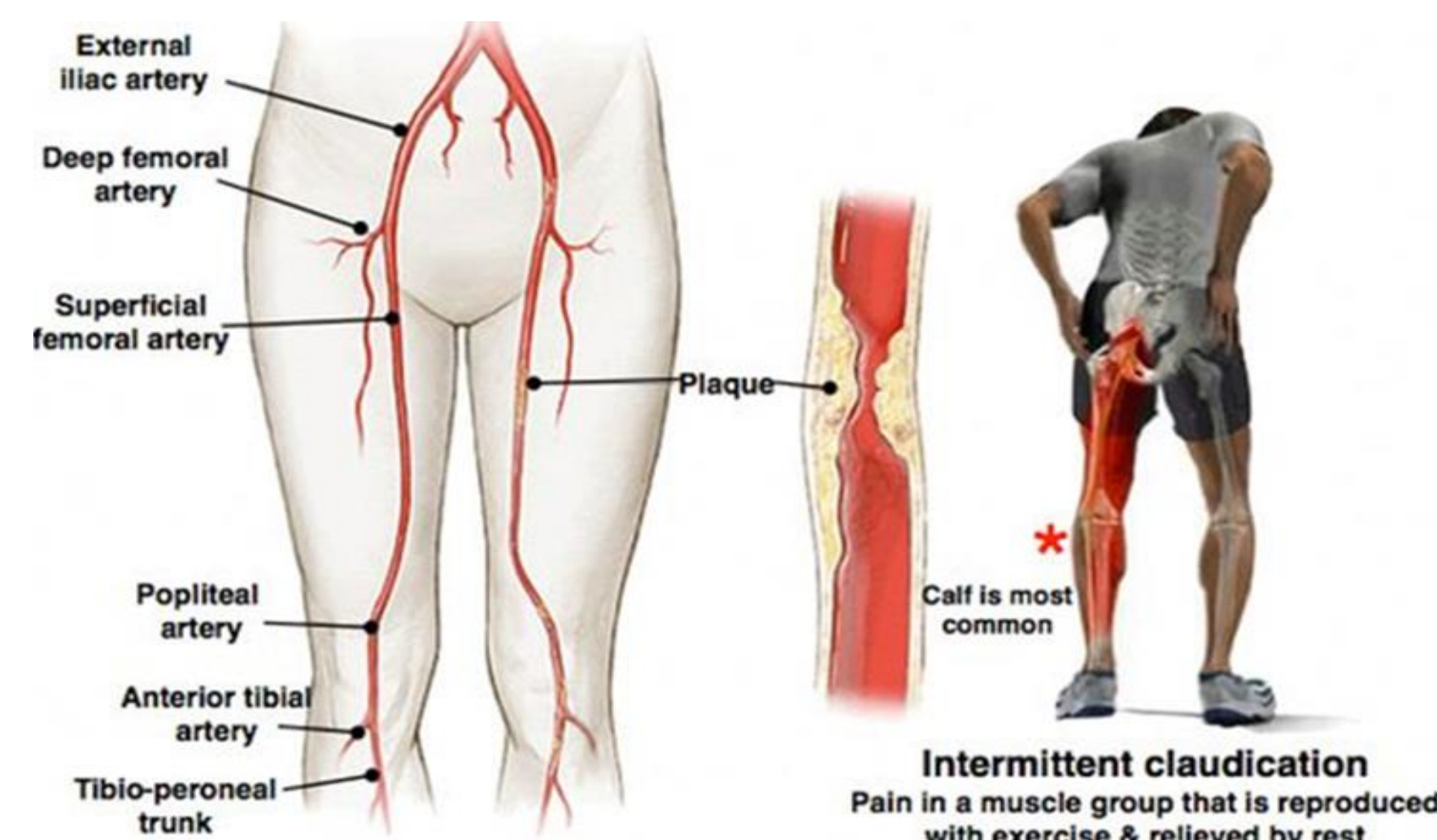


Objective

This research proposes a non-invasive drug delivery system, which aims to provide an innovative approach for delivering therapeutic agents to target ischemic muscles. Through the incorporation of targeting moieties to PLGA nanoparticles, we seek to maximize the bioavailability of the therapeutic payload, thereby enhancing its therapeutic efficacy. This multifaceted approach holds significant promise not only for PAD therapy but also for addressing a wide range of inflammatory diseases, cancer treatment, and wound healing.

Background

- PAD is a progressive vascular disorder characterized by atherosclerosis in lower extremities. Globally, >200 million people are affected, of which 80 million in the United States (1). Intermittent claudication can progress to chronic limb ischemia and tissue necrosis (2), that has a 25% mortality rate and a 45% risk of limb amputation.
- Conventional management of PAD focuses on relieving symptoms, preventing disease progression, or invasive procedures (angioplasty, surgical bypass, or amputation). However, while effective, they often do not address the underlying drives of atherosclerosis.



Experimental Test Plan

➤ Nanoparticle fabrication and characterization:

- Poly (lactic-co-glycolic acid) (PLGA) nanoparticles loaded with Cyanine 7 were synthesized using a standard emulsion technique and conjugated to the targeting moiety through covalent coupling. Sizing, charge, and polydispersity (PDI) of NPs were quantified using Dynamic Light Scattering (DLS).

➤ Biodistribution of targeting NPs *in vivo*:

- PAD mouse model:** Ischemic hindlimb injury was induced by ligating the left femoral artery and its branches, while the right hindlimb was left untreated as a control. The nanoparticles (NPs) were administered intravenously via the tail vein.
- Ex vivo image:** Organ and muscle tissues were harvested 1-hour post-injection, and NPs accumulation was assessed using the Kodak Pro System.
- Homogenization:** Muscle were homogenized, lyse and studied for NPs uptake using fluorescence spectrophotometer.

Experimental Test Plan

Table 1. Characterization of Nanoparticles.

Nanoparticles	Size (nm)	PDI	Charge (mV)
PLGA NPs	190 ± 65.7	0.162 ± 0.016	-28.48 ± 2.13
PLGA NPs Conjugated Targeting Moiety	205.3 ± 79	0.161 ± 0.033	-24.49 ± 2.67

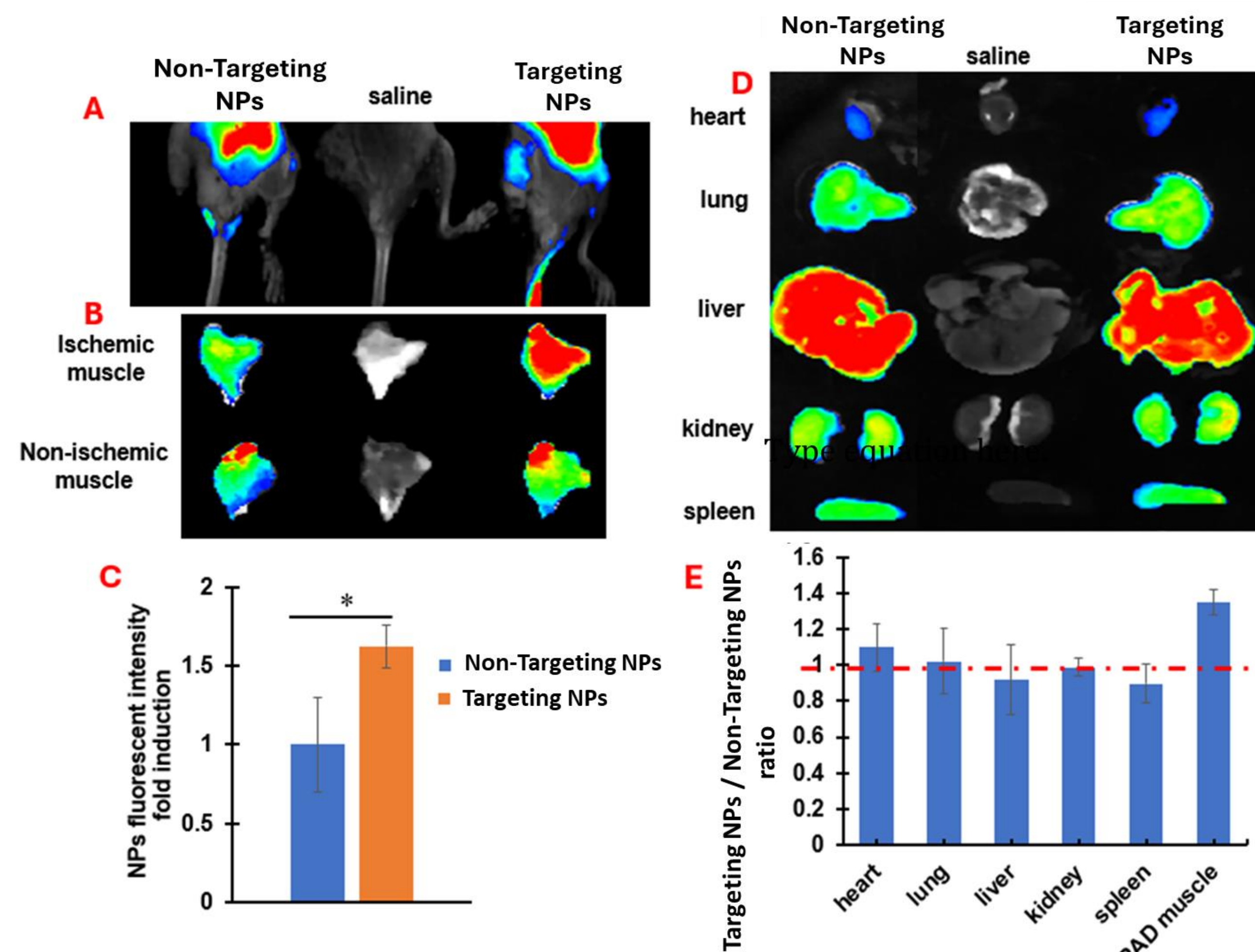


Figure 1. *In vivo* and *ex vivo* biodistribution study.

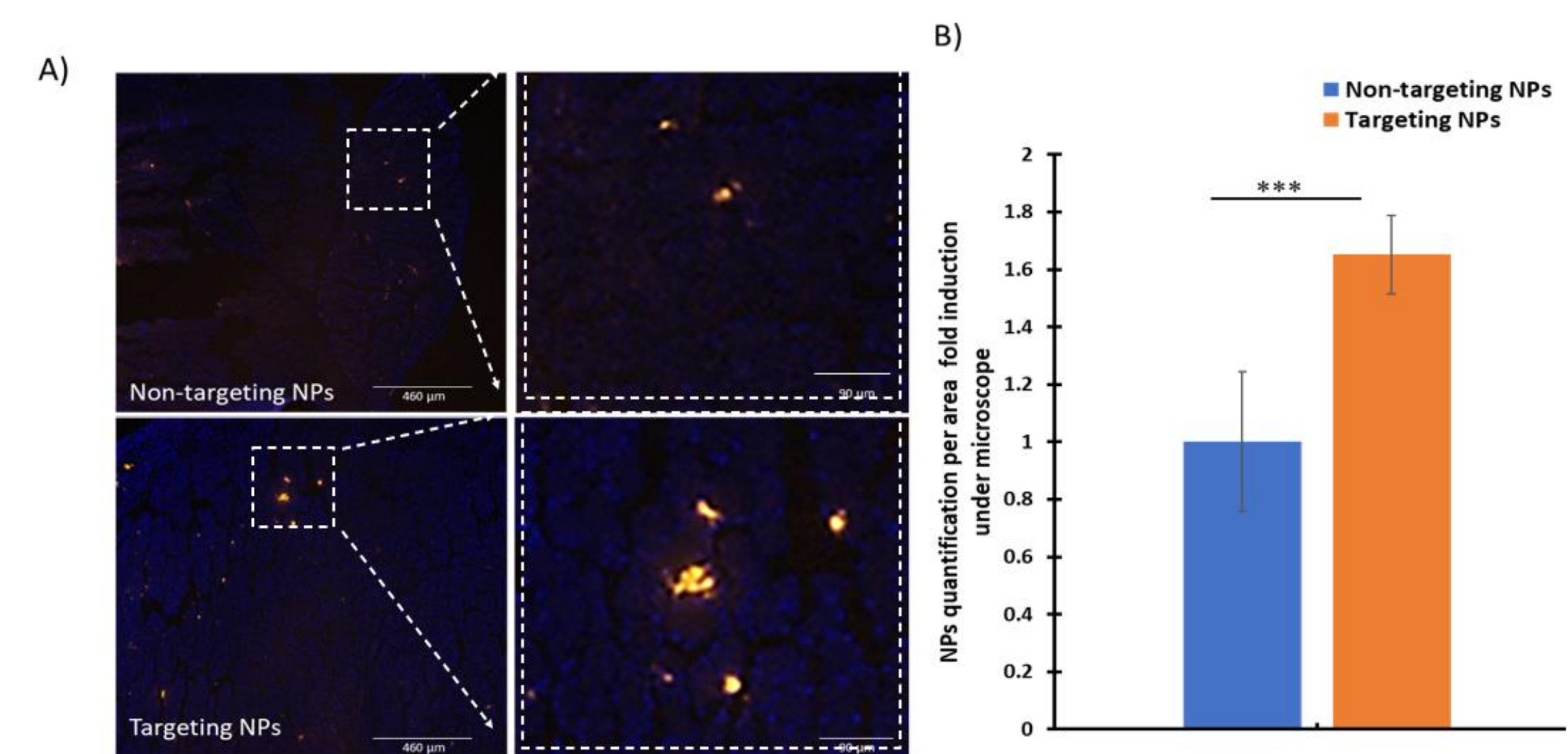


Figure 2 . Biodistribution study of NPs under fluorescent microscope. **A)** Representative images of NPs (orange). **B)** Data analysis of NPs quantification per area of fluorescent 4x image using ImageJ.

Discussion

- We have successfully engineered and incorporated targeting moiety to PLGA NPs which have uniform, average diameters of around 200 nm, and negative surface charges (Table 1).
- The *in vivo* and *ex vivo* bio-distribution results show that targeting nanoparticles significantly enhance targeting to ischemic tissue in the PAD mouse model (Figure 1). *In vivo* imaging revealed greater fluorescence localization in the ischemic hindlimb of mice treated with targeting NPs than in those treated with non-targeting NPs. While very few NPs localized in non-ischemic muscle (Figure 1 A). *Ex vivo* analysis further confirmed the preferential accumulation of targeting NPs in ischemic muscle relative to non-ischemic muscle, demonstrating effective targeting of the inflamed endothelium (Figure 1A). Quantitative data from overall NPs fluorescent intensity showed an approximate 1.6-fold increase in nanoparticle accumulation in ischemic tissue with targeting NPs, supporting improved delivery efficiency (Figure 1C).
- Importantly, bio-distribution across major organs (heart, lung, liver, kidney, spleen) remained comparable between targeting and non-targeting NPs, suggesting minimal off-target effects (Figure 1D, E).
- After we collected the gastrocnemius muscles of NPs treated mice, the samples were fixed, wax-embedded, sectioned, and imaged under a fluorescence microscope. The distribution of NPs observed under a fluorescence microscope further supports their targeting (Figure 2). Together, these findings highlight the specificity and potential therapeutic advantage of targeting the NPs system for enhancing nanoparticle delivery to ischemic regions in PAD.

Conclusions

Our *in vitro* and *in vivo* studies show that PLGA nanoparticles effectively target the inflamed endothelium and selectively accumulate in ischemic tissue in a PAD mouse model. Biodistribution and quantitative analyses confirmed enhanced localization of targeting nanoparticles to the ischemic hindlimb compared to non-targeting controls. Importantly, minimal differences in off-target organ accumulation suggest a favorable specificity profile. Future studies will focus on evaluating cytocompatibility and long-term safety through both *in vitro* assays and *in vivo* toxicity assessments, including monitoring of organ function biomarkers and systemic responses.

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