Blood Outgrowth Endothelial Cell Response to Culture on Novel Ferromagnetic Flow Diverting Stent

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INTRODUCTION

- Intracranial aneurysm is a life-threatening condition that affects roughly 1 in 50 people in the United States (1). Rupture rate is 1.6% and associated with extraordinary rates of mortality and morbidity.
- Currently, the use of flow diverters is widely considered the primary minimally invasive endovascular aneurysm therapy with ~30% of aneurysms treated using flow diverters.

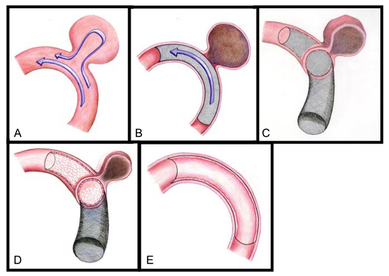


Figure 1. Flow diverting stents are placed across the aneurysm neck within the parent artery to divert flow away from the aneurysm, promote intra-aneurysmal thrombosis, reduce rupture risk, and accelerate healing (2).

- The lack of rapid endothelialization on current flow diverting stents (FDS) necessitates antiplatelet therapy during aneurysm treatment. This limits the clinical utility of flow diverters, creating a need to balance bleeding risk against device-related thrombosis risk.
- Novel ferromagnetic flow diverters were developed to capture and retain magnetically labelled patient-specific cells for quicker endothelialization and healing of the aneurysm.

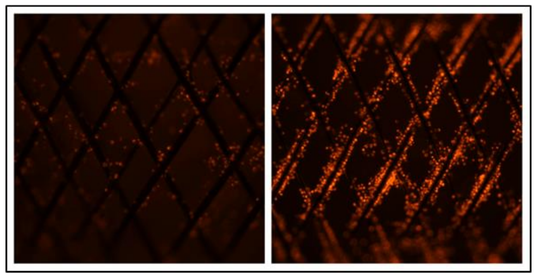


Figure 2. Magnetically-labeled endothelial cell capture to non-magnetic Pipeline (left) and magnetic prototype 2205 stainless steel (right) flow diverters. Cells are fluorescently labeled red.

- **The goal of this study is to characterize the response of blood outgrowth endothelial cells (BOECs) *in vitro* to being seeded onto a novel ferromagnetic flow diverter designed for the *in situ* capture and retention of patient-specific BOECs after stent placement.**

I. RABBIT BOEC CHARACTERIZATION

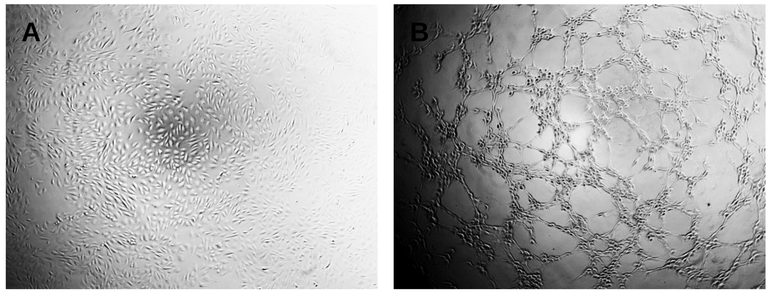


Figure 3. BOECs isolated from rabbit blood exhibit cobblestone morphology (A) and form capillary-like networks when cultured on Matrigel (B). Peripheral blood mononuclear cells were isolated from rabbit blood through density gradient centrifugation and cultured in endothelial cell media until the appearance of BOEC colonies. P4 rabbit BOECs were seeded at 5000 cells/well onto ibidi angiogenesis plates with 10 µl Matrigel per well after 24 hr serum reduction and observed after 4 hours.

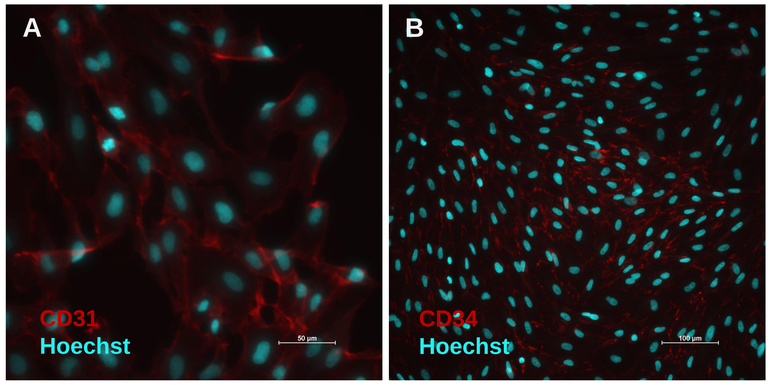
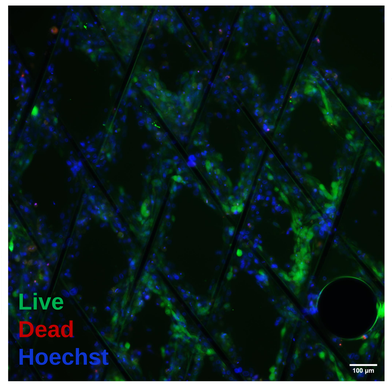


Figure 4. Rabbit BOECs stain positively for endothelial cell markers CD31 (A) and CD34 (B).

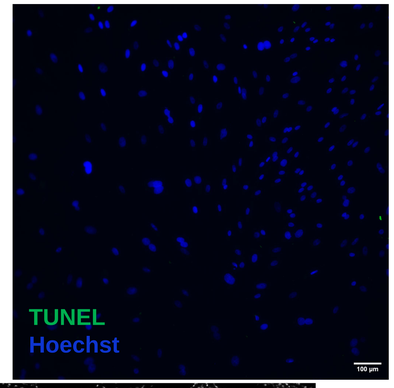
II. CYTOCOMPATIBILITY OF NOVEL FDS



For all following experiments, P4-P7 BOECs were seeded rotationally onto flow diverters at 25 rpm for 1 hour at room temperature. They were then transferred to individual wells of a 96 well plate and cultured statically for up to 36 days.

Figure 5. BOECs remain mostly alive after 14 days culture on FDS. Cells were treated with 1 µM Calcein AM for cytoplasm staining of live cells (green) and 1.6 µM ethidium homodimer-1 for nuclear staining of dead cells (red). Nuclei are visualized by Hoechst (blue).

Figure 6. BOECs cultured on FDS for 6 days show a similar percentage of apoptotic cells to those cultured in a monolayer on tissue culture plastic. Cells were evaluated using the Click-iT TUNEL Alexa Fluor 488 apoptosis detection kit. 0.46% ± 0.09% 2D cultured BOECs were apoptotic (green) as compared to 0.45% ± 0.33% of BOECs cultured on novel FDS devices. Nuclei were stained blue with Hoechst.



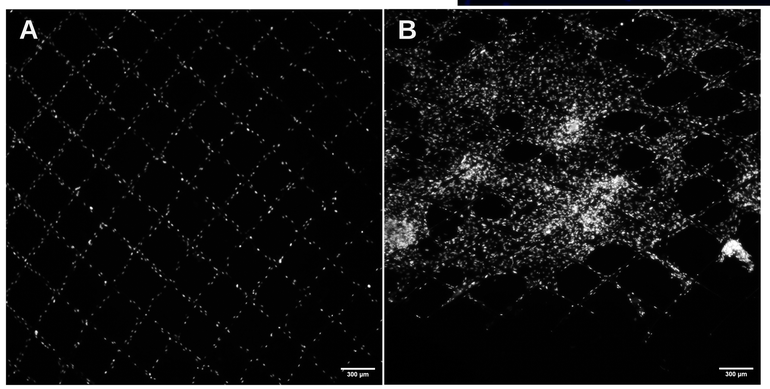


Figure 7. BOECs proliferate on FDS as observed by Hoechst staining of nuclei on day 7 (A) and day 36 (B) of culture.

III. BOECS FILL IN BETWEEN STRUTS OF FDS AND DEPOSIT COLLAGEN IV, LAMININ, FIBRONECTIN

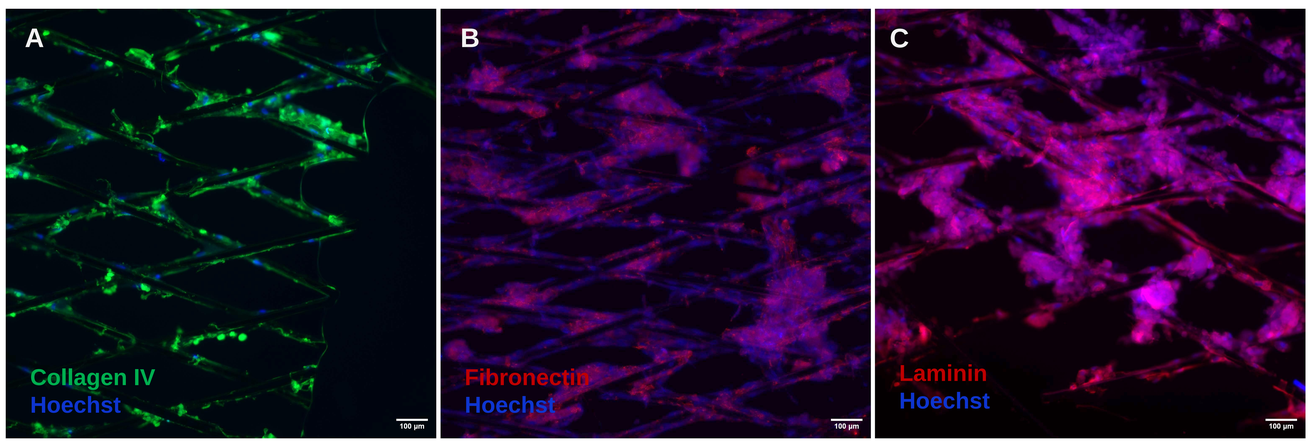


Figure 8. Rabbit BOECs seeded onto FDS proliferate, filling in gaps between the wires of the stent and depositing extracellular matrix proteins crucial to the blood vessel wall as shown by immunofluorescence staining. A) Collagen IV staining (green) after 20 days culture, B) Fibronectin staining (red) after 36 days culture, C) Laminin α2 staining (red) after 36 days culture. Nuclei are labeled blue with Hoechst.

CONCLUSIONS AND FUTURE DIRECTIONS

- Novel ferromagnetic flow diverting stents are compatible with BOECs as assessed by viability, proliferation, and apoptosis assays.
- The deposition of ECM proteins by cells demonstrates their potential to properly heal the aneurysm site, preventing thrombosis and aneurysm rupture.
- Next, the safety and feasibility of rapid endothelialization of magnetic flow diverters will be tested using a rabbit aneurysm model.

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2. Jiang B, Paff M, Colby GP, Coon AL, Lin LM. Cerebral aneurysm treatment: modern neurovascular techniques. *Stroke Vasc Neurol*. 2016;1(3):93-100. Published 2016 Oct 25.