

Development of Dual Pro-Angiogenic Agents Loaded Nanoparticles for Treatment of Peripheral Arterial Disease

REU awardee: Anthony Gomez, Do Dang Khoa

REU mentors: Na Nguyen, Khiem Le, Tam Nguyen, and Kytai T. Nguyen

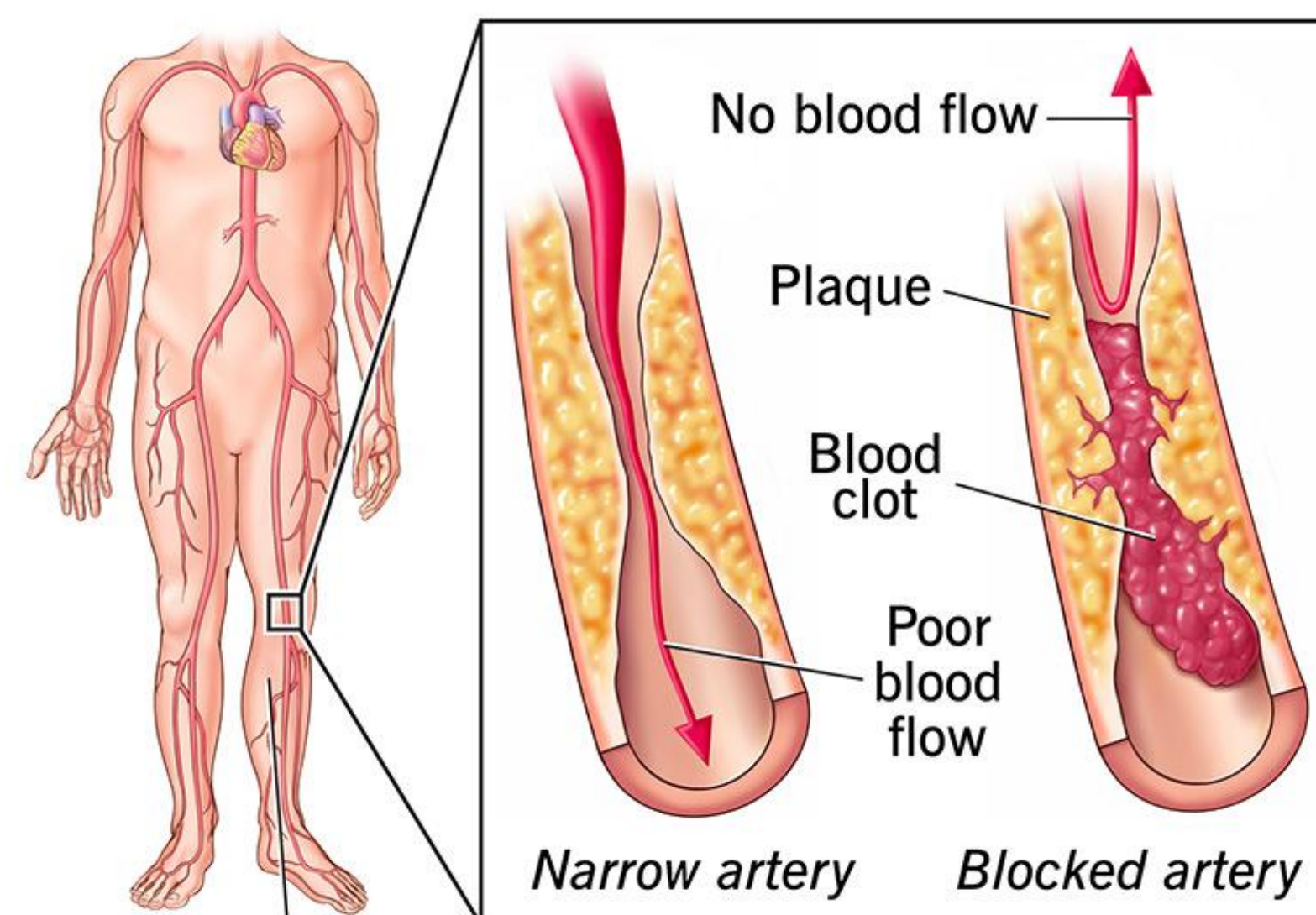
Department of Bioengineering, University of Texas at Arlington, Arlington, Texas, USA

Executive Summary

This project focuses on developing poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with dual pro-angiogenic agents for controlled therapeutic delivery. These nanoparticles are designed to promote angiogenesis, enhance blood perfusion, and reduce ischemic tissue damage. By leveraging controlled-release mechanisms, this approach aims to provide a minimally invasive and effective alternative to improve vascular regeneration and treatment outcomes in PAD.

Background

Peripheral artery disease



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- Peripheral arterial disease (PAD) is a common circulatory condition in which narrowed arteries reduce blood flow to the limbs, most commonly affecting the lower extremities (1,2). This reduced perfusion leads to ischemia, resulting in muscle degeneration, delayed wound healing, and in severe cases, limb loss (1,2). PAD is particularly prevalent in aging populations and individuals with risk factors such as diabetes and smoking.
- Current treatment approaches, including surgical revascularization and pharmacological therapies, are often limited by invasiveness, cost, and inconsistent long-term outcomes.
- Biomaterial-based delivery systems, such as polymeric nanoparticles, have emerged as promising platforms for targeted and controlled release of pro-angiogenic factors to enhance vascular repair and improve clinical outcomes (2).

Experimental Test Plan

- Poly(lactic-co-glycolic acid) (PLGA) nanoparticles encapsulating dual pro-angiogenic agents were synthesized using a standard double emulsion technique to enable controlled therapeutic delivery.
- To evaluate therapeutic efficacy, an *in vivo* peripheral arterial disease (PAD) model was established in BALB/c mice through femoral artery ligation, inducing hindlimb ischemia, with the contralateral limb serving as a non-ischemic control.
- The therapeutic performance of the dual-loaded PLGA nanoparticles was evaluated by comparing multiple treatment groups, including saline (control), blank nanoparticles, free drug, and dual-loaded nanoparticles.
- Blood perfusion was monitored using laser speckle contrast imaging (LSCI), and tissue-level effects were assessed through histological analysis, including hematoxylin and eosin (H&E) staining for muscle morphology and collagen IV immunostaining to evaluate angiogenesis.

Experimental Results

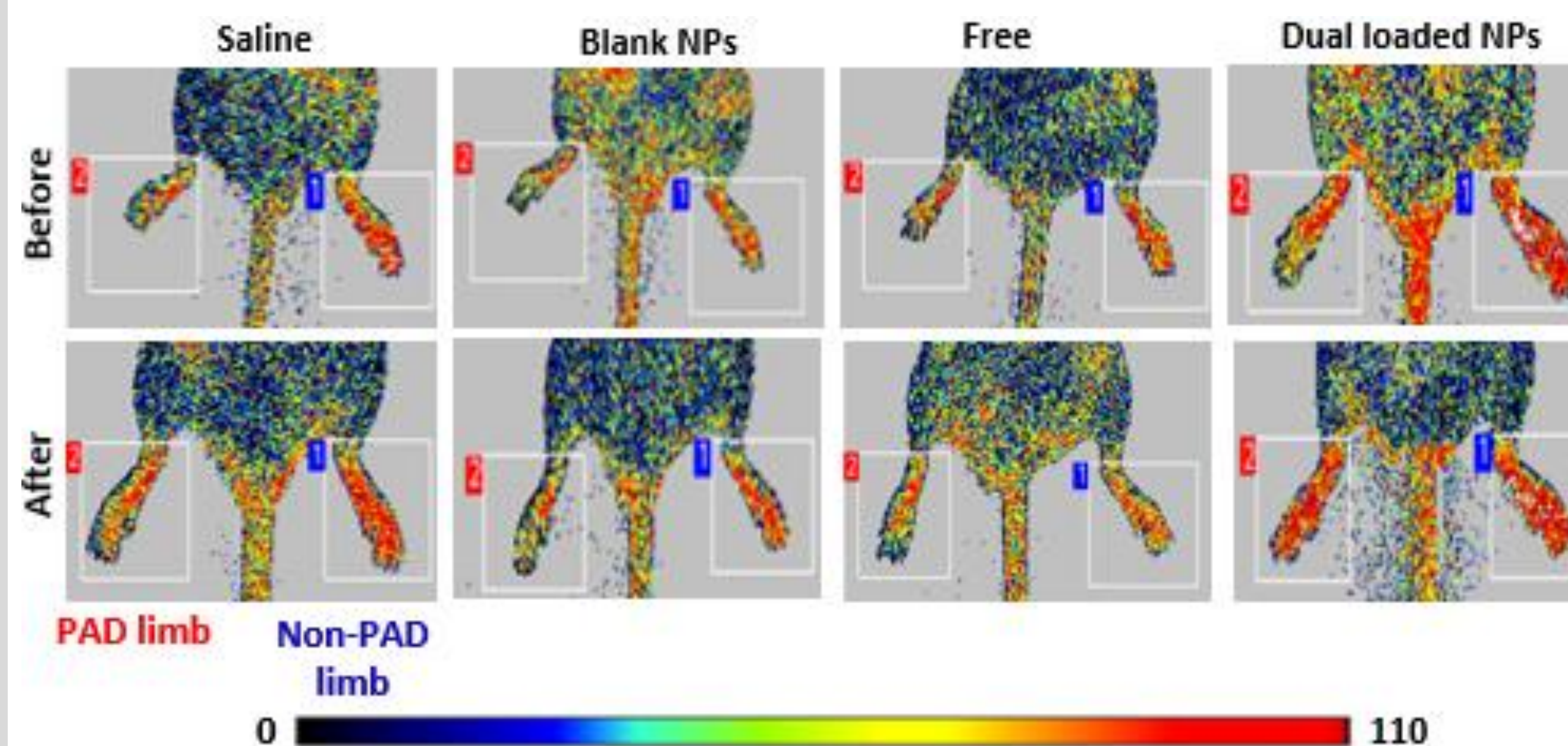


Figure 1. *In vivo* therapeutic study of NPs on PAD mice model. A) Representative images of LSCI showed Dual NPs prevent mice from necrosis and enhanced blood reperfusion on ischemic hindlimb. Color spectrum illustrated different blood perfusion level: red for high blood perfusion and blue for reduced blood perfusion.

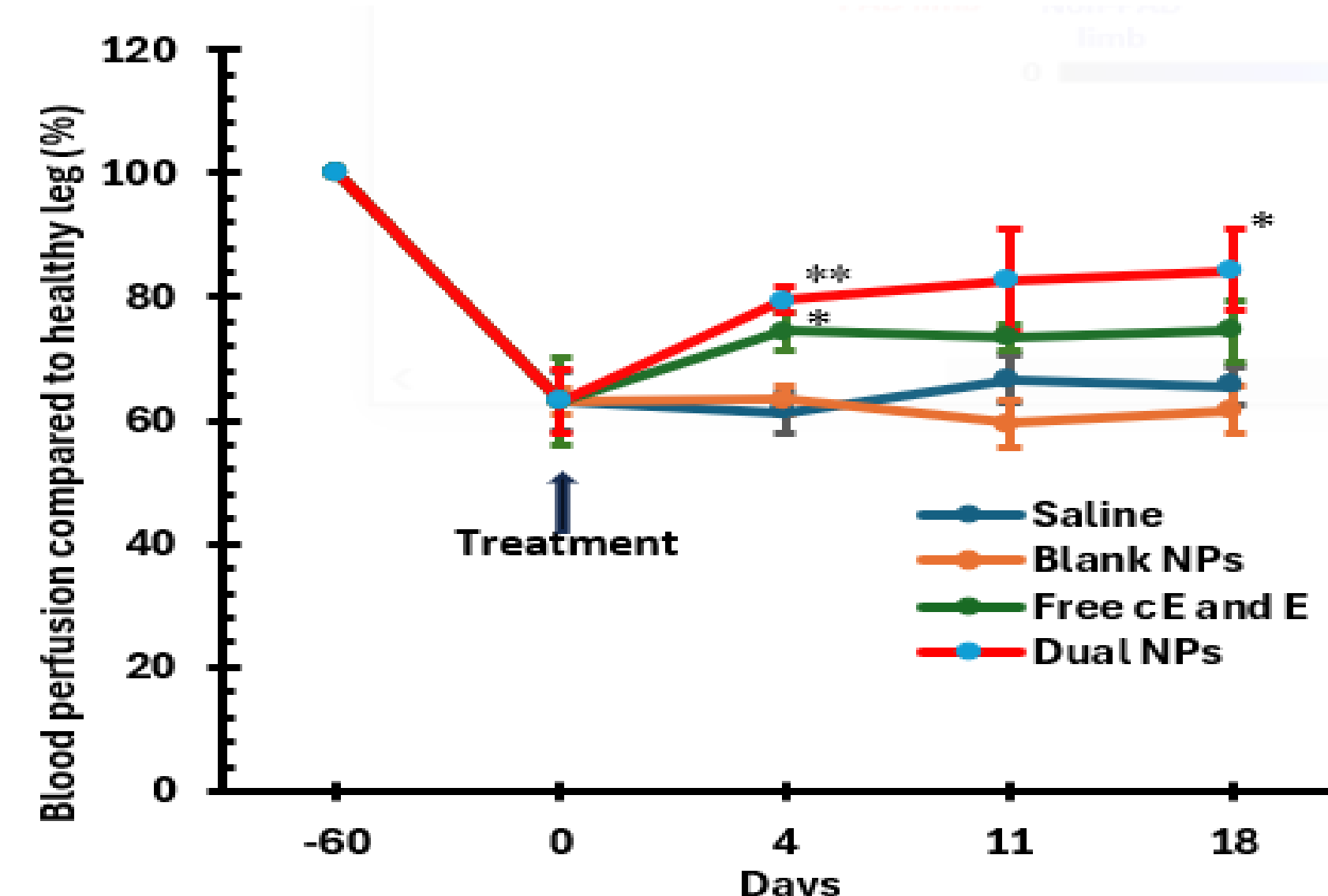


Figure 2. Data analysis of blood perfusion improvement of treated PAD mice via non-ischemic leg versus ischemic leg blood perfusion from day 0 to day 18. Error bars indicated the standard error of mean. The statistical significance of fluorescent intensity was assessed using a student's t-test. * $p < 0.05$, ** $p < 0.01$

Experimental Results

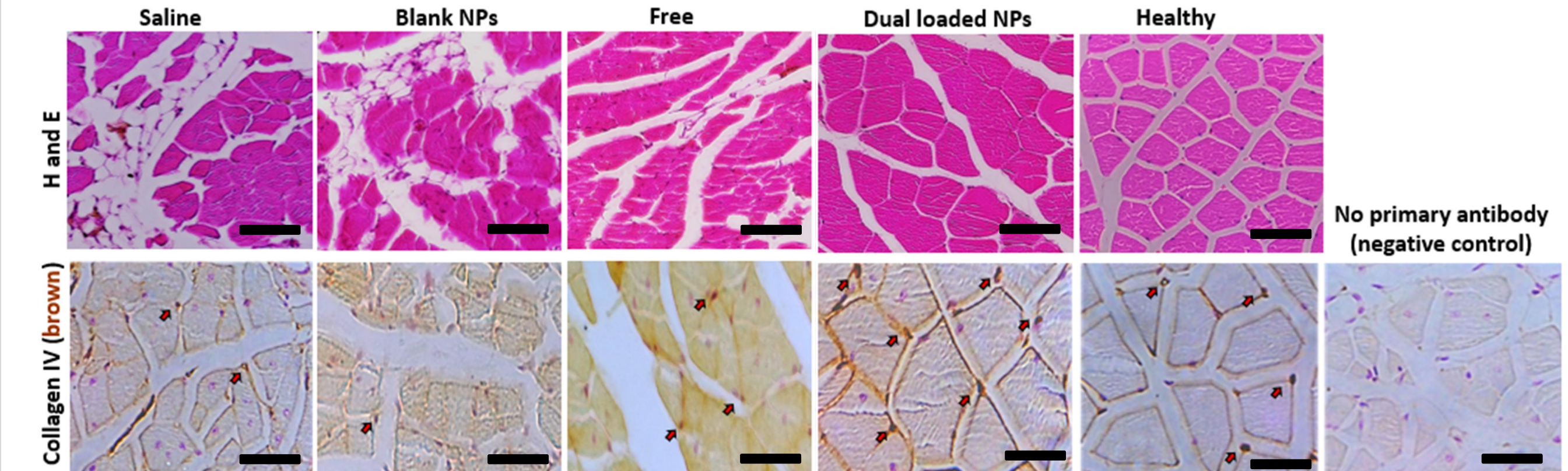


Figure 3. Histology of the gastrocnemius muscle on treated PAD mice. Hematoxylin and Eosin staining and collagen IV immunohistochemistry of PAD muscles demonstrated Dual NPs protected muscular fiber morphology and reduced adipocyte infiltration. Dual NPs enhanced capillary formation and angiogenesis (red arrow) compared to other groups. Scale bars are 100 μm .

Discussion

PAD model in Balb/c mice was established by femoral artery ligation through the reduction in blood perfusion to 60%, which was measured by LSCI. Our *in vivo* therapeutic studies showed that Dual NPs can significantly restore blood flow by up to 85%, the highest among all groups (Figures 1 and 2). The dual-NP-treated mice outperformed the free group, which initially showed benefits but no enhancement due to rapid degradation and inadequate delivery without a carrier (Figure 2). Histological analysis further revealed that Dual NPs protected muscle bundles from hypoxia-induced damage, reducing necrosis and monocyte infiltration compared with the saline and blank NPs groups, which exhibited muscle fiber separation, atrophy, and increased adipocytes (Figure 3). Immunostaining for collagen IV showed increased capillary density and angiogenesis in our NPs group, supporting their efficacy in restoring blood perfusion in ischemic limbs (Figure 3).

Conclusion

The results of this study demonstrate that dual pro-angiogenic agent-loaded PLGA nanoparticles provide a promising therapeutic strategy for the treatment of peripheral arterial disease. In the PAD mouse model, treatment with dual-loaded nanoparticles significantly improved blood perfusion, reduced ischemic tissue damage, and preserved muscle morphology. Histological analysis further confirmed enhanced angiogenesis through increased capillary formation. More *in vivo* studies will be conducted to verify cellular recruitment and toxicity of our NPs. Overall, this approach highlights the potential of controlled nanoparticle-based delivery systems as a minimally invasive and effective solution for improving vascular regeneration in PAD.

References

- U.S. Department of Health and Human Services. (2022). *What is Peripheral Arterial Disease?* National Heart, Lung, and Blood Institute.
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