Endothelial Myelin Debris Clearance and Its Contribution to Secondary Spinal Cord Injury

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Introduction

Spinal cord injury (SCI) occurs when trauma damages the spinal cord, causing loss of movement and sensation. It develops in two phases: a brief primary injury from immediate mechanical damage, followed by a longer secondary injury phase. During the secondary phase, a toxic biological cascade, including inflammation and cell death, spreads damage to surrounding healthy tissue over weeks or months.

During secondary injury, damaged nerve cells release myelin debris, a lipid-rich material that normally insulates nerve fibers. This debris accumulates at the injury site, promotes inflammation, and limits nerve regeneration. While immune cells are known to help clear myelin debris, recent research suggests that endothelial cells — the cells lining blood vessels — may also respond to and process this material. Endothelial activation can disrupt blood vessel stability by altering proteins that maintain cell structure and connections, potentially contributing to chronic inflammation after injury. In this study, we examined how endothelial cells respond to myelin debris by analyzing markers of inflammatory activation and structural changes using immunocytochemistry. Understanding this response may help identify new strategies for controlling inflammation and improving recovery after spinal cord injury.

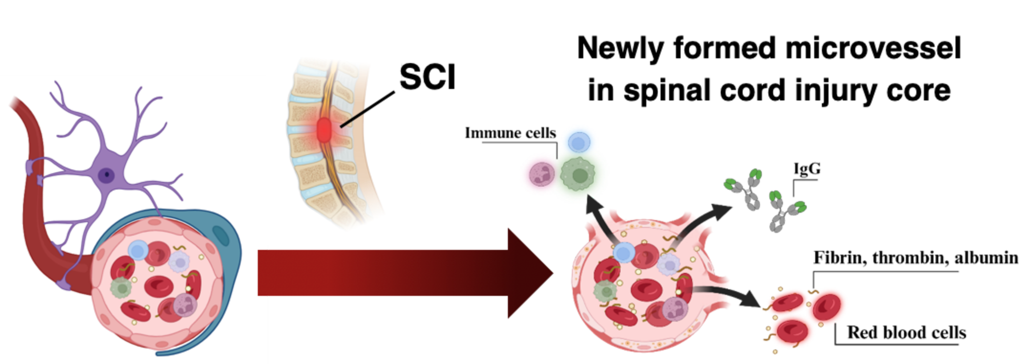

Figure 1. Overview of secondary spinal cord injury and myelin debris accumulation
Schematic illustration of spinal cord injury showing the secondary injury phase, during which myelin debris accumulates and contributes to inflammation and microvascular changes. This model provides the biological context for examining endothelial cell responses to myelin debris.
Image provided by Grace Hammel, Nowakowski Lab.

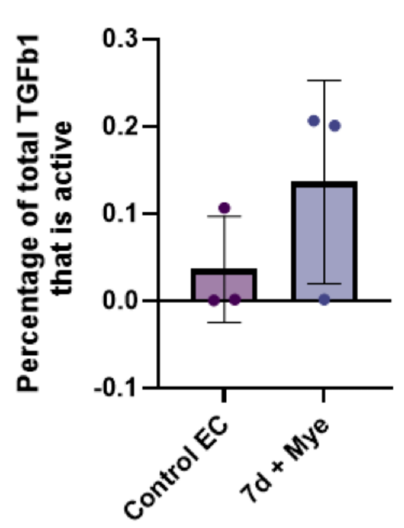

Figure 2. ELISA measurement of TGF-β1 secretion from endothelial cells following myelin debris exposure. No significant difference was detected between control and treated groups ($p > 0.05$, $n = 4$), suggesting endothelial activation may occur independently of measurable cytokine increases at this stage.

Methods

Endothelial cells grown in culture were exposed to isolated myelin debris to model conditions following spinal cord injury and examine how vascular cells respond to injury-related debris. Cellular responses were analyzed using immunocytochemistry, a fluorescent staining technique used to visualize specific proteins within cells.

- Markers analyzed:
  - CD31 — confirms endothelial cell identity
  - αSMA — indicates cellular activation and stress response
  - VE-cadherin — evaluates stability of connections between neighboring cells
  - DAPI — stains cell nuclei for structural reference

Fluorescent microscopy was used to observe changes in cell morphology and protein organization after myelin exposure. Inflammatory signaling was assessed using an ELISA assay measuring TGF-β1, a cytokine associated with endothelial activation and inflammation. Analysis of four biological replicates showed no significant difference between control and treated groups ($p > 0.05$). These findings were also interpreted alongside existing literature to better understand endothelial contributions to post-injury inflammation.

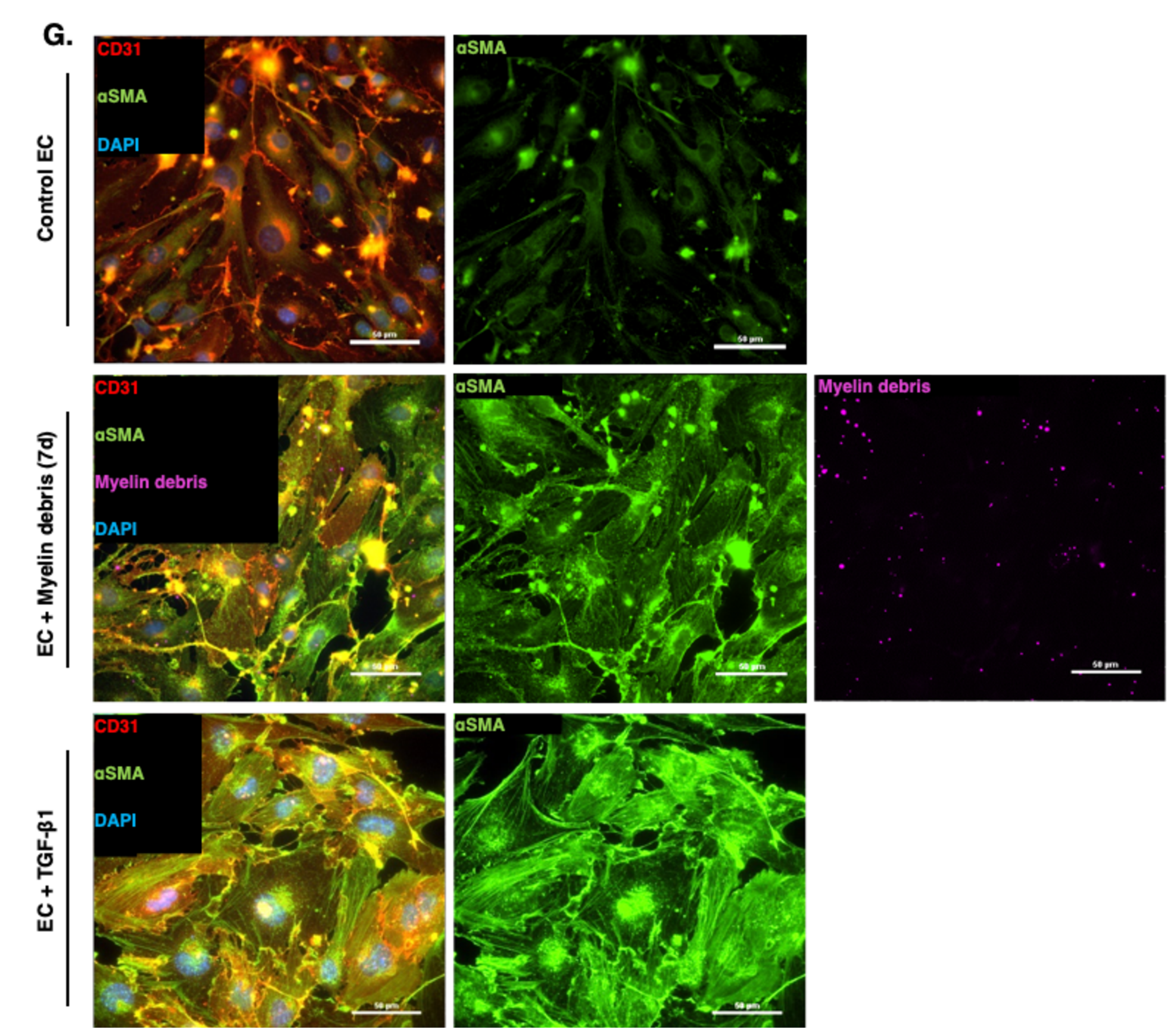

Figure 3. Endothelial activation and structural changes following myelin debris exposure
Immunocytochemistry images comparing control cells, TGF-β1–treated cells (activation control), and myelin debris–treated cells. CD31 (green) confirms endothelial identity, αSMA (red/orange) indicates activation, and DAPI (blue)labels nuclei. Myelin exposure increased αSMA staining and altered cell morphology, demonstrating endothelial activation.
Key finding: Myelin debris induces endothelial activation similar to known inflammatory signaling.

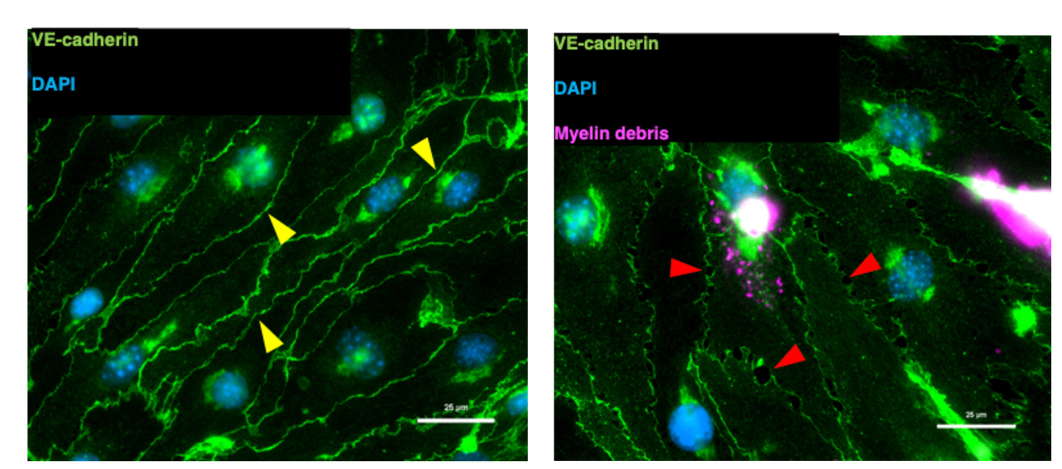

Figure 4. Endothelial activation following myelin debris exposure
(A) Control endothelial cells show low αSMA expression and normal morphology.
(B) Myelin-treated cells display increased αSMA staining and structural changes consistent with cellular activation.

References

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Results

Exposure of endothelial cells to myelin debris produced measurable structural and protein-level changes consistent with cellular activation while preserving endothelial identity.

- Endothelial Activation
  - Myelin debris exposure resulted in increased αSMA expression compared to control cells.
  - Treated cells displayed cytoskeletal rearrangement and altered morphology, indicating cellular activation.
  - TGF-β1–treated cells showed similar changes, validating αSMA as a marker of endothelial activation.
- Disruption of Cell–Cell Junctions
  - Control cells exhibited continuous VE-cadherin localization along cell borders.
  - Myelin-treated cells showed disrupted VE-cadherin organization.
  - These changes suggest reduced endothelial junction stability and impaired microvascular integrity.
- Interaction With Myelin Debris
  - Fluorescent imaging demonstrated endothelial cells interacting with and internalizing myelin debris.
  - Debris exposure was associated with structural remodeling, indicating activation of intracellular response pathways.
- Inflammatory Signaling Analysis
  - ELISA measurement of TGF-β1 secretion showed no statistically significant difference between control and myelin-treated groups ($p > 0.05$, $n = 4$).
  - Structural and protein-level changes were observed despite the absence of detectable increases in cytokine release.

Discussion & Future Direction

Myelin debris accumulation is a major driver of secondary injury and inflammation following spinal cord trauma. While debris clearance is necessary, our results suggest that how endothelial cells process myelin debris influence recovery outcomes. Exposure to myelin debris produced clear signs of cellular activation, including increased αSMA expression and structural remodeling, while CD31 staining confirmed that endothelial identity was maintained. Disruption of VE-cadherin organization further indicated reduced stability of endothelial cell–cell junctions and altered vascular function. We also observed endothelial cells interacting with and internalizing myelin debris, supporting an active role in post-injury immune responses rather than a purely structural function. Although ELISA analysis did not detect a significant increase in TGF-β1 secretion, the observed structural and protein-level changes suggest that endothelial activation may occur before measurable cytokine release or involve alternative signaling pathways.

Together, our findings and existing literature indicate that debris processing is not uniformly beneficial. Depending on intracellular signaling pathways, myelin debris clearance may promote tissue repair or contribute to prolonged inflammation and fibrotic remodeling. These results highlight the complexity of immune regulation during the secondary injury phase and the importance of controlled inflammatory signaling. Future studies could focus on identifying signaling pathways activated during endothelial debris uptake and determining how these pathways influence inflammation, vascular stability, and tissue remodeling over time. Understanding these mechanisms may help guide targeted therapeutic strategies aimed at improving recovery following spinal cord injury.