Endothelial Trauma From Mechanical Thrombectomy in Acute Stroke
In Vitro Live-Cell Platform With Animal Validation

Dayu Teng, PhD; Jeffrey Scott Pannell, MD; Robert C. Rennert, MD; Jieying Li, MS; Yi-Shuan Li, PhD; Victor W. Wong, MD; Shu Chien, MD, PhD; Alexander A. Khalessi, MD, MS

Background and Purpose—Endovascular thrombectomy has shown promise for the treatment of acute strokes resulting from large-vessel occlusion. Reperfusion-related injury may contribute to the observed decoupling of angiographic and clinical outcomes. Iatrogenic disruption of the endothelium during thrombectomy is potentially a key mediator of this process that requires further study.

Methods—An in vitro live-cell platform was developed to study the effect of various commercially available endovascular devices on the endothelium. In vivo validation was performed using porcine subjects.

Results—This novel in vitro platform permitted high-resolution quantification and characterization of the pattern and timing of endothelial-cell injury among endovascular thrombectomy devices and vessel diameters. Thrombectomy devices displayed heterogeneous effects on the endothelium; the device performance assessed in vitro was substantiated by in vivo findings.

Conclusions—In vitro live-cell artificial vessel modeling enables a detailed study of the endothelium after thrombectomy and may contribute to future device design. Large animal studies confirm the relevance of this in vitro system to investigate endothelial physiology. This artificial vessel model may represent a practical, scalable, and physiologically relevant system to assess new endovascular technologies.

Key Words: endothelial cells ■ stroke

Cerebrovascular disease affects >6 million US adults,1 with these numbers expected to increase as the population ages in the coming decades.2 The cerebrovascular system is particularly sensitive to injury, with disruptions in cerebral perfusion being the fourth leading cause of death in the United States.1 Timely restoration of normal blood flow and reconstitution of cerebral macrovasculature is critical in patients with cerebrovascular pathology. Endovascular interventions have shown promise for a variety of cerebrovascular diseases, and thrombectomy procedures continue to be a subject of ongoing research and trials for the treatment of ischemic stroke resulting from large-vessel occlusion.3–7

Early clinical trials studying mechanical embolectomy demonstrated a decoupling of clinical outcomes with the degree of angiographic reperfusion4–10; these findings may reflect challenges in patient selection, inadequate reperfusion with first-generation devices, or reperfusion-related injury.3,7 Clinical trials with second-generation devices are currently ongoing to elucidate the role of adequate, timely mechanical reperfusion in patients with large-vessel strokes.4,5 Given this clinical need, vascular endothelial cell (EC) damage resulting from altered flow dynamics, reperfusion injury, or iatrogenic trauma represents a potentially important source of secondary neuronal injury that merits further study.11,12 As modern thrombectomy devices focus on rapid and complete revascularization, considerations of the degree of EC injury have substantial clinical and design implications.

The cerebral endothelium is a dynamic biological system that regulates blood brain barrier (BBB) permeability, auto-regulates cerebral microcirculation via nitric oxide pathways, and mediates cerebral inflammation via release of tumor necrosis factor-α, interleukin 1β, and interleukin 6. We have previously demonstrated that physiological blood flow and resultant shear stress are crucial for EC maintenance and remodeling after injury13–22 and that locally disrupted blood flow contributes to inflammatory cascades.23 Many of the proposed mechanisms of reperfusion injury after acute ischemic stroke also originate at the EC level.

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1099
Breakdown of the BBB in ischemic stroke begins after the thrombotic disruption of pulsatile flow over the endothelium. This causes decreased EC expression of vascular endothelial cadherin and β-catenin, which are critical components of BBB tight junctions. Thrombosis also causes EC release of proinflammatory cytokines, including tumor necrosis factor-α, interleukin 1β, and interleukin 6. Together, these processes facilitate resident glial–cell activation and infiltration of circulating leukocytes, thus increasing the local release of cytotoxic molecules and exacerbating ischemic cerebral injury. Decreased nitric oxide production by injured ECs can also adversely affect distal collateral flow, on which penumbral tissue relies in the setting of large-vessel occlusion.24–26

Importantly, the timely restoration of pulsatile flow over the endothelium without mechanical disruption can promote rapid endothelial repair and BBB restoration within 24 hours.27,28 However, direct EC injury during mechanical thrombectomy may exacerbate the activation of deleterious pathways, highlighting the necessity for devices designed to minimize EC injury.

Thrombectomy devices are currently assessed in glass or plastic tubing in vitro and in vivo models before clinical trials. Although transparent glass and plastic tubing provides direct luminal visualization, dimension consistency, and ready availability, these materials are acellular and fail to recapitulate blood vessel biology. In vivo animal studies are performed on realistic vessels, but vessel opacity prevents direct observation of the interaction between the devices and ECs; furthermore, these vessels vary in dimensions and animal studies are costly. In vivo vessels must also be sectioned for histopathologic analysis, and therefore, they cannot provide 3-dimensional (3D) patterns of injury and are prone to processing artifacts. To address these problems, we have developed a novel in vitro live-cell platform that allows for direct visual characterization of EC injury patterns and mechanisms in the setting of mechanical thrombectomy for acute stroke. The validity of this platform was confirmed through in vivo porcine experiments. As such, this in vitro model could ultimately augment or supplant animal experiments as a new approach to assess endovascular technology.

Materials and Methods

Creation of In Vitro Live-Cell Platform

The in vitro live-cell platform was created briefly as follows (full depiction is given in the Materials and Methods section in the online-only Data Supplement). Vessels for the in vitro live-cell platform were created from optically clear and biocompatible tubular silicone substrates custom-manufactured by Specialty Silicone Fabrications (Paso Robles, CA), with a thickness of 0.25 mm and inner diameters of 2.5, 3.5, and 4.5 mm. A cell suspension of $10^5$ cells/mL of bovine artery ECs was perfused into the platform, with a monolayer of $10^3$ cells/cm² achieved before being subjected to bioreactor flow. Vessels were mounted into the bioreactor chamber (Figure 1A) and connected to a media reservoir generating peristaltic flow at 30 mL/min. This flow rate approximates 30% of the normal middle cerebral artery flow, consistent with partial flow restoration during stent-retriever deployment.29 To confirm the presence of a confluent monolayer and EC alignment in the direction of flow, cells were stained with a live-cell tracking agent, MitoTracker Red (Life Technologies), and a nuclear counterstain, Hoechst dye (Sigma-Aldrich). Bright field fluorescent MitoTracker Red and Hoechst images were then acquired (Figure 1B–1D).

In Vitro Platform

Porcine blood clots were introduced into the lumen of the in vitro live-cell platform under flow stasis and allowed to integrate for 10 minutes. The integrity of the ECs was confirmed before and after the introduction of the clots (Figure 2A). Various clot-retrieval devices were then deployed, and after device integration into the clot, the clots and devices were removed (Figure 2B). The features of the devices interacting with the ECs in the in vitro platform were visualized (Figure 2C).

Six thrombectomy devices were tested across 3 vessel diameters (2.5, 3.5, and 4.5 mm) in vitro: (1) 5MAX ACE (Penumbra, Inc, Alameda, CA) with A Direct Aspiration first Pass Technique (ADAPT), (2) MERCI (3.0 firm; Concentric Medical Inc/Stryker Corp, Kalamazoo, MI), (3) 5MAX with Separator (Penumbra, Inc), (4) 5MAX with Separator 3D (Penumbra, Inc), (5) Solitaire FR (4x20 mm; Covidien, Ltd, Mansfield, OH), and (6) TREVO (Stryker Corp), n=4 for all experimental conditions and n=3 for controls. A full description of these experiments is provided in the Materials and Methods section in the online-only Data Supplement.

In Vivo Vessels

All animal work was performed in accordance with Institutional Animal Care and Use Committee protocols (surgical details are available in the Materials and Methods section in the online-only Data Supplement). Two healthy adult pigs were used for in vivo validation of the live-cell model. After anesthetic induction, femoral artery cut downs were performed and a large porcine blood clot was introduced using an 8-French balloon guide catheter into the common carotid after temporary balloon occlusion.

After the restoration of flow to allow thrombus embedding, a single pass was made with each thrombectomy device tested. Four thrombectomy devices were tested in vivo: (1) 5MAX ACE (Penumbra, Inc) in the ADAPT technique, (2) 5MAX with Separator 3D (Penumbra, Inc), (3) Solitaire FR (4x20 mm; Covidien, Ltd), and (4) TREVO (Stryker Corp). The Trevo was deployed with the Trevo Pro 18 L microcatheter, and the Solitaire device was deployed through a Rebar microcatheter. After thrombectomy, the affected vessel was isolated and excised, and the pigs were euthanized in accordance with Institutional Animal Care and Use Committee protocols. Importantly,
the excised portion of vessel was never exposed to wire or a guide catheter during thrombus placement, and the entirety of the thrombectomy pass was centered within the vessel segment that was ultimately excised for analysis. For thrombectomy procedures, representatives from each company were present to certify the proper use of each device.

**Histochemistry**

**In Vitro Platform**

After in vitro thrombectomy, vessels from the live-cell platform were fixed with 4% paraformaldehyde in phosphate buffered saline and stained with rhodamine phalloidin (Life Technologies) at 1 U/mL in antibody-diluting buffer containing a nuclear Hoechst dye (Sigma-Aldrich) at 5 μg/mL in phosphate buffered saline.

**In Vivo Vessels**

After excision, vessels from in vivo experiments were fixed in 4% paraformaldehyde in phosphate buffered saline. The fixed vessels were embedded in optimal cutting temperature compound and cryosectioned at 10-μm thickness. Sections were stained with a Hoechst nuclear dye (Sigma-Aldrich) at 5 μg/mL in phosphate buffered saline and an antibody for the EC marker, von Willebrand factor, at 1:100 dilution (Abcam, Cambridge, MA).

**Image Acquisition**

**In Vitro Platform**

A novel rotational-scanning image system was designed to image the entire intact artificial vessel using a scanning microscope (Nikon, Melville, NY; Figure 3A). The vessel was sandwiched between a cover glass and a substrate glass. The opposite but equal motion of the 2 glasses rotated the vessel over the microscope lens, allowing a complete scan of the entire vessel without cutting the vessel open.

**In Vivo Vessels**

Stained sections of vessels from in vivo experiments were imaged using a scanning fluorescent microscope (Nikon).

**Data Analysis**

Consecutive images from the scanning of in vitro constructs and in vivo vessel sections were montaged and reconstructed for 3D visualization using Matlab (Mathworks, Natick, MA). The number of cells in each in vitro construct was quantified in an unbiased fashion using pattern recognition software (CellProfiler; Broad Institute,
Cambridge, MA) and normalized to control, uninjured constructs. A blinded pathology assessor manually counted the number of endothelial nuclei in the in vivo vessel cross-sections.

Statistics
Data are presented as mean±SEM.

Results
Novel In vitro Live-Cell Platform Enables Controlled Study of EC Physiology and Real-Time Injury Monitoring
To reproducibly study EC biology in the setting of physiological flow, a novel in vitro live-cell platform was developed (Figure 1A). The viability and behavior of ECs in this system were subsequently tested, with confluent sheets of ECs shown to align in the direction of bioreactor-induced flow (Figure 1B–1D). These findings are in line with native EC physiology, indicating successful recapitulation of in vivo EC characteristics with this model.

The transparent nature of this platform allowed for real-time visualization of ECs (Figures 1 and 2). Such monitoring is critical for confirming the EC viability before and after the introduction of the clots (Figure 2A) and determining specific locations of EC damage resulting from thrombectomy devices (Figure 2C).

Device-Specific EC Injury Patterns After Thrombectomy are Visible Using In vitro Live-Cell Modeling
We next assessed the effect of commercially available thrombectomy devices on the endothelium using the in vitro live-cell platform. Constructs with various tube diameters (2.5, 3.5, and 4.5 mm) were tested to provide information on the spectrum of vessel sizes likely to be encountered in the clinical setting. Whole-construct fluorescent scans after thrombectomy with 6 different devices (5MAX ACE, Separator, Trevo, Solitaire, Separator 3D, and Merci) showed clear differences in the character and degree of EC damage (Figure 3A and 3B). 3D-vessel reconstructions further demonstrated this inter-device variability (Figure 4A; Movie I in the online-only Data Supplement). In these experiments, total cell area was used to quantify EC density via the cytosolic stain Mitotracker Red, which is compatible with long-term live-cell experiments. The direct proportionality of the cell area to nuclei counts suggests that cell area is an effective method to quantify EC density (Figure 4B). The in vitro model thus accurately quantified the magnitude of EC damage and provided important technical information about the patterns of injury to guide device design and performance.

The differential effect of specific devices on the endothelium was even more apparent when compared across vessel sizes (Figure 4C), with the differences being most prominent in vessels with smaller calibers. Specifically, the Merci device denuded the largest EC area and served as a positive control. The Separator 3D denuded the second largest area of ECs on average. The stent retrievers (Solitaire and Trevo) demonstrated a similar, medium degree of EC denudation across all vessel diameters; the 5MAX ACE with the ADAPT technique and the Separator 3D led to the lowest cell areas denuded.

Characteristic injury patterns were observed for different devices. The Merci retriever caused a nearly complete denudation of the endothelium, whereas the Separator 3D resulted in a severe linear EC damage pattern. Both injury patterns were

Figure 4. Thrombectomy devices heterogeneously effect the endothelium. A, Representative 3D reconstructions of full vessel scans. B, Confirmation of the correlation between fluorescent whole-vessel scans and endothelial cell density. C, Cumulative in vitro post-thrombectomy procedure, cell area comparisons.
most prominent in smaller caliber vessels. Thrombectomy with each of the stent retrievers resulted in a linear pattern of excoriation. There were nonetheless differences among these devices, as the Trevo resulted in a predominantly distal pattern of denudation that was most prominent in smaller vessels. Conversely, thrombectomy with the Separator 3D resulted in solitary thin linear excoriations that were similar for all vessel diameters. Finally, thrombectomy with the 5MAX ACE using the ADAPT technique resulted in a focal circumferential injury that was similar at each diameter.

These findings highlight the heterogeneity of endothelial injuries caused by different thrombectomy devices and support the use of the in vitro live-cell model for translational EC study and device improvements to minimize EC trauma.

In Vitro Live-Cell Modeling of Device-Specific EC Injury Patterns Can Predict In Vivo Injury

The predictive value of the in vitro live-cell platform was assessed through similarly designed in vivo experiments (Figure 5A; Movies II and III in the online-only Data Supplement). Specifically, the number of intact ECs was quantified in porcine vessels after thrombectomy procedures using cross-sectional histology and immunohistochemical staining for the EC marker von Willebrand factor, with nuclei separately stained with Hoechst dye.

In agreement with our in vitro findings, quantification of intact ECs in in vivo experiments revealed that the density of intact ECs was high in vessels treated with the 5MAX ACE but lower in the Solitaire, Trevo, and Separator 3D. Also in agreement with our in vitro experiments, qualitative differences in intact EC patterns were visible across devices in vitro cross-sectional analysis (Figure 5B and 5C), although it was harder to visualize larger injury patterns in vivo. These data validate the in vivo predictive power of our in vitro model and highlight the use of high-resolution 3D-vessel reconstructions for the study of EC injury.

Discussion

Mechanical thrombectomy remains an important area of stroke research for patients with large-vessel occlusion who are ineligible or refractory to systemic thrombolytic therapy. Endothelial injury during mechanical thrombectomy may contribute to the observed decoupling of clinical and angiographic outcomes in ischemic stroke. Mechanical disruption of the BBB, loss of large-vessel intermediate filament compliance as a functional unit, and the resultant inflammatory responses initiated at the EC level remain potential sources of secondary neuronal injury.26–28

Our in vitro model provides novel quantitative and qualitative information about the degree and pattern of injury as it pertains to device design and vessel diameter. In this model, we were able to quantitatively evaluate the degree of EC injury in an intact vessel based on cell area and characterize the pattern of injury with 3D-rotational scanning microscopy. Subsequent in vivo validation of device-specific endothelial injuries was consistent with previous reports examining a limited subset

Figure 5. In vivo experimental design and confirmation of the effect of thrombectomy devices on the endothelium. A, Depiction of in vivo experimental setup. The common carotid was accessed with an 8-French balloon guide catheter via a femoral puncture, with radio-opaque markers used to denote the distal vessel segment not traversed with wire or a guide catheter (i). The balloon was then inflated to interrupt arterial flow, and a large porcine blood clot was injected through the guide catheter (ii). The balloon was then deflated to restore arterial flow and allow thrombus embedding before thrombectomy and subsequent vessel excision for processing (iii). B, Immunohistochemical staining of vessel cross-sections for the endothelial cell (EC) marker von Willebrand factor (vWF; green; nuclei stained blue) redemonstrates the heterogeneity of devices. Whole-vessel imaging is nonetheless challenging with in vivo modeling. Scale bar, 500 μm. C, In vivo post-thrombectomy EC counts display heterogeneity across devices predicted by in vitro studies. The number of section samples quantified for 5Max, Trevo, Solitaire, and Separator 3D are n=8, 21, 20, and 24, respectively.
of these devices. These studies also highlight the limitations of animal work for discerning larger patterns of injury and their relationship with the design and use of each device. This is because the multicell layer nature and opacity of the in vivo vessels make transluminal imaging nearly impossible. Hence, EC evaluation in animal experiments commonly relies on cross-sectional imaging of the vessels. In such histological studies, factors such as embedding, slicing, and staining can all induce damage to the ECs and obscure underlying patterns of injury. The sample size, frequency, and location of the cross-sections can further introduce artifact. Advanced technologies, such as confocal and laser ablation imaging, may potentially provide full vessel images, but the vessels would have to be cut or destroyed during the process. The use of these technologies would further exaggerate the labor-intensive and costly nature of in vivo experimentation. In contrast, our in vitro model is readily available and cost-effective, while providing quantitative imaging on live ECs in intact whole vessels (Table).

In addition, the mechanical information about microscopic EC injury during device application provided by this platform cannot be achieved with any existing in vivo methodology. For example, using our in vitro model, we determined that the sharp distal taper of the Trevo device results in greater distal EC injury during device deployment. The device is typically machined from a tube, and therefore, it harbors a taper that is eccentric to one side. Our results suggest that a midline taper and more graduated angle to the shoulders of the device would reduce its EC trauma profile.

Our data also indicate that the degree of EC injury is related to the vessel diameter in a device-specific manner (Figure 4). For example, we found that the EC trauma profile for the Solitaire FR device varied dramatically across in vitro vessel sizes. This finding illustrates the importance of applying devices that properly match the dimension of the vessels to reduce EC damage. Although all thrombectomy methods led to greater EC injury in smaller vessels, the unconstrained distal tines of the Solitaire FR carried comparatively high EC trauma consequences. These results suggest that the Solitaire could particularly benefit from the development of smaller and intermediate diameter devices.

Injury pattern data also provide important information for future trial designs. Although it is intuitive that the minimal linear injury caused by the Separator 3D is preferable to the large, circumferential injury caused by the positive control MERCI, the functional effects of such injuries on downstream neuronal survival remain largely unknown. The implications of the more subtle pattern-based differences, such as the minimal linear injury associated with the Separator 3D when compared with the minimal circumferential injury from the 5MAX ACE using the ADAPT technique, also remain to be explored. The authors hypothesize that circumferential injuries may exert greater effects on the performance of the large intracranial vessel segment as a functional unit in the maintenance of a stable end-organ pressure head between systolic and diastolic phases. However, the significance of the pattern and degree of EC injury remain important areas of future research.

Because this platform studies only the endothelium, the effect of thrombectomy-related damage to underlying vessel structures, such as the internal elastic lamina, media, and adventitia, may be missed. Nonetheless, our in vivo work and previous studies testing thrombectomy devices confirm that damage to deeper structures is rare. Moreover, as secondary neuronal injury results in part from activation of deleterious cytokine cascades, the effect of thrombectomy devices on the highly bioactive endothelium likely outweighs that of deeper arterial wall structures. The relatively reduced presence of these deeper arterial structures within the cerebral arterial system further diminishes their likely overall effect on clinical outcomes. Recapitulating the native EC–substrate adhesion forces under in vitro conditions is also challenging. Although fibronectin-coated silicon (and other synthetic substrates) are established methods for in vitro assessment of EC behavior, comparisons of in vitro to in vivo EC adhesion forces are beyond the scope of this work. The true value in this platform is nonetheless in determining relative differences between thrombectomy devices. Our in vitro device-specific data were highly reproducible and were confirmed through in vivo experiments; this rigorous quantitative approach affirms the translational potential of this platform for iterative device testing and improvement. These methods hold similar promise for assessing the effect of other endovascular devices, such as stents, on the endothelium. Ultimately, the tight coupling of in vivo and in vitro results implies a practical test of adhesion forces and model fidelity comparable with live vessels.

### Table. Advantages of In Vitro Live-Cell Platform for Endovascular Device Testing

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<th>Glass/Plastic Tubing</th>
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EC indicates endothelial cell.

### Summary

EC injury is an important, but incompletely understood, source of proinflammatory factors and BBB breakdown in ischemic stroke, which has been implicated in reperfusion injuries. In our in vitro live-cell model permits high-resolution quantitative study of the pattern and degree of EC injury in mechanical thrombectomy. This platform was validated through in vivo animal work and provides new insights into the pattern and timing of EC injury not readily accessible in in vivo studies. Irrespective of the implications of injury characteristics, EC disruption can be minimized based on lessons from our in vitro findings. With recommendations, including the elimination of eccentric tapering and the use of appropriately sized thrombectomy devices, this in vitro platform offers valuable lessons for the engineering of future devices. Investigations on the downstream effects of EC injury are needed, and they may fundamentally alter the techniques and devices used for thrombectomy in acute ischemic stroke. Ultimately, the current high degree
of technical success in clot removal affords the luxury of pursuing similar angiographic results while minimizing endothelial trauma and perturbation of a complex biological system. Artificial vessel assessments may ultimately reduce the need for animal testing and limit a potentially important source of secondary neuronal injury.

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**Disclosures**

A.A. Kahlessi holds consulting arrangements for physician training with Stryker Neurovascular, Covidien, and Penumbra. A.K. Kahlessi has no direct financial interest in the outcome of this line of research inquiry. The other authors report no conflicts.

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Endothelial Trauma from Mechanical Thrombectomy in Acute Stroke: An In Vitro Live Cell Platform with Animal Validation

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Supplemental Video I: Representative full 3D reconstructions of vessel scans following thrombectomy using the in vitro live cell platform.

Supplemental Video II: Representative angiographic visualization of porcine left common carotid artery with complete occlusion following clot injection.

Supplemental Video III: Representative angiographic visualization of left common carotid artery immediately following thrombectomy with the 5 MAX with 3D Separator, demonstrating complete restoration of flow.

Key Words: stroke, endovascular thrombectomy, endothelial cell

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Supplemental Materials and Methods:
Creation of In Vitro Live Cell Platform
Silicone Tubing Treatment
Vessels for the in vitro live cell platform were created from optically clear and biocompatible tubular silicone substrates custom-manufactured by Specialty Silicone Fabrications (Paso Robles, CA), with thickness of 0.25 mm and inner diameters of 2.5, 3.5, and 4.5 mm. To improve cell attachment, the inner surfaces of the substrates were first soaked in 10% (3-aminopropyl) trimethoxysilane (Sigma-Aldrich, St. Louis, MO) in 100% ethanol overnight; then crosslinked with 0.1 mM sulfo-SANPAH (Bioworld, Dublin, OH) in water via UV activation (8 min x 2 treatments; 365 nm, 84W, at a distance of 8 cm). After washing in PBS, the substrates were coated with collagen I and fibronectin in PBS at 200 µg/ml and 20 µg/ml, respectively. The substrates were rinsed with PBS and sterilized under UV light immediately before cell seeding.

Cell Culture and Endothelial Monolayer Formation
Bovine artery endothelial cells (BAECs) were cultured under standard condition (37°C, 5% CO2) with high glucose Dulbecco’s modified eagle medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Omega Scientific, Tarzana, CA). A cell-suspension at 10^5 BAECs/ml was perfused into the protein-coated silicone substrates, which were rotated 90 degrees every 20 minutes to promote even cell attachment. A monolayer of 1000 cells/cm^2 were achieved before subjecting to subsequent bioreactor flow.

Bioreactor Setup
Bioreactor chambers were fabricated using PDMS (Dow Corning, Midland, MI) and glass plates. Two vessels were mounted to each chamber (Figure 1A) and connected to a media reservoir. The media flow in the closed bioreactor system was generated using a peristaltic pump at 30 ml/min. To confirm the presence of a confluent monolayer and endothelial-cell alignment in the direction of flow, cells were stained with a live-cell tracking agent, MitoTracker Red (Life Technologies) at a concentration of 62.5 nM in DMEM, and a nuclear counter stain, Hoechst dye (Sigma-Aldrich) at 5 µg/ml in PBS. Bright field, fluorescent MitoTracker Red and Hoechst images were then acquired (Figure 1B, C & D).

Thrombectomy Procedure
In Vitro Platform
Porcine blood clots were formed by allowing previously collected porcine blood samples to coagulate for 24 hours at 4°C. Under flow stasis, size matched clots (of larger diameter than vessel constructs to ensure circumferential occlusion) were introduced into the desired luminal site of the in vitro live cell platform using a guide catheter, and allowed to integrate for 10 minutes. The integrity of the endothelial cells was confirmed before and after the introduction of the clots via microscopy (Figure 2A). Various clot-retrieval devices were then deployed, and the features of the devices interacting with the ECs in the in vitro platform were visualized (Figure 2B,C).

Artificial vessels were generated with 2.5, 3.5, and 4.5 mm diameters. Six thrombectomy devices were tested in vitro with four trials per vessel size: (1) 5MAX ACE (Penumbra, Inc., Alameda, CA) with A Direct Aspiration first Pass Technique (ADAPT), (2) MERCI (3.0 firm) (Concentric Medical Inc/Stryker Corp., Kalamazoo, MI), (3) 5MAX with Separator (Penumbra, Inc.), (4)
5MAX with Separator 3D (Penumbra, Inc.), (5) Solitaire FR (4 x 20 mm) (Covidien, Ltd, Mansfield, OH) and (6) TREVO (Stryker Corp.). Controls were run in triplicate. In vitro thrombectomies were performed by one of two surgeons, both of whom possessing previous clinical experience in the use of all devices tested. All thrombectomy systems were used with adjunct catheter and wires consistent with their manufacturer’s specifications. Microcatheters were positioned distal to the clots, except for the Penumbra 5MAX and 5MAX ACE catheters, which were positioned proximally. The Trevo was deployed utilizing the Trevo Pro 18 L microcatheter, and the Solitaire device was deployed through a Rebar microcatheter.

**In Vivo Vessels**

All animal work was performed in accordance with Institutional Animal Care and Use Committee (IACUC) protocols. Two healthy adult hybrid pigs (S&S Farms, Ramona, CA) were utilized for in vivo validation of the live cell model, using an adaptation of a previously described technique for selective arterial thrombus placement via catheter injection ([Figure 5A](#)) [1]. Briefly, each pig was placed in the supine position and anesthetized, first with an intramuscular injection of pre-anesthetic cocktail of Ketamine 30-35 mg/kg, Xylazine 2mg/kg, and Atropine 0.05 mg/kg. Venous access was then attained via a suitable ear, leg, or central vein. General anesthesia was induced by intravenous injection of 2 mg/kg of propofol, followed by intubation and mechanical ventilation. Anesthesia was maintained with a combination of 1-2% isoflurane and propofol. No paralytics were administered.

Following induction of complete anesthesia, femoral artery cutdowns were performed. An 8-French long sheath was placed through the common femoral artery into the distal abdominal aorta, through which an 8-French balloon guide catheter was introduced. The common carotid artery was then catheterized utilizing the guide catheter without a wire, to avoid EC damage in the distal artery (radio-opaque clamps were used as markers of the desired vessel site that had not been traversed with any endovascular instrumentation). The distal guide catheter tip was placed just proximal to the desired location, and the balloon was inflated to interrupt arterial flow. Following balloon inflation, a large porcine blood clot (generated as described above) was injected through the guide catheter and reproducibly lodged within the desired arterial segment. The balloon was then deflated to restore arterial flow and allow thrombus embedding prior to thrombectomy.

A single surgeon (senior author AAK) with clinical experience with all devices tested performed the in vivo thrombectomies. A single pass was made with each thrombectomy device tested, with representatives from each company present to certify proper use of each device. Following thrombectomy, the affected vessel was isolated and excised, and the pigs were euthanized in accordance IACUC protocols (administration of 1 ml/10 lbs of a veterinary euthanasia solution consisting of 390 mg/ml pentobarbital).
Supplemental References:

Supplemental Video Legend:

**Supplemental Video I:** Representative full 3D reconstructions of vessel scans following thrombectomy using the *in vitro* live cell platform.

**Supplemental Video II:** Representative angiographic visualization of porcine left common carotid artery with complete occlusion following clot injection.

**Supplemental Video III:** Representative angiographic visualization of left common carotid artery immediately following thrombectomy with the 5 MAX with 3D Separator, demonstrating complete restoration of flow.
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