NAD+ Depletion in Vein Grafts Contributes to Intimal Hyperplasia and Failure: A Potential Therapeutic Target

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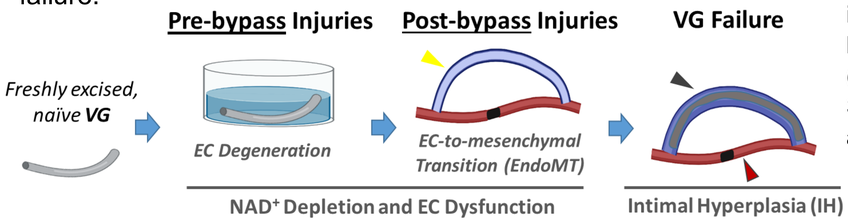
Introduction

- Autologous **vein graft (VG)** is the most commonly used conduit for bypass procedures.
- Up to 50% of VG fail within 10 years, yet no clinically established treatment available currently.
- The pathophysiology of VG failure primarily involves **intimal hyperplasia (IH)**.
- Suboptimal pre-bypass storage of VG induces **endothelial cell (EC) dysfunction**, causing IH.
- Nicotinamide adenine dinucleotide (**NAD+**) is a co-enzyme critical to metabolism and redox signaling.
- **NAD+** dysregulation and depletion have been implicated in diseases, including EC dysfunction.
- **The association between NAD+** dysregulation and VG bypass failure remains completely undescribed.

Hypothesis

NAD+ homeostasis is vital to EC dysfunction underlies IH and VG failure.



Methods

- Bioinformatics: We use Seurat v5.0 for the re-analysis of snRNA-seq (GSE263280) and spatial transcriptomics data (GSE263281). Single R was utilized for cluster identification. Expression level of several NAD+ related genes and EC dysfunction associated genes were explored between each group.
- VG bypass animal model: The rat's common jugular vein was harvested and stored in different solutions. The common carotid artery was exposed, and blood flow was temporarily blocked at both ends. One end of the severed artery was passed through an anastomotic sheath, clamped, and fixed. The artery was then everted to expose the intima, which was tied off at the distal end. A section of the harvested jugular vein was placed over the everted artery, and an intima-intima anastomosis was created by tying a knot on the anastomotic sheath. After ensuring the knot was secure, the vascular clamp was released, and the slipknots were opened to check for normal blood flow and pulsation, ensuring no leakage.

Results

SnRNA-seq and Spatial Transcriptomics data Re-analysis Reveals Transcriptomic Changes in EC: *EC Dysfunction and NAD+ biosynthesis*

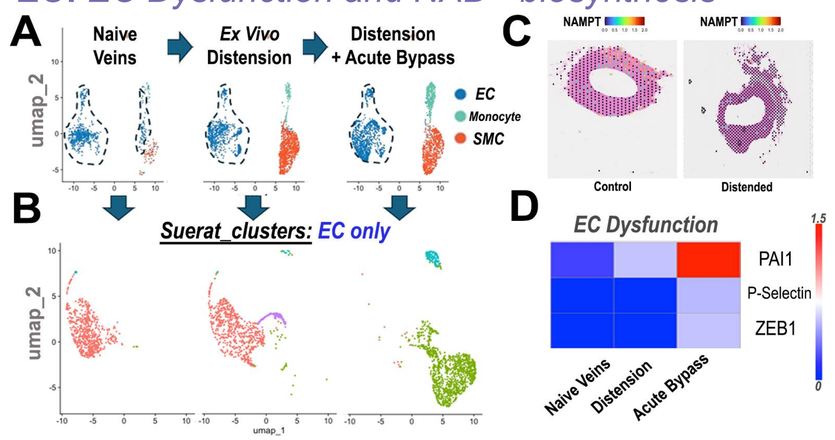


Fig 1: EC dysfunction during pre-bypass VG insults (30min saline distension) and acute bypass injuries (24hr post-bypass). Re-analysis of publicly deposited single-nuclei RNA sequencing (snRNA-seq) data and spatial transcriptomics data. (A) UMAP plots highlighting major cell populations, with EC emphasized in dotted circles. (B) Seurat sub-clustering of EC unveils clusters unique to healthy and injured groups, corresponding to healthy (red) vs dysfunctional EC (purple and green), respectively. (C) Spatial expression of NAMPT (NAD+ Synthetase) in control and distended veins. (D) Heatmap of relative mRNA abundance of EC dysfunction pathways.

Pre-bypass Storage Depletes NAD+ in VG

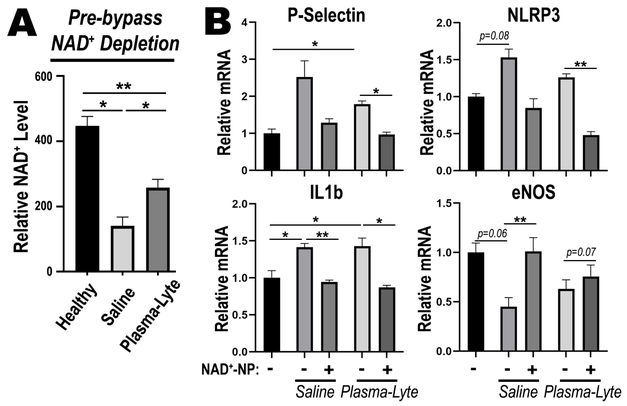


Fig. 2. *Ex vivo* VG storage in non-buffered (*saline*) and buffered (*Plasma-Lyte*) solutions led to rapid NAD+ depletion and EC dysfunction. (A) Storage in saline or Plasma-Lyte (30min) reduced NAD+ in human saphenous VG (n=4). (B) Supplementation of 10μM NAD+-NP reduced the mRNA expression of dysfunctional EC (EC degeneration) markers P-Selectin and inflammasome genes (NLRP3 and IL1b) and rescued the expression of EC function marker eNOS after 6hr *ex vivo* exposure. Mean±SEM. Paired One-Way ANOVA followed by Tukey test. *p<0.05.

Results

Ex Vivo NAD+ Repletion Improves Post-bypass VG Patency

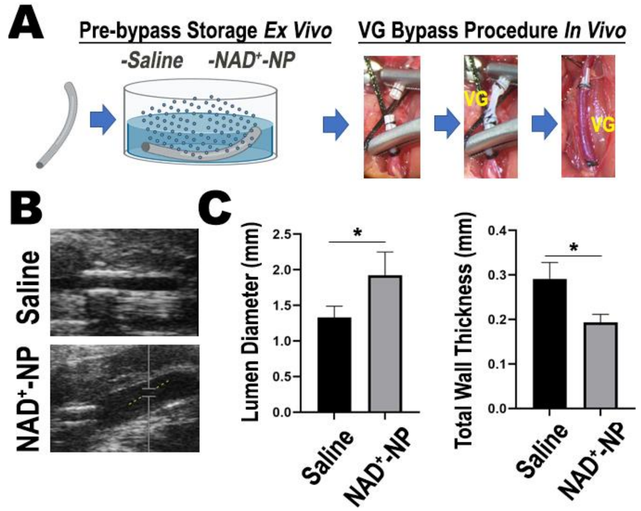


Fig. 3. (A) Schematic of the pre-bypass VG storage and subsequent bypass procedure in rats. (B) Representative ultrasound imaging of VG 2 months post-bypass, following 3h intraoperative storage in saline with or without 10 μM NAD+-NP at room temperature. (C) Quantification of lumen diameter and distal wall thickness. Mean±SEM. n=4-7. Unpaired Student's t-test. *p<0.05.

Conclusion

NAD+ dysregulation plays a crucial role in EC dysfunction, contributing to IH and VG failure.

References & Acknowledgements

References:

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