolong joint residence time of Zol
- Increase ligand density for specific targeting
- Promote sustain release of Zol
  - Tuning polymer degradation rate

CaZol: Calcium-Zoledronate; NiM: Nanoparticles(NP) in Microparticles(MP); FA: Folic Acid

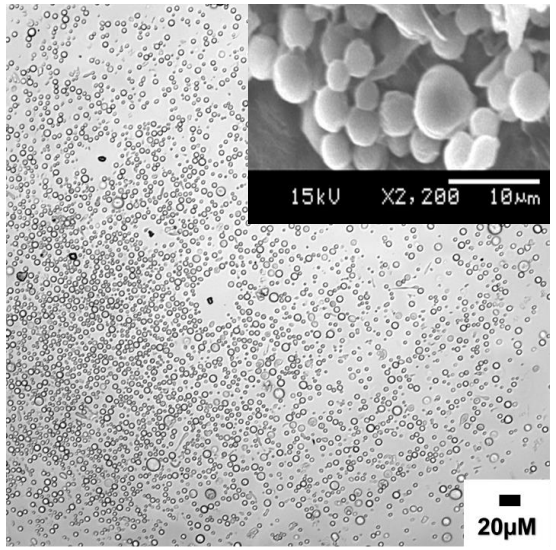
Figure created in Biorender.com

Loading Zol as NiM maintains desirable particle size and promotes higher encapsulation efficiency

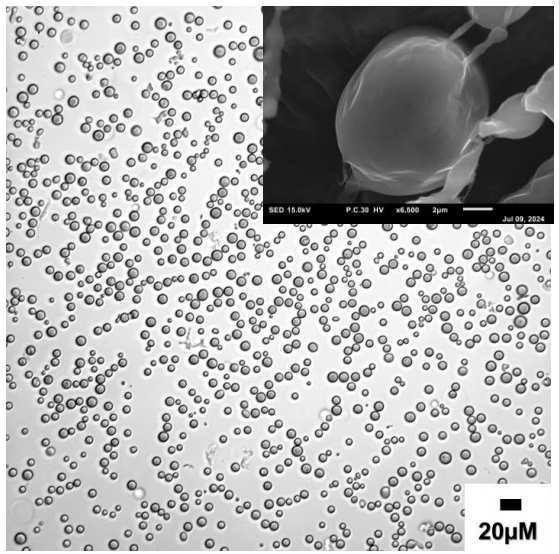


NiM promotes higher Zol encapsulation while maintaining a desirable particle size ideal for uptake by macrophage

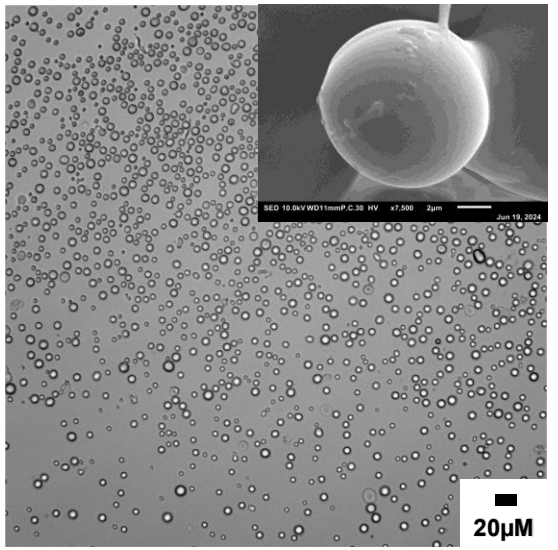
PEG-PLGA MP

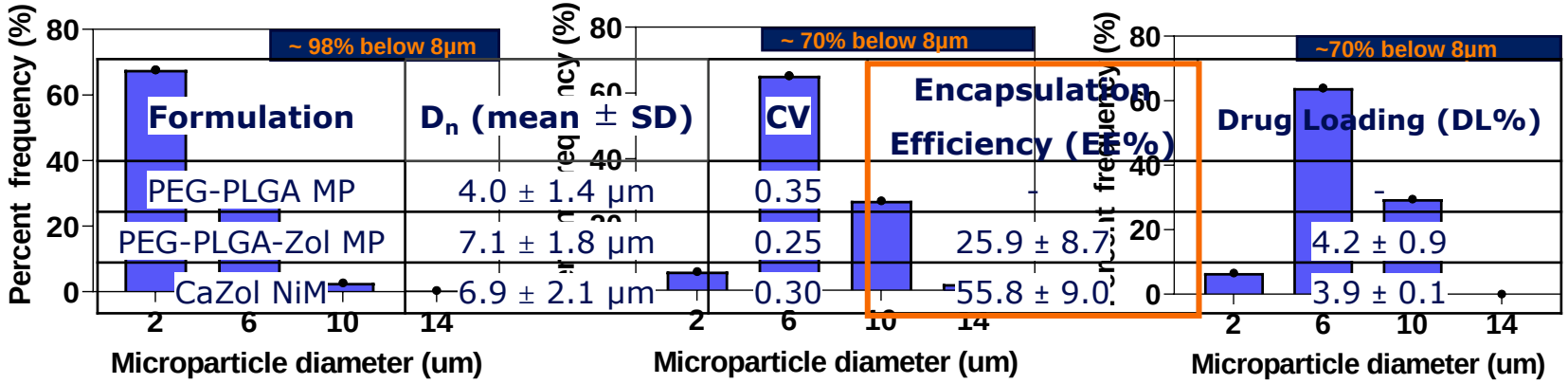


CaZol NiM

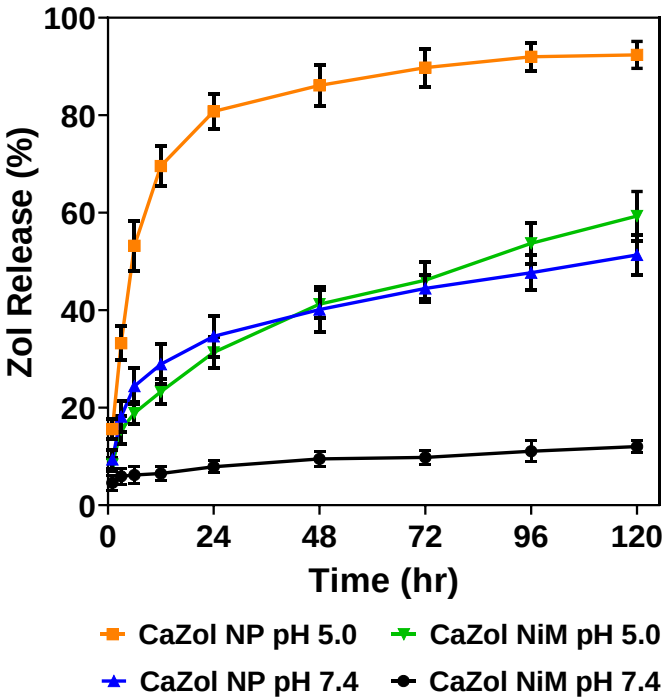


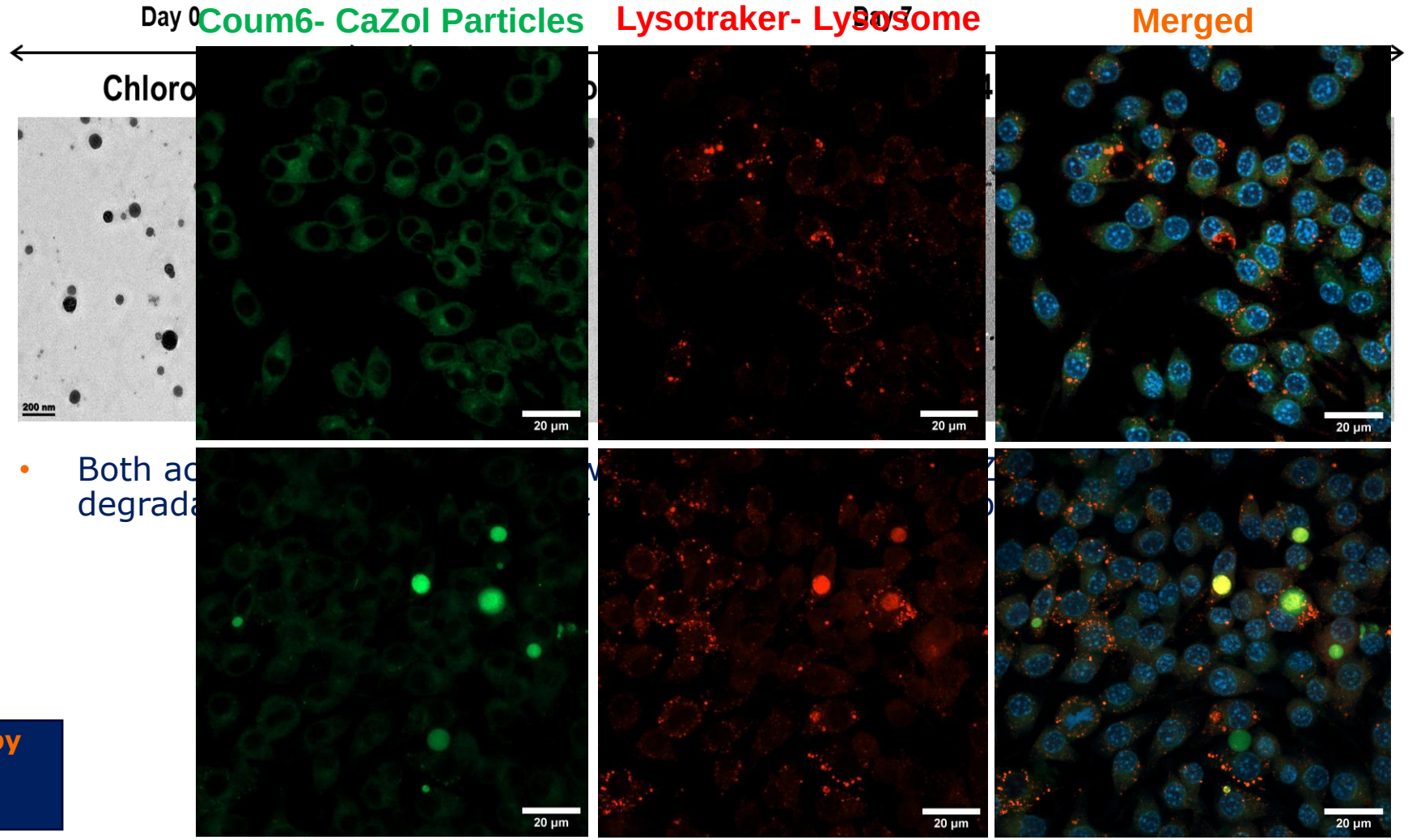
PEG-PLGA MP-Zol





NiM-FA allows for sustained release of pH-responsive CaZol NPs and facilitates lysosomal localization



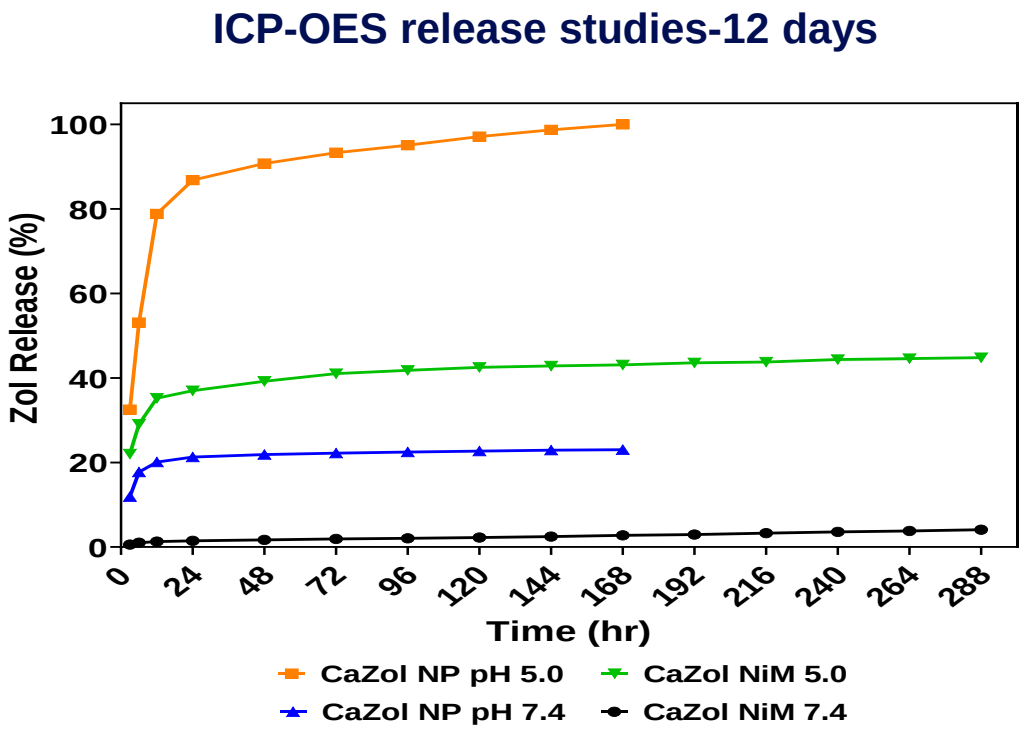


- Both ac
  degrada

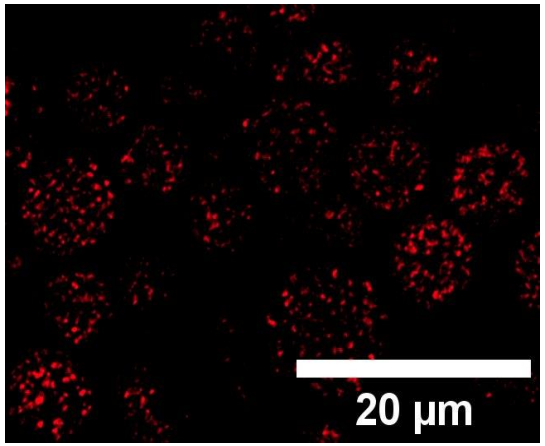
NiM could support intra-articular delivery by minimizing the rapid release of Zol during synovial circulation until cellular uptake

Nucleus stained with DAPI while particles colocalization with lysosomes was confirmed by orange coloration.

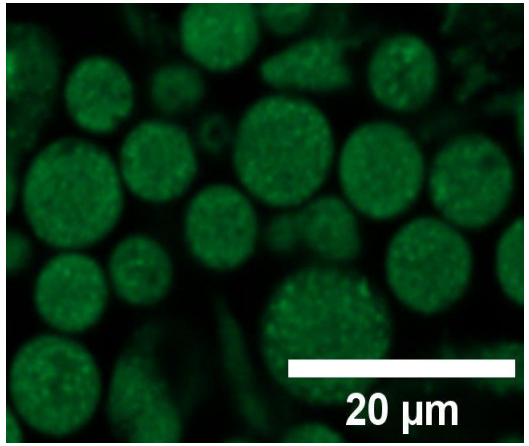
Extended Release Studies via ICP-OES



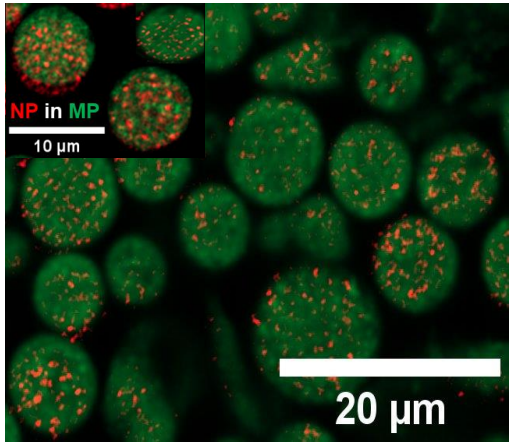
DiD - CaZol NP

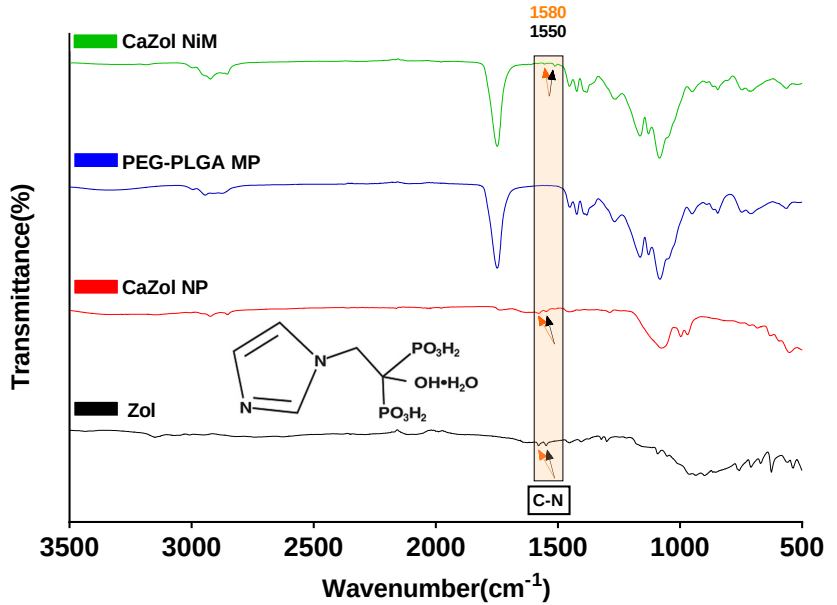


Coum 6 - PEG-PLGA MP

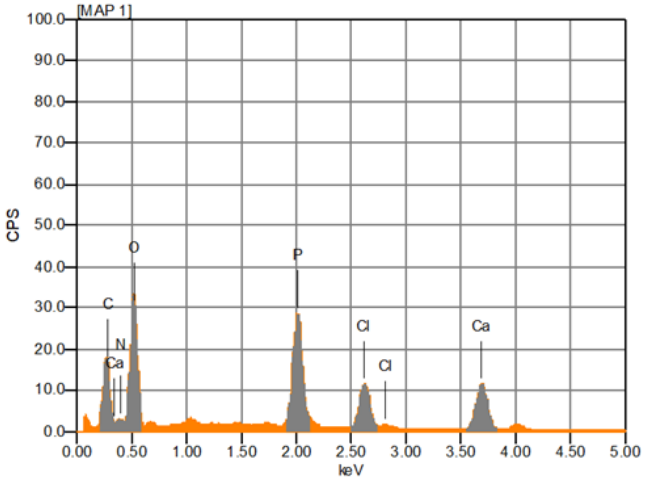


Merge - CaZol NiM

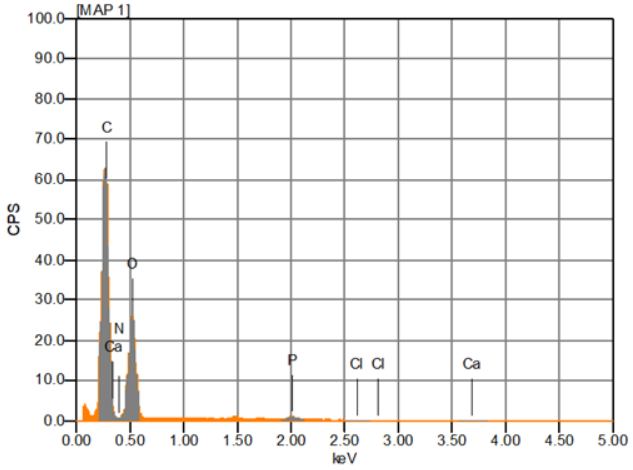




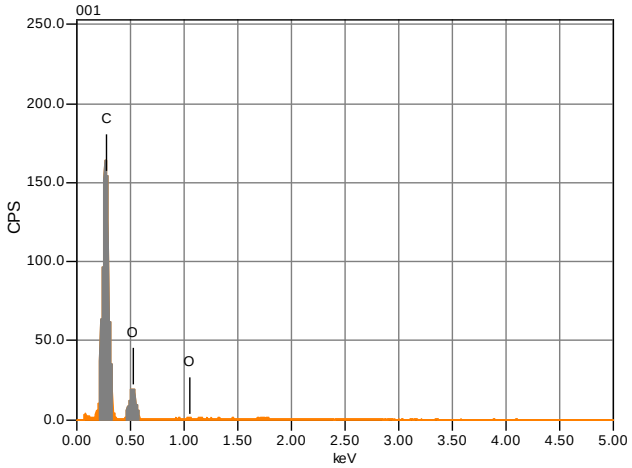
CaZol NP



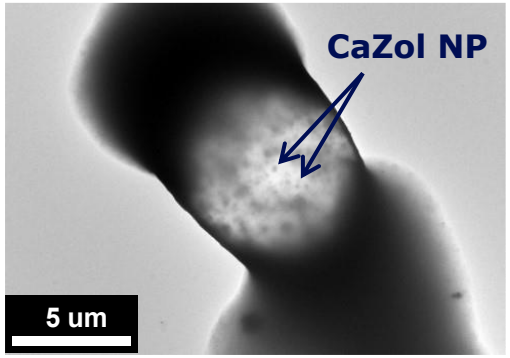
CaZol NiM



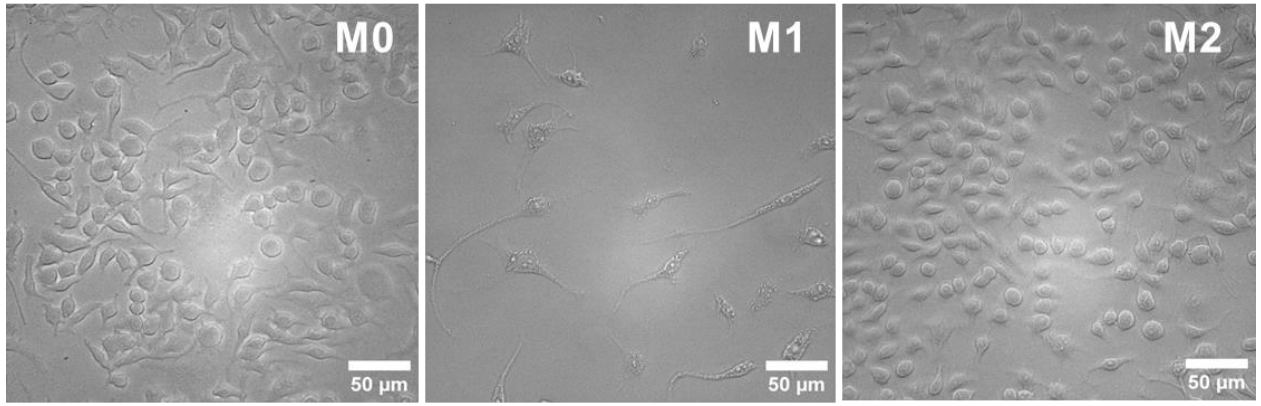
PEG-PLGA MP



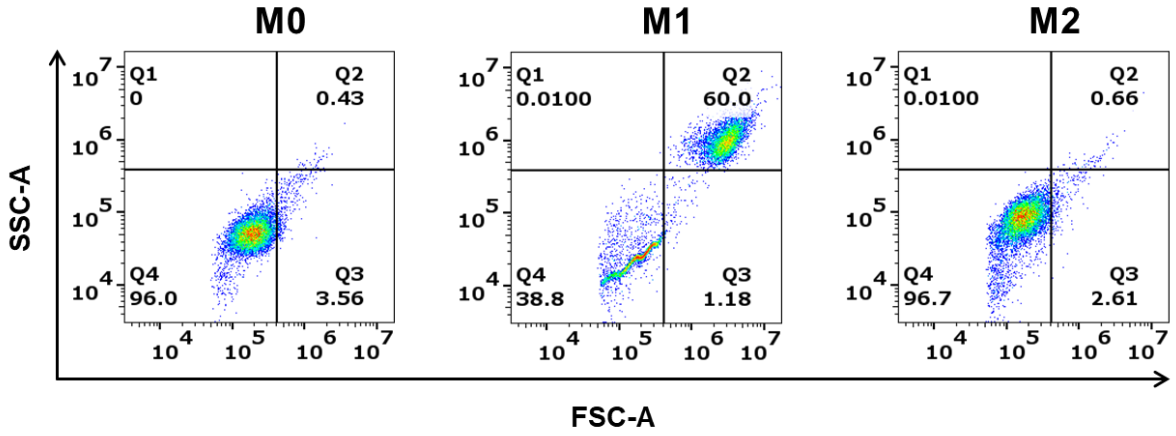
TEM Image of NiM



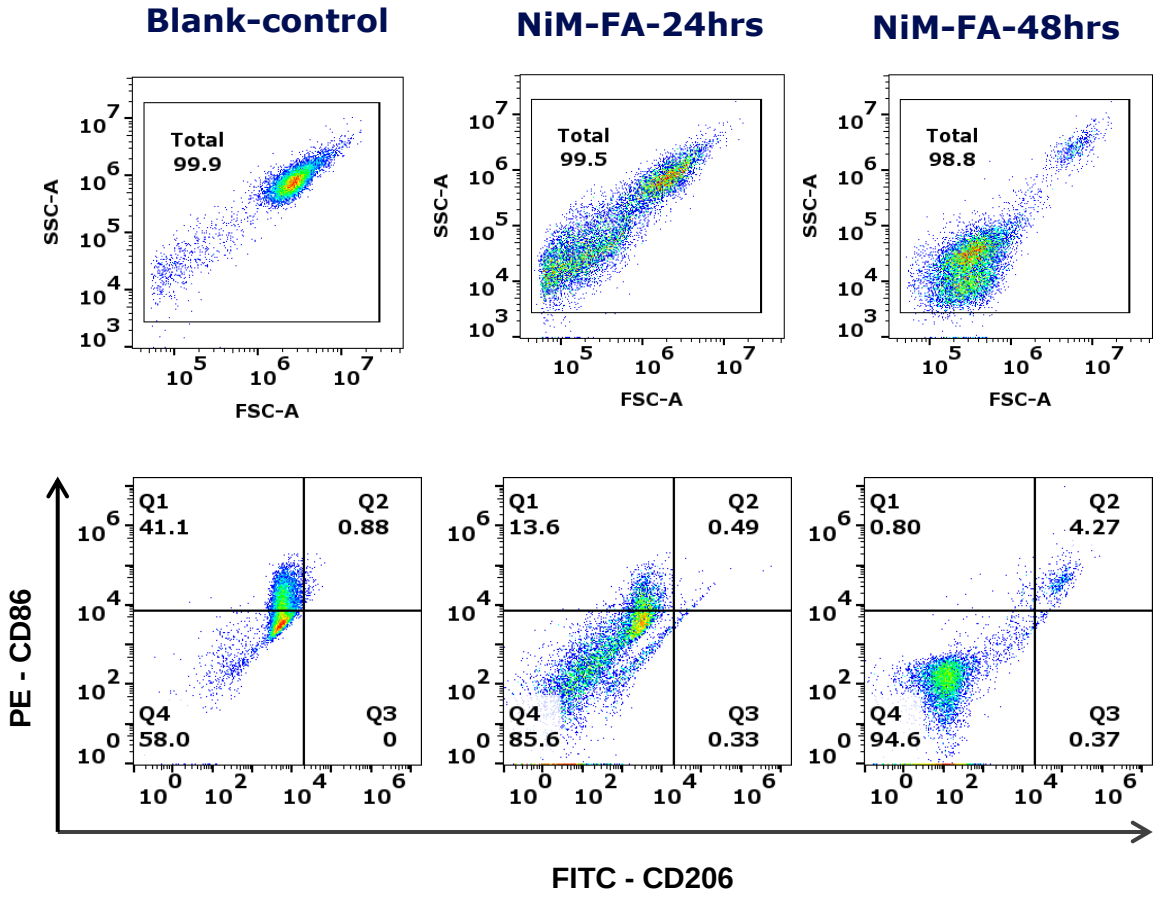
NiM-FA shows anti-inflammatory effect by reprogramming macrophages from pro-inflammatory state



M1 macrophages exhibit oval, flattened and elongated morphology while **M0 and M2** are rounded.

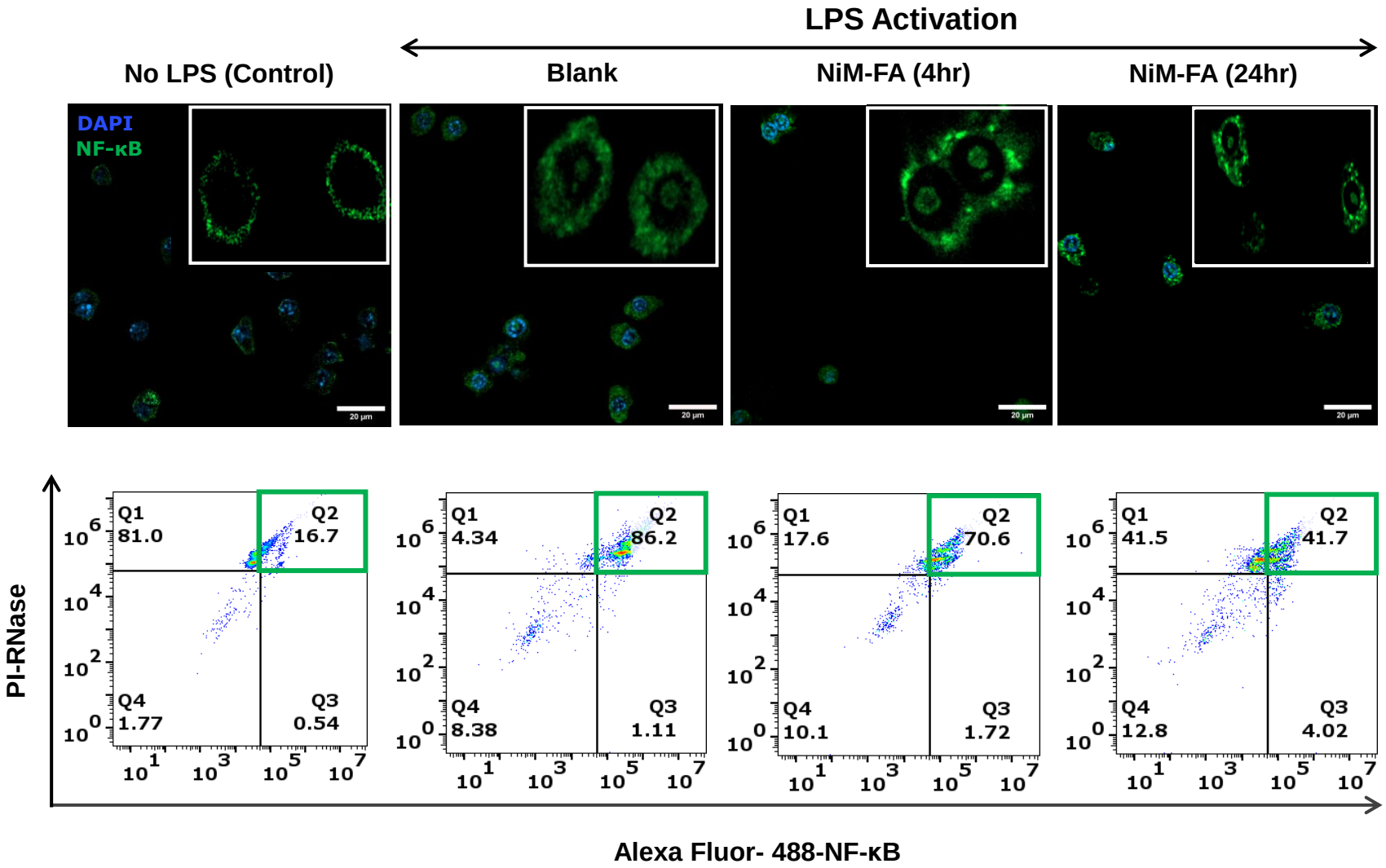


M1 macrophages are complex and larger than **M0 and M2**

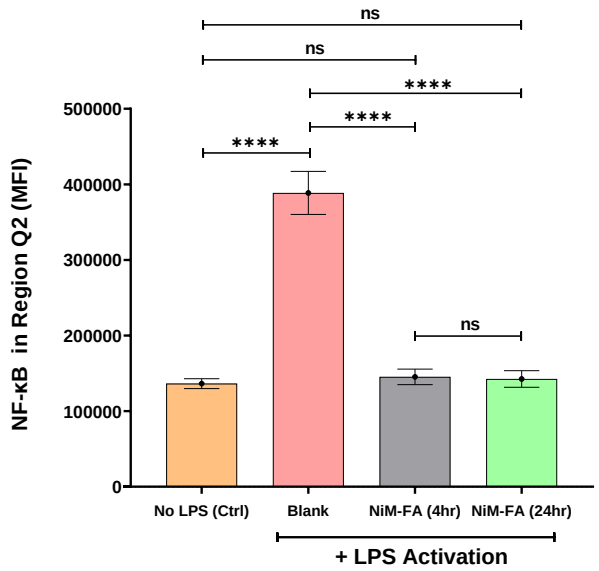


- **M1:** LPS(100ng/ml) and IFN-γ(10ng/ml)
- **M2:** IL-4 and IL-13 (10ng/ml each)

NiM-FA can modulate inflammation in macrophages by suppressing NF-κB

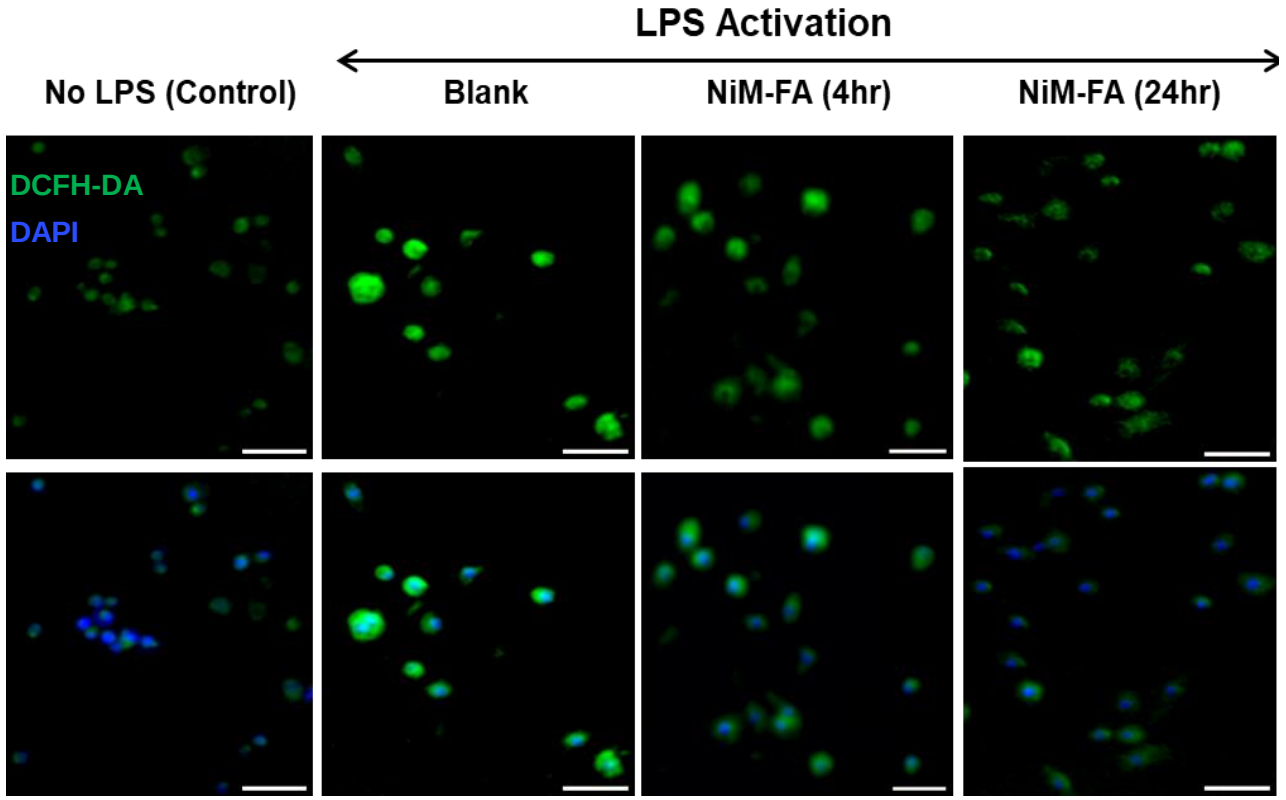


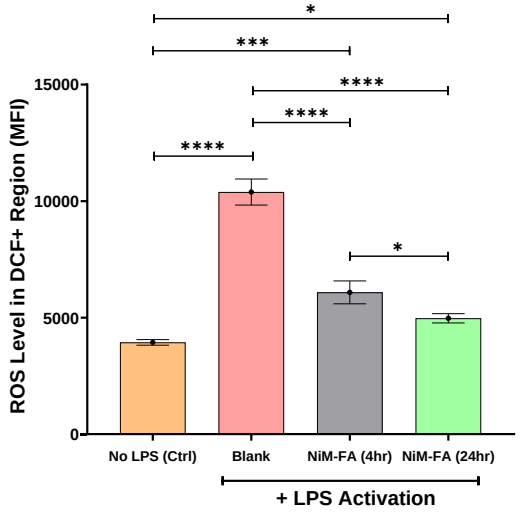
- **NF-κB** is a master regulator of inflammation
- **NF-κB** was activated by LPS stimulation and confirmed by nuclear translocation in **Q2**.

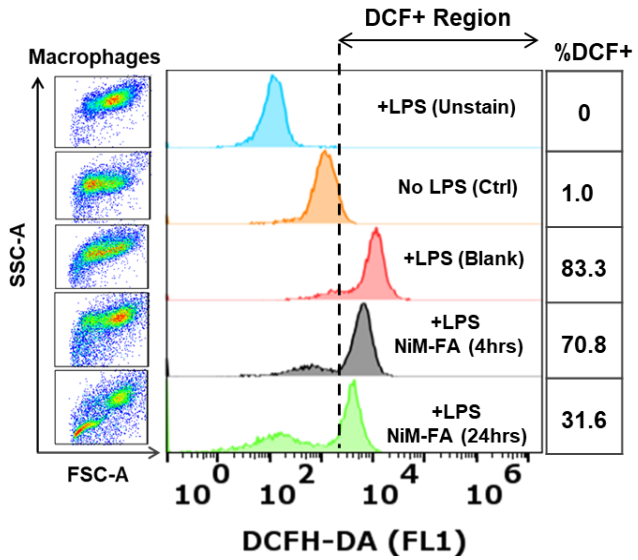


- NiM-FA suppressed **NF-κB,** closely matching the MFI of non-LPS control

NiM-FA shows antioxidant effect by scavenging ROS produced by inflammatory macrophages

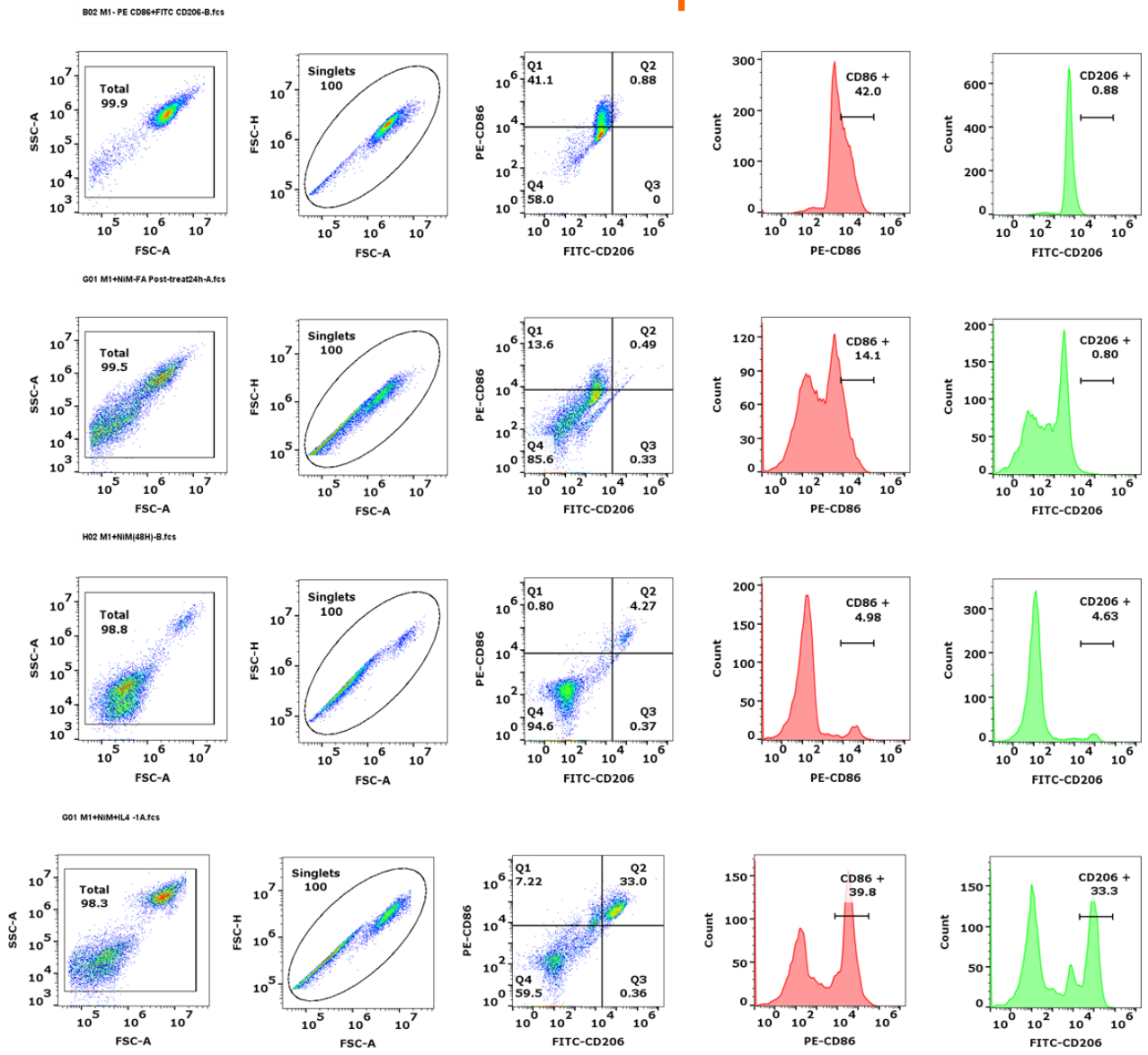






- **ROS** was induced by LPS stimulation and confirmed by DCFH-DA fluorescent probe.

NiM-FA and IL-4 co-delivery fosters M1 macrophage repolarization towards M2



Blank – No NiM-FA (M1 Activation Only)

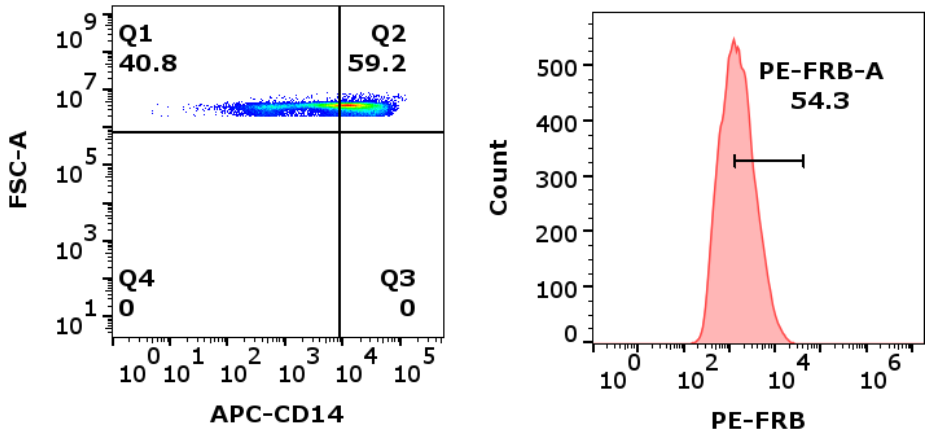
NiM-FA – 24 hrs (Post M1 Activation)

NiM-FA – 48 hrs (Post M1 Activation)

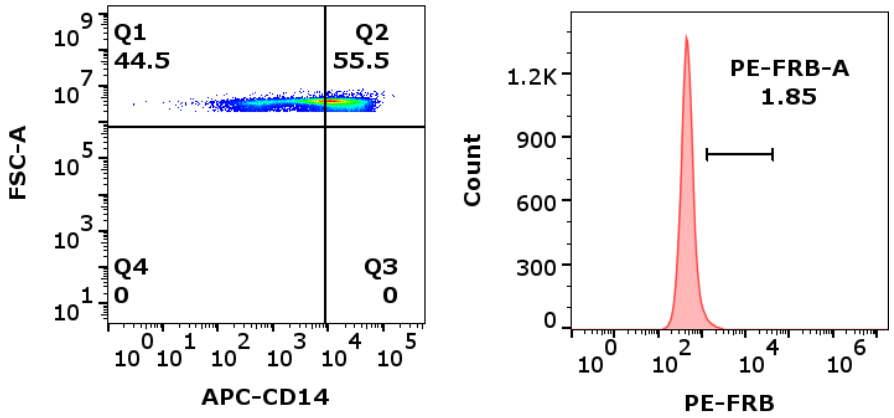
NiM-FA – 24 hrs+ IL-4 24 hrs (Post M1 Activation)

Monocytes from PBMCs express FR-β

Primary



PE- Isotype Control



CaZol NP particle concentration

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Total Number Concentration (particles/ml)	1.373E+13	2.153E+12	15.68	1.145E+13	1.572E+13
Particle Concentration Peak 1 (particles/ml)	1.373E+13	2.153E+12	15.68	1.145E+13	1.572E+13
Size Concentration Peak 1	80.45	0	0	80.45	80.45
D 10 (nm)	75.56	0	0	75.56	75.56
D 50 (nm)	77.73	0	0	77.73	77.73
D 90 (nm)	79.9	0	0	79.9	79.9