



# Scalable Production of Diclofenac Lauryl Ester Prodrug Nanoparticles for Targeted Therapy in Intervertebral Disc Disorders

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## Abstract

Intervertebral disc degeneration (IVDD) and related inflammatory pain conditions require innovative therapeutic approaches for effective management. In this study, a lipidic prodrug of diclofenac, Diclofenac Lauryl Ester (L-DCF), was synthesized and formulated into nanoparticles (Nanofenac-L) using the ethanol injection method followed by microfluidization. The physicochemical characterization of Nanofenac-L confirmed high encapsulation efficiency (%EE > 97%) and sustained drug release, reaching approximately 65% diclofenac acid release within 48 hours. In vitro efficacy studies demonstrated a significant downregulation of pain marker Substance P by Nanofenac-L, whereas COX-2 expression was more effectively reduced by diclofenac sodium (DCF-Na). Antioxidant markers (GPX1, PRDX1, and SOD1) were upregulated in nucleus pulposus cells (NPCs), with Nanofenac-L showing a more pronounced effect on PRDX1. Although preventive treatment showed limited efficacy, the curative treatment regimen demonstrated significant therapeutic potential. These findings suggest that Nanofenac-L is a promising anti-inflammatory, analgesic, and antioxidant candidate for IVDD and other pain-related conditions. Further preclinical and clinical studies are warranted to validate its translational potential.

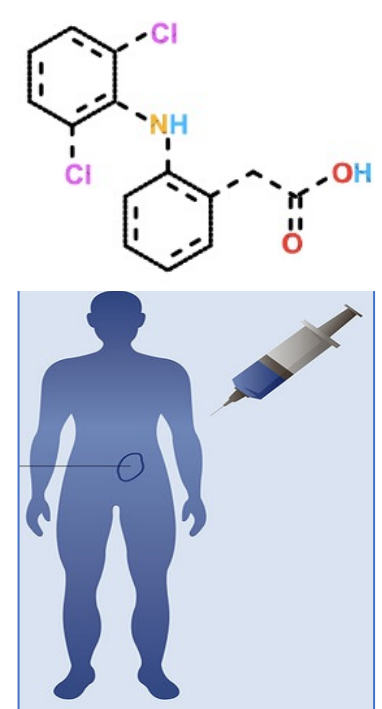
## Introduction

### Diclofenac

Potent Non-Steroidal Anti-Inflammatory Drug (NSAID) used as a painkiller and an anti-rheumatic drug

Diclofenac leads to severe side effects due to undesirable pharmacokinetics, like

- Short half-life, Large volume of distribution
- Time-dependent dosage

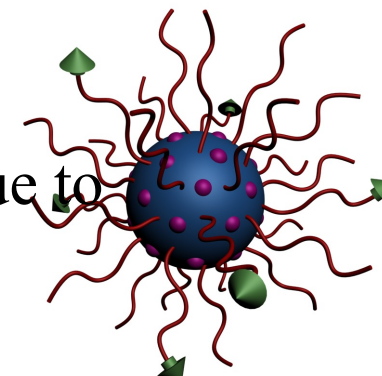


### Nanoparticles of diclofenac

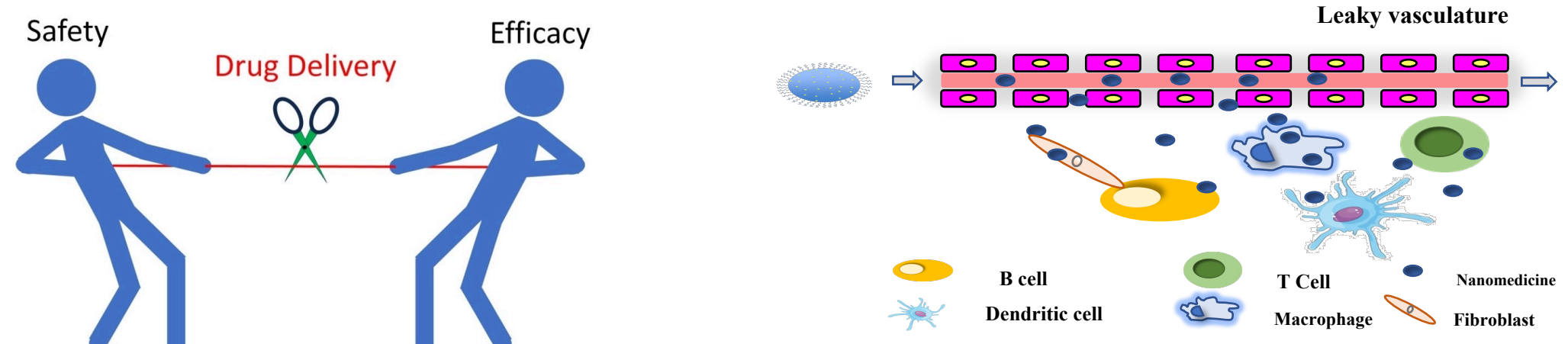
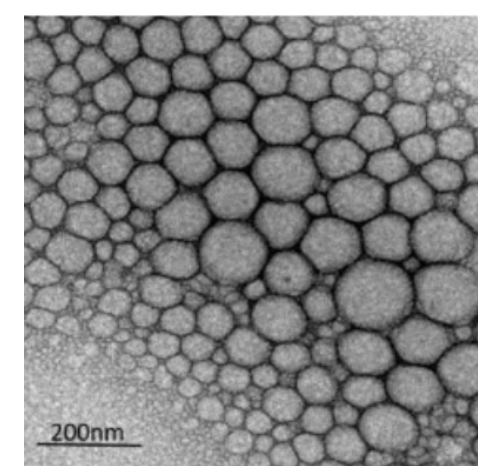
- Lower dose administration
- Limited side effects and Increased efficacy

Nanoparticles lack in rate of clinical translation due to

- Limited drug loading
  - Premature burst release
  - Lacking Scalability
- Prodrugs of diclofenac & lipid polymer
- Scale-up production of Prodrug NPs
  - Long-circulating nanoparticles



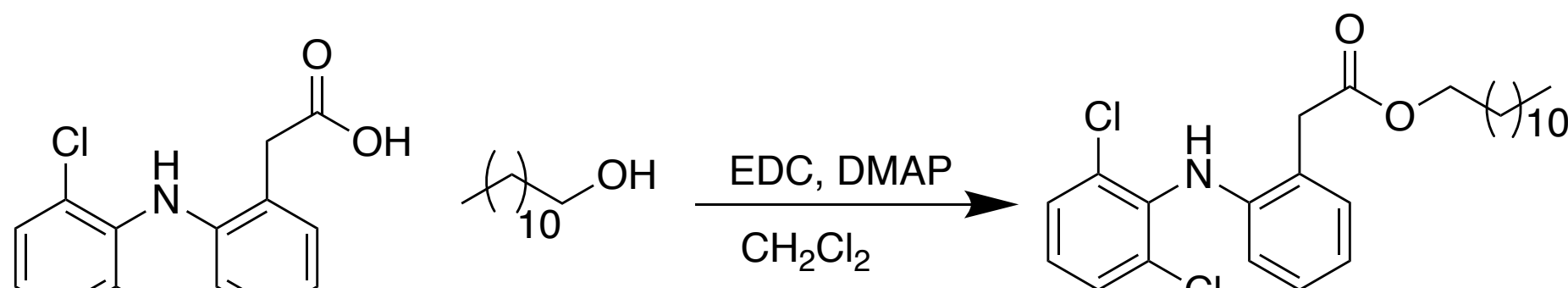
## Objectives



To design stable NPs with higher %EE and stability

To design Safer and efficacious prodrug NPs Extravasation through Leaky Vasculature / EPR Effect

## Synthesis of Prodrug



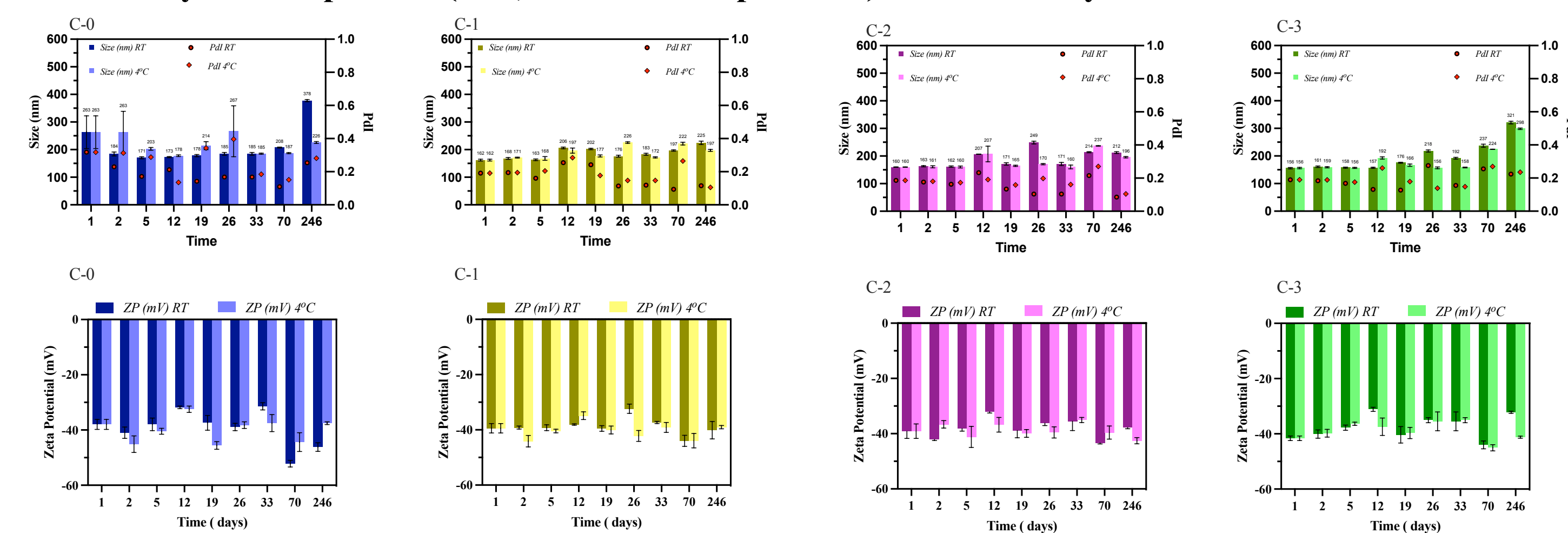
The Prodrug synthesized was structurally characterised by FT-IR, <sup>1</sup>H-NMR, Mass Spectrometry, <sup>13</sup>C-NMR for structural elucidation and confirmation of molecular formula.

## Scale up production of Diclofenac Prodrug Nanoparticles (NPs)



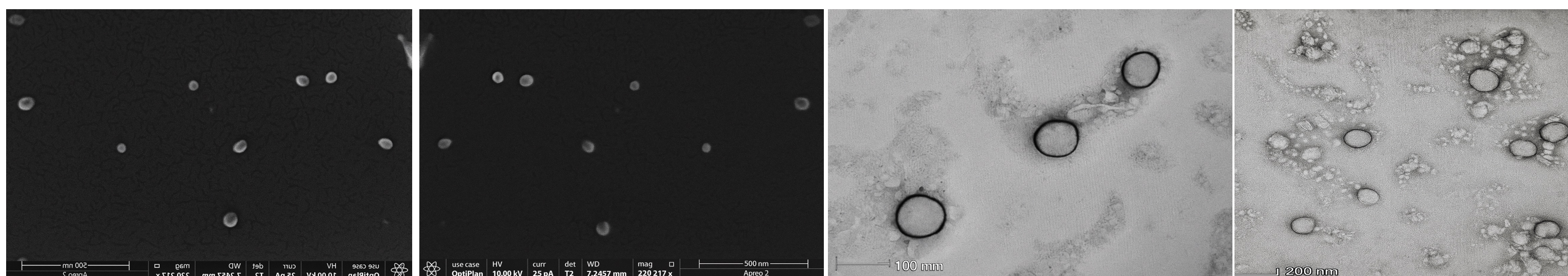
## Physicochemical Characterization of Prodrugs NPs

### Stability of Nanoparticle (Size, PdI and Zeta potential) determined by Malvern Nanozetasisizer over time



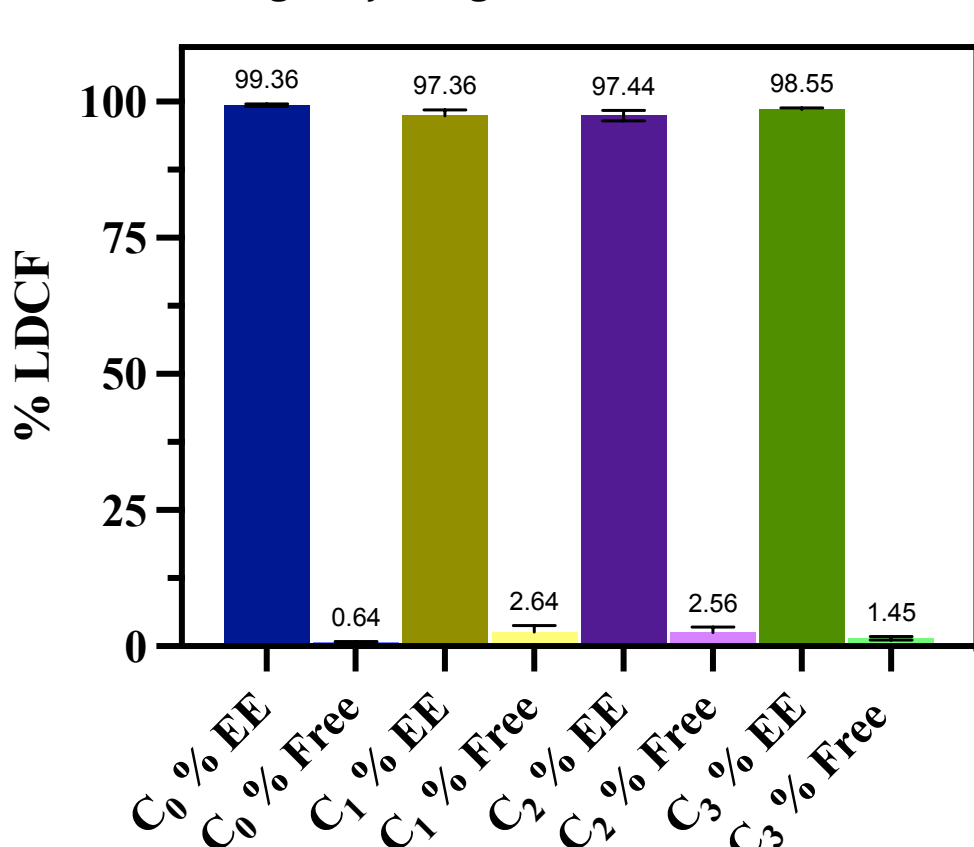
Size (d<sub>H</sub>), Polydispersity index (PdI) and Zeta potential of Prodrug NPs over time (246 days) at Room temperature and 4°C storage conditions.

## Scanning Electron Microscopy & Transmission Electron Microscopy Analysis

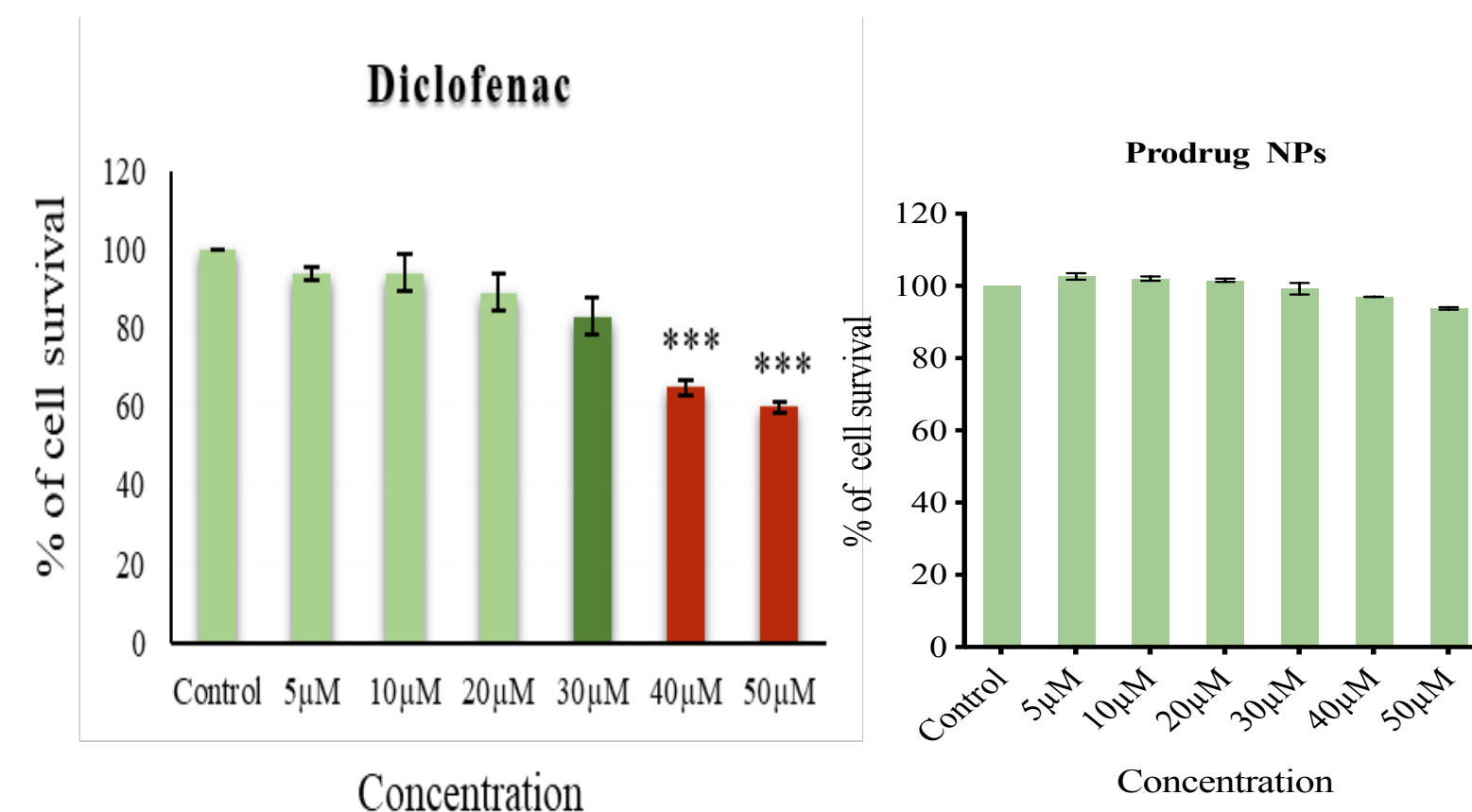


## Determination of % EE of Prodrug Nanoparticles

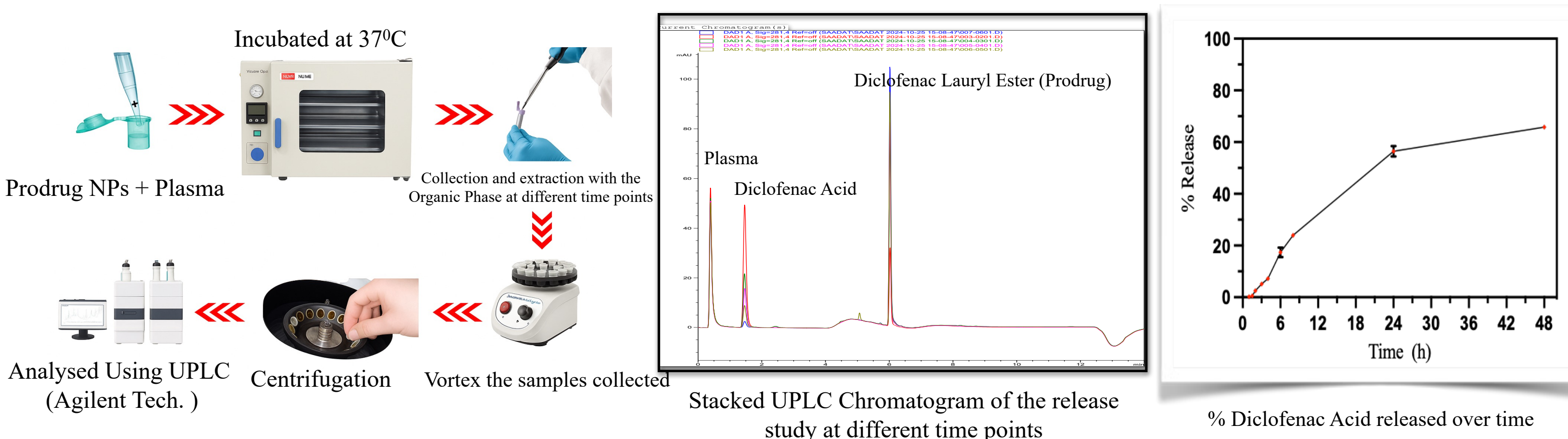
% Encapsulation Efficiency =  $\frac{\text{Amount of prodrug in NPs} - \text{Amount of free prodrug in NPs}}{\text{Weight of drug in NPs}} \times 100$



## Cell Viability Assay of Prodrug Nanoparticles

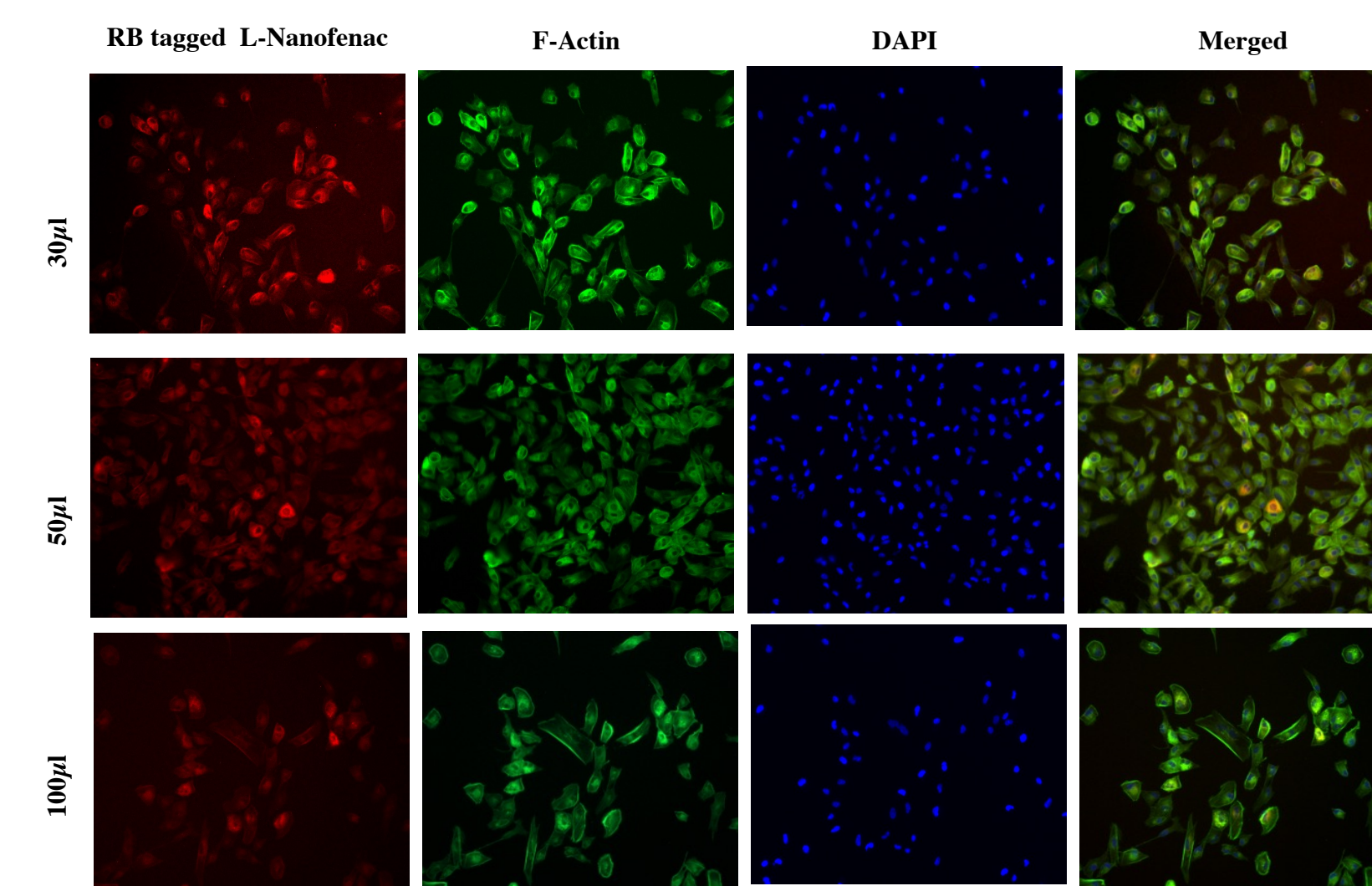


## Diclofenac Release Profile in Murine Plasma from Prodrug NPs

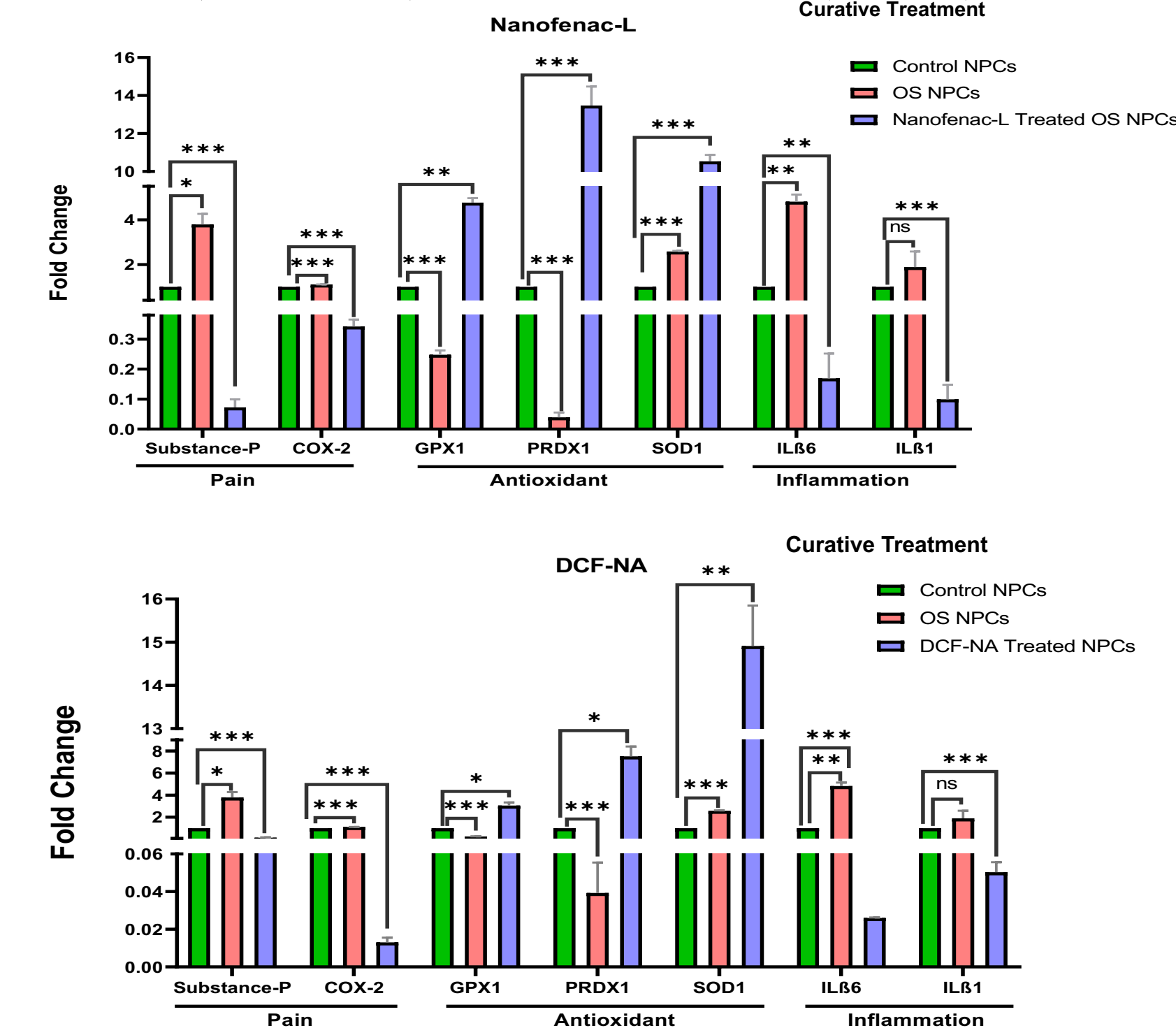


## In Vitro Efficacy of Diclofenac Prodrug Nanoparticles

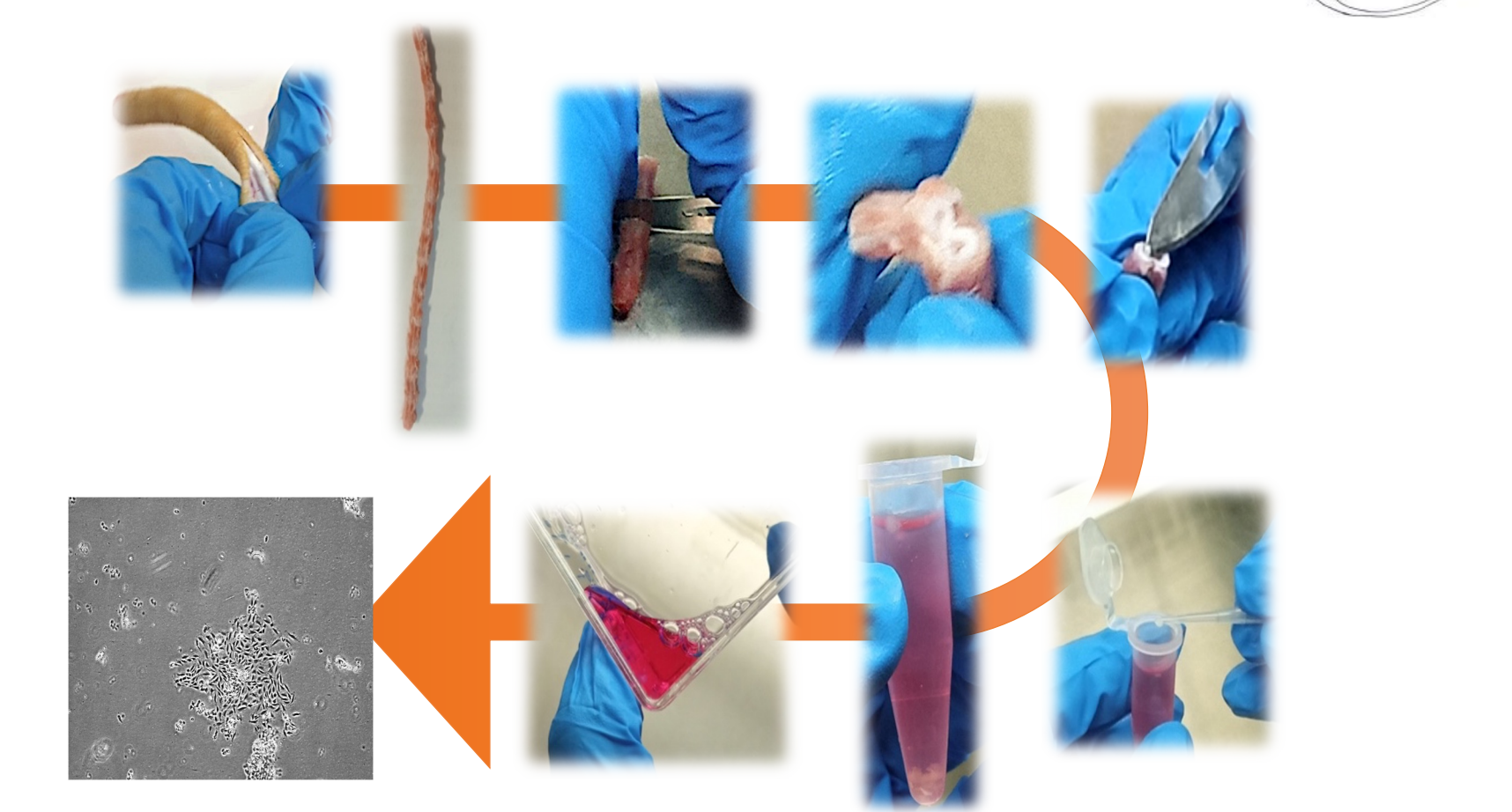
### In vitro cellular uptake study of prodrug NPs



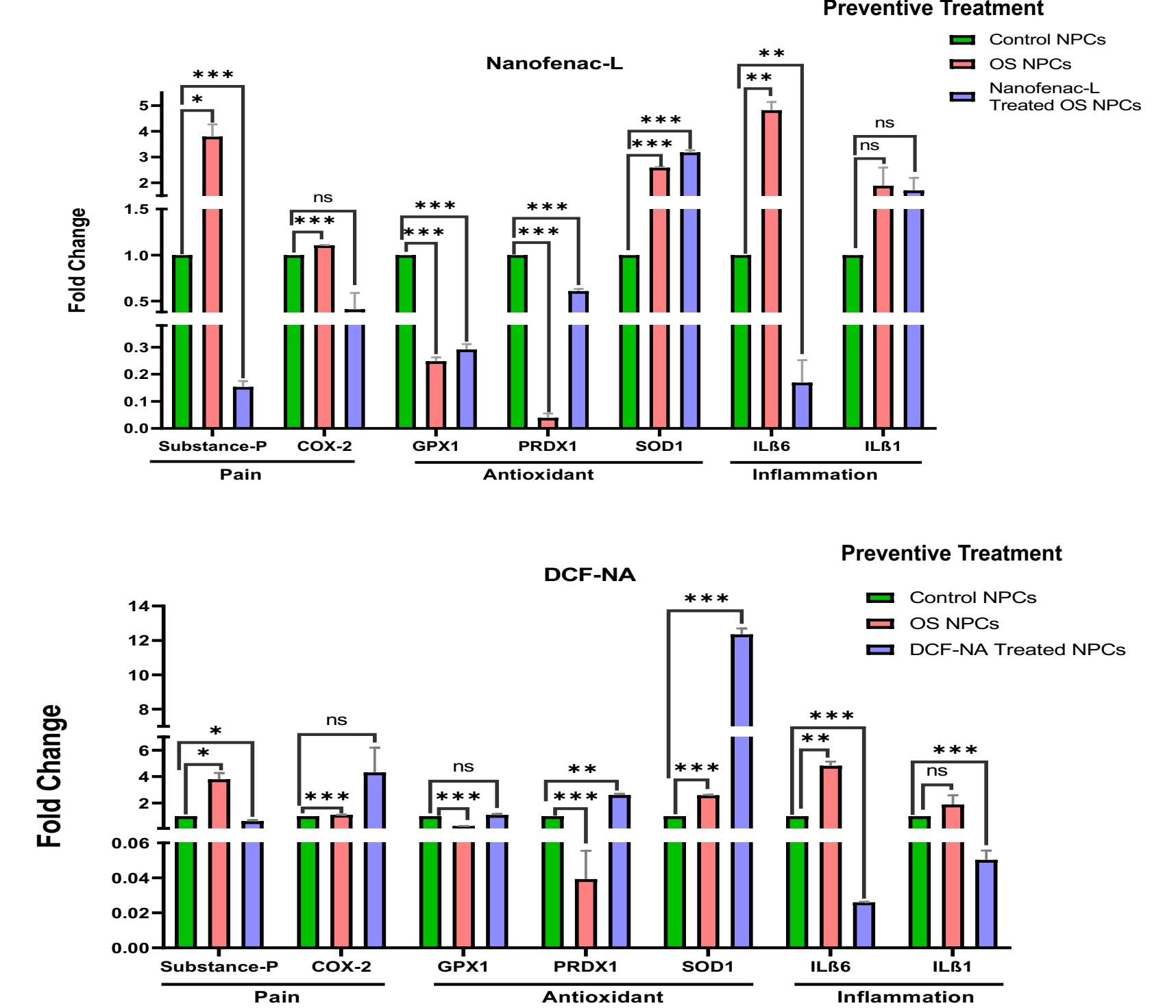
### Gene Expression Analysis of Pain, Antioxidant and Inflammation Markers for Compound Curative Treatment using Prodrug Nanoparticles (Nanofenac-L) and Diclofenac Sodium (DCF-Na)



### Nucleus Pulposus Cell Isolation from Rat Intervertebral Disc



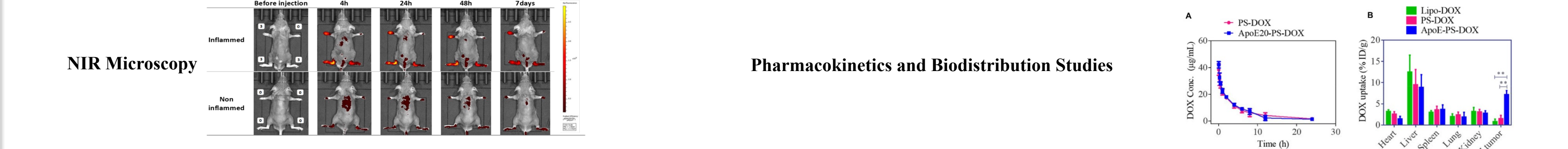
### Gene Expression Analysis of Pain, Antioxidant and Inflammation markers to evaluate preventive Treatment using Prodrug Nanoparticles (Nanofenac-L) and Diclofenac Sodium (DCF-Na)



## Conclusion

Diclofenac Prodrug NPs (Nanofenac-L) exhibited promising therapeutic potential for treating inflammatory and pain related complications, (including intervertebral disc degeneration). This promise stems from their multifaceted properties, encompassing anti-inflammatory, antioxidant, and analgesic effects. Further research is warranted to fully explore Nanofenac-L as a potential drug candidates in future pre-clinical and clinical trials.

## Future Prospects



## References

Hussain, S., Ur-Rehman, M., Arif, A., Cailleau, C., Gillet, C., Saleem, R., & Tsapis, N. (2023). Diclofenac prodrugs nanoparticles: An alternative and efficient treatment for rheumatoid arthritis?. *International Journal of Pharmaceutics*, 643, 123227.