ORIGINAL RESEARCH

High-Resolution Imaging of Interaction Between Thrombus and Stent-Retriever in Patients With Acute Ischemic Stroke

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***Background*—**Currently, acute ischemic stroke is still a leading cause of mortality and morbidity. Approximately 2 years ago, mechanical thrombectomy was proven beneficial as a revolutionary new therapy for stroke in the MR-CLEAN trial (A Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands). However, the mechanisms by which the thrombectomy device, or stent-retriever, interacts with the thrombus are largely unknown. A better understanding could lead to improved efficacy of mechanical thrombectomy devices.

***Methods and Results*—**Seven stent-retrievers with thrombi still entrapped were collected directly after thrombectomy. The stent-retrievers were studied using micro computed tomography, followed by scanning electron microscopy and light microscopy. Two independent observers rated interaction type and thrombus surface structure (porous filamentous or dense) at the interaction sites. A total of 79 interaction sites between thrombus and stent-retriever were categorized. Thrombus-stent-retriever interaction was found to be adhesive (n=44; 56%) or mechanical (n=35; 44%). Adhesive interaction was most frequently observed at interaction sites with a dense surface, compared with interaction sites with a porous filamentous fibrin surface (38/58; 66% versus 6/21; 29%, P=0.011).

***Conclusions*—**The interaction between thrombus and stent-retriever was predominantly adhesive, not mechanical. Adhesive interaction was strongly associated with the presence of a dense thrombus surface without a porous filamentous fibrin network. **(*J Am Heart Assoc*. 2018;7:e008563. DOI: 10.1161/JAHA.118.008563.)**

Key Words: ischemic stroke • mechanical thrombectomy • scanning electron microscopy • stent-retriever • thrombus

Ischemic stroke is one of the most important causes of neurologic morbidity and mortality. Recently it was shown that intra-arterial treatment (IAT) is highly effective in patients with acute ischemic stroke caused by a proximal intracranial arterial occlusion, and it has revolutionized stroke treatment.[1–6]

Currently, the standard approach of IAT consists of intravenous thrombolysis followed by either mechanical thrombectomy with stent-retrievers or by aspiration of thrombus with microcatheters.

While angiographic reperfusion has been reported in 70% to 90% of patients, good clinical outcome is observed only in ≈45%.[7] A better understanding of the interaction between thrombus and stent-retriever could lead to the design of strategies aimed to increase efficacy of mechanical thrombectomy.

Successful reperfusion with stent-retrievers has been associated with the extent of thrombus integration in the stent-retriever mesh.[8] Pilot in vitro data indicate that thrombus interaction may be influenced by stent-retriever design, specifically mesh size and the degree of expansion after 5 minutes.[9] Thrombus characteristics also seem to influence IAT efficacy, because erythrocyte-rich thrombi have been associated with successful reperfusion.[10–13] As such, efficacy of mechanical thrombectomy devices seems to be influenced by properties of both thrombus and stent-retriever. Based on scanning electron microscopy (SEM) and light microscopy, it was suggested that different types of interaction occur

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Accompanying Tables S1, S2, and Figures S1 through S7 are available at http://jaha.ahajournals.org/content/7/13/e008563/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- This is the first report studying the interaction between thrombus and stent-retriever after thrombectomy, in patients with acute ischemic stroke, using high-resolution imaging.
- Stent-retrievers with thrombi still entrapped were studied using micro computed tomography, scanning electron microscopy, and light microscopy.

What Are the Clinical Implications?

- We found that the interaction between thrombus and stent-retriever was predominantly adhesive, not mechanical.
- Adhesive interaction was strongly associated with a dense thrombus surface without a porous filamentous fibrin network.
- A better understanding of the interaction between thrombus and stent-retriever enables developments to optimize mechanical thrombectomy for patients with ischemic stroke.

between thrombus and stent-retriever.[14] However, not much is known about the mechanisms of interaction between thrombus and stent-retriever. The appearance of these interactions seemed not merely mechanical but often displayed thrombus coalescing around the stent-retriever struts with smooth covers of fibrin overlying erythrocyte-rich areas.[14]

In this study we aimed to explore the characteristics of thrombus-stent-retriever interaction by stent-retrievers with thrombi still entrapped, at a microscopic level using both light microscopy and SEM. We hypothesized that a correlation exists between specific thrombus surface characteristics and type of thrombus-stent-retriever interaction.

Materials and Methods

Sample Collection and Preparation

Seven stent-retrievers with thrombus material still entrapped were prospectively collected from 7 patients treated with mechanical IAT for acute ischemic stroke in 2 different stroke centers in the MR-CLEAN registry (A Multicenter Clinical Registry of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands). This registry was approved by the medical ethical committee of our hospital as a substudy of the MR-CLEAN registry (IRB approval: MEC-2014-235). All patients were provided with a written explanation of the study. The patients or their representatives were given the opportunity to refuse participation. Clot presence after initial intravenous thrombolytic treatment was confirmed by digital subtraction angiography. Clot position was unchanged compared with initial CTA (*Computed tomography angiography*) in all patients, confirming failure of intravenous thrombolytic therapy to achieve recanalization. Thrombectomy was performed by passing the thrombus with the microcatheter, followed by deployment of a stent-retriever. After a delay of 5 minutes to optimize integration of the thrombus into the stent-retriever, the stent-retriever was gently pulled back in the guiding catheter in the internal carotid artery. During stent retrieval, flow reversal in the internal carotid artery was established by balloon inflation and manual aspiration over this balloon-guiding catheter. Directly after thrombectomy, the stent-retrievers were cut from the wire, carefully rinsed in non-heparinized saline, fixed in buffered formaldehyde-glutaraldehyde for at least 48 hours in preparation for electron microscopy, rinsed and stored in 0.1 mol/L cacodylate buffer (pH 7.4, Sigma Aldrich, Zwijndrecht, The Netherlands). Samples were then photographed and stored until further processing (Figure 1A).

Micro Computed Tomography

Micro computed tomography (micro-CT) was performed on each stent-retriever with 90 kV, 200 μA, and a field of view between 20 and 40 mm (Caliper Quantum FX, Perkin Elmer, Waltham, MA). Semiautomated analysis software was used to determine the total volume of the retrieved thrombi (AnalyzeDirect v11.0, Biomedical Imaging Resource; Mayo Clinic, Rochester, MN) (Figure 1B).

Scanning Electron Microscopy

Samples were dehydrated in a graded ethanol series, dried with hexamethyldisilazane (Sigma Aldrich, Zwijndrecht, The Netherlands), and sputter coated with gold (Agar Auto sputter coater; Agar Scientific, Stansted, UK). Samples were then studied at 3 kV (JSM 6100LV, JEOL, Japan) and all identifiable interactions between thrombus and stent-retriever-struts were photographed at both high- and low-level magnification for later analysis. In a preliminary analysis in the first stent-retriever (Figure S1), the following types of thrombus surface and thrombus-stent-retriever interaction were predominantly found:

Thrombus-Stent-Retriever Interaction

1. Mechanical Interaction: Thrombus entwining around the struts, leaving spaces between strut and thrombus material (Figure 2A).
2. Adhesive Interaction: Thrombus coalescing to a stent-retriever strut, much like adhesion of a drop of water to a thread (Figure 2B).

Thrombus Surface

1. Porous filamentous surface: Thrombus having a visible porous filamentous surface at a magnification factor of at least 200× using SEM, resembling fibrin networks described in previous publications[15,16] (Figure 3A).

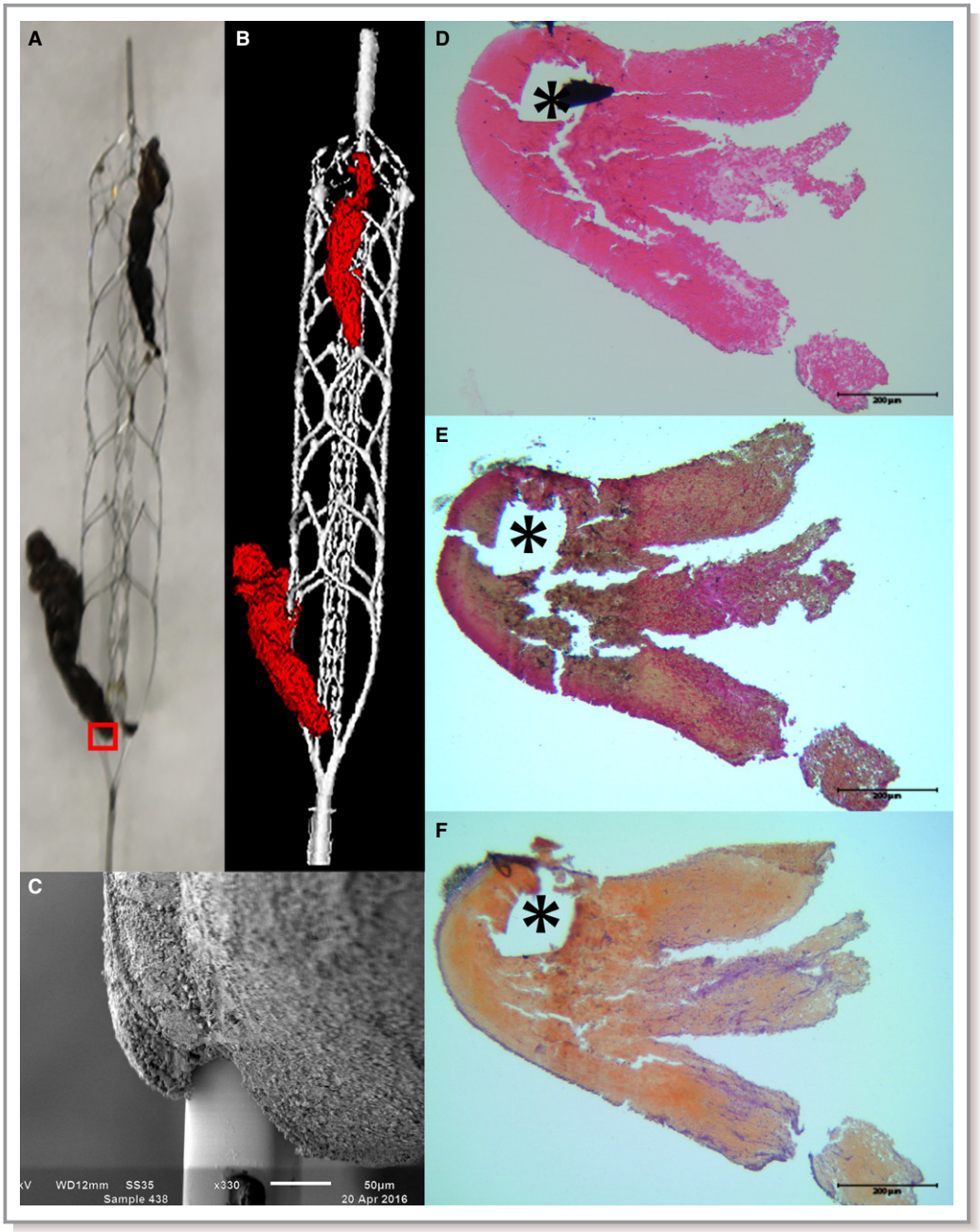

Figure 1. Macroscopy, micro-CT, and microscopy of a stent retriever. After retrieval, thrombi were photographed (A) and imaged using micro-CT (B). Each interaction site was visualized on a microscopic scale using SEM (C). Subsequently, thrombi were prepared for histology for assessment of thrombus composition: hematoxylin-eosin (D), Resorcin-Fuchsin (E), and Okajima (F). Erythrocytes are pink (D), beige (E), and orange (F). The small red box in 1A marks the location of the SEM (C) and LM images (D-F). Stent strut voids are marked with an asterisk (*). LM indicates light microscopy; Micro-CT, micro computed tomography; SEM, scanning electron microscopy.

2. Dense thrombus surface: Thrombus at which no porous filamentous fibrin network could be distinguished using SEM with a magnification factor of least 200× (Figure 3B).

Then, all stent-retrievers with thrombus still entrapped were examined for sites of interaction between thrombus and stent-retriever. These interaction sites were then evaluated for interaction type and surface properties based upon the aforementioned categories. Per thrombus, multiple interaction sites were found and rated independently. Each interaction site was allowed to have only 1 of the 2 predefined interaction and surface types.

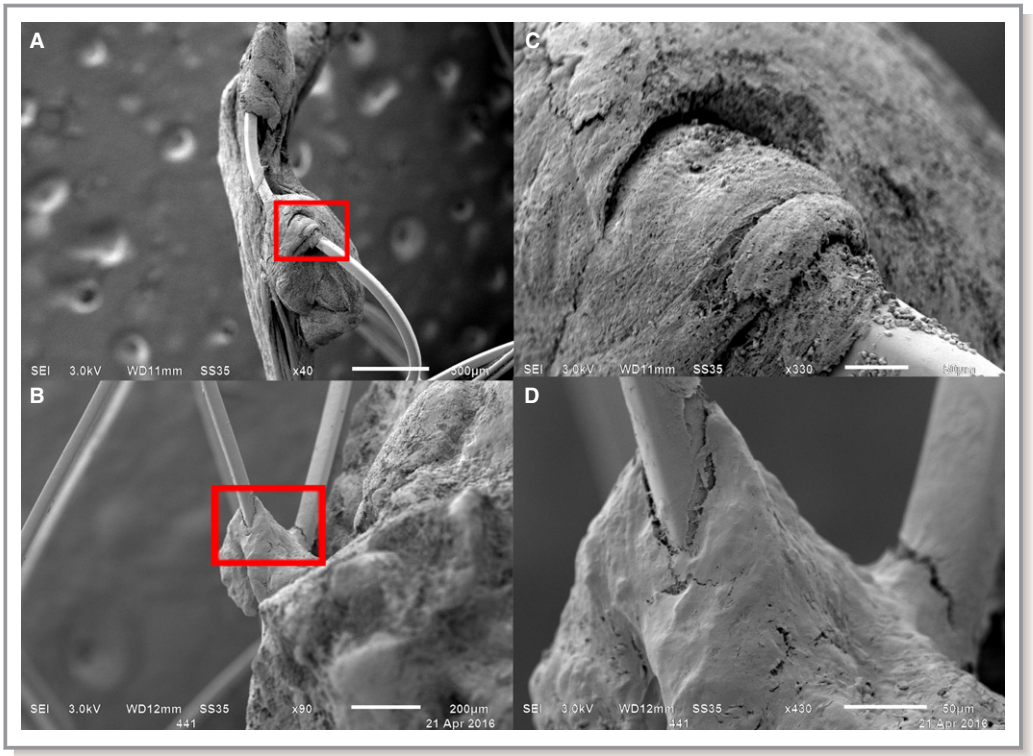

Figure 2. Stent–thrombus interaction. Per stent-retriever, generally 2 interaction types were observed: mechanical (A) or adhesive (B). C and D, magnifications of the small red boxes in (A and B), respectively.

Both interaction type and thrombus surface were independently classified by 2 researchers (A.A., H.B.) who specialized in thrombus histology. In case of disagreement, samples were reevaluated and consensus was reached.

Histologic Analysis

After SEM analysis, samples were placed in 100% ethanol and embedded in methyl methacrylate (Merck KgaA, Darmstadt, Germany) at −17° as published before.[17] Following polymerization, thin 5-µm sections were cut using a microtome (Microm 355S; Thermo Scientific, Walldorf, Germany) and mounted using butylglycol (Sigma Aldrich, Zwijndrecht, The Netherlands). Sections were dried overnight at 37°C, then deplasticized in xylene-chloroform (1:1) and stained using hematoxylin-eosin as an overview stain (Figure 1D), Resorcin-Fuchsin as an elastin stain (Figure 1E), and Okajima (Figure 1F) as a hemoglobin stain.

Thrombus composition was assessed semiquantitatively by 2 researchers (H.B. and A.E.), estimating the amount of

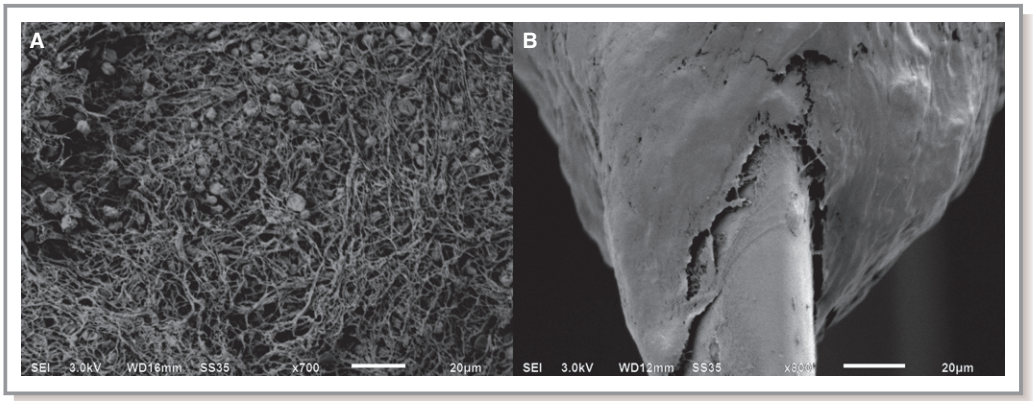

Figure 3. Thrombus surface by SEM. Two types of surfaces were recognized using SEM. A porous filamentous fibrin network was observed (A) vs a denser surface with the lack of such a network (B); magnification factors are 700 and 800, respectively. SEM indicates scanning electron microscopy.

erythrocytes versus fibrin/platelets content at multiple locations in each thrombus.

Study Data

The data, analytic methods, and study materials will not be made available to other researchers for purposes of reproducing the results or replicating the procedure. The patient consent forms do not allow for data and material sharing.

Statistical Analysis

Descriptive statistics are given as median with range for continuous variables, and as count with percentages for categorical variables. A multilevel regression analysis was performed since multiple observations originated from the same samples. A $P<0.05$ was considered statistically significant. Statistical analysis was performed using both Stata (StataCorp. 2017. Stata Statistical Software: Release 15. StataCorp LLC, College Station, TX) and SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. IBM Corp., Armonk, NY) software.

Results

Thrombus Surface Characteristics and Thrombus-Stent-Retriever Interaction

In total, 94 interaction sites (median number of interaction sites per stent-retriever 12 [5–32]) were identified and photographed in the 7 stent-retrievers using SEM. Upon analysis, 15 interaction sites were excluded, either because the specific interaction or surface type could not be established based on the photographs (n=13), or because they could not be classified under the prespecified distinctive categories (n=2), leaving 79 interaction sites (median per stent-retriever 11 [4–27]) for further analysis.

The thrombus-stent-retriever interaction was of the adhesive type at 44 (56%) interaction sites (median per stent-retriever 6 [1–12]) and of the mechanical type at 35 (44%) interaction sites (median per stent-retriever 6 [0–15]).

Overall, a porous filamentous thrombus surface was observed at 21 (27%) interaction sites, with a median of 3 (0–7) interactions per stent retriever. A dense thrombus surface, lacking such a porous structure, was observed at 58 (73%) interaction sites, with a median of 5 (3–21) interactions per stent retriever.

Adhesive interaction was more frequently observed at interaction sites with a dense surface (38 of 58), as compared with adhesive interaction at sites with a porous filamentous surface (6 of 21) (P=0.011). Surface characteristics and interaction types for each individual stent-retriever are shown in Table (see also Table S1 and Figures S1 through S7).

Table. Thrombus Characteristics

Thrombus	Volume (mm^3)	Nr. Interaction Sites	Nr. Dense Surface at Interaction Sites (%)	Nr. Adhesive Interactions (%)
1	40.9	14	11 (79)	7 (50)
2	75.3	27	21 (78)	12 (44)
3	31.4	4	4 (100)	3 (75)
4	48.4	11	4 (36)	6 (55)
5	18	11	10 (91)	10 (91)
6	11.9	5	5 (100)	5 (100)
7	10.3	7	3 (43)	1 (14)
No. or avg±SD	33.7±23	79	58 (73)	44 (61)

Thrombus volume, number of interaction sites, thrombus surface type, and interaction type per stent-retriever.

Thrombus Composition

The retrieved thrombi consisted predominantly of erythrocytes (median 87%, range 47%–95%, Table S2), but also contained platelets, fibrin, and leukocytes. Erythrocytes showed either an intact discoid shape or loss of shape. Zahn lines, which indicate the formation of thrombi under arterial flow conditions, were observed in 2 thrombi. Areas with extracellular DNA on hematoxylin-eosin staining with the same morphology as neutrophil extracellular traps suggested the presence of neutrophil extracellular traps and were observed in 5 thrombi. These areas were found in different patterns, either diffusely spread through the thrombus, in hotspots, or lining the thrombus. There was no predominant pattern observed. Cholesterol crystals were not observed. Nuclear fragmentation was present in all but 1 thrombus, indicating thrombi were older than 1 day according to the criteria by Rittersma et al.[18] Hematoxylin-eosin staining of thrombi revealed areas of different age with fibrin networks of variable density and both intact leukocytes and nonintact leukocytes.

Thrombus surface most often resembled fibrin, even if the thrombus predominantly consisted of erythrocytes (Figure S4H). A statistically significant correlation between inner thrombus composition and thrombus surface by light microscopy was not found, most likely because of the small number of cross sections per thrombus. Also, because of the limited number of cross sections, exactly corresponding SEM photographs and light microscopy histology were available at only a few interaction sites. At these sites, a clear correlation was seen between thrombus surface structure on both imaging modalities, with corresponding porous filamentous and dense surfaces and pits that tended to contain erythrocytes (Figure 4).

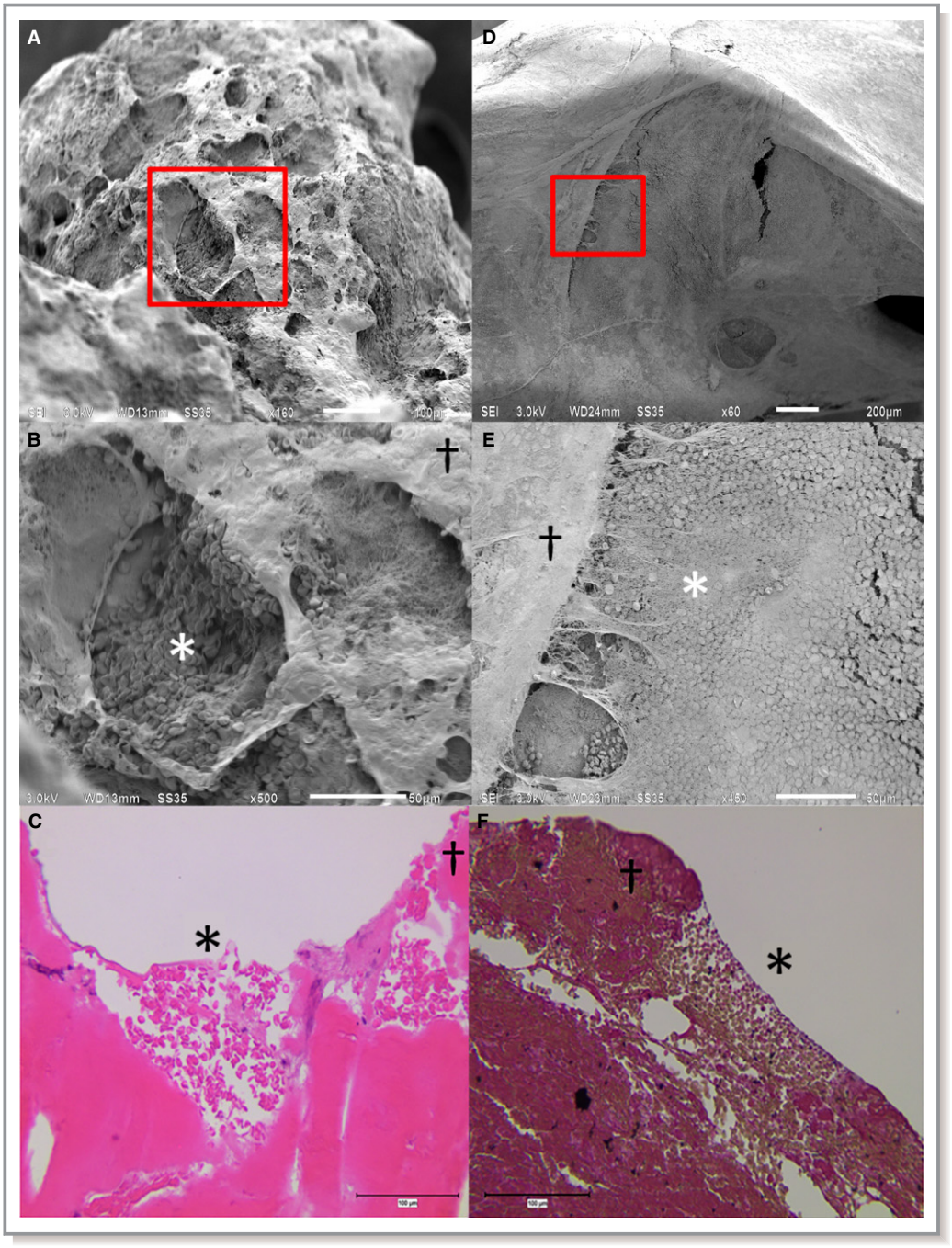

Figure 4. SEM and corresponding thrombus histology. Two examples of SEM and their corresponding LM shed light on the relation between the surface and interior of these thrombi. SEM is given as an overview (A, D). B and E, magnifications of the small red boxes in (A and B), respectively, and corresponding LM (C, F). SEM revealed both dense and porous filamentous networks on the surface of thrombi. Porous filamentous (*) and dense (†) surface areas on SEM corresponded with porous filamentous and dense surfaces on LM. Porous filamentous networks and pits (*) tended to contain erythrocytes. LM indicates light microscopy; SEM, scanning electron microscopy.

Discussion

We found that thrombus-stent-retriever interaction was predominantly adhesive, not merely mechanical. Previous studies hypothesized that the main capture mechanism is direct engagement of the clot between the crossings of stent-retriever struts, resembling merely a mechanical interaction.[8,9,19] This apparent contradiction is possibly because of the use of much higher magnifications in this study, at which these different types of interaction can be distinguished, and possibly also

because of inherent differences among in vivo, in vitro, and our in situ study design.

Secondly, we found that adhesive interaction was associated with a dense thrombus surface. The density of fibrin networks is determined by many factors, among others the initiators of thrombus formation such as thrombin, tissue factor and calcium chloride, and their concentration.[20] A dense fibrin network reduces the generation of plasmin activators and therefore reduces the degradation of the fibrin network.[21] Whether sites of adhesive interaction with a dense thrombus surface consist of original thrombus or whether they are newly formed thrombi remains to be determined. If the observed surface of the retrieved thrombus reflects the original surface of the embolized thrombus, it might reflect resistance to thrombolysis.

It remains to be determined whether thrombus composition, and specifically fibrin content, affects thrombus behavior during mechanical thrombectomy. Recent in vitro data seem to suggest that fibrin-rich thrombi containing relatively low amounts of erythrocytes are more resistant to thrombectomy, possibly because of their frictional properties.[22,23]

Since our study was limited by sample size, it might represent only a small subgroup of the acute ischemic stroke population, and future studies will have to confirm our findings. Furthermore, a selection bias was inevitable because successful thrombolysis before IAT excluded thrombi sensitive to intravenous thrombolysis therapy from analysis. Hypothetically, thrombi fully resistant to mechanical IAT are also not included because they were not retrieved.

Although the interventional radiologist was aware of study inclusion at the time of the procedure, it is of course possible that thrombus surface characteristics have been altered by thrombolytic therapy before mechanical thrombectomy or by the thrombectomy procedure itself. However, thrombi were retrieved using a large-bore guiding catheter in order to reduce mechanical strain on thrombus and stent-retriever. Also, thrombi were immediately rinsed after retrieval to prevent new clot formation from aspirated blood outside of the patient. Of course, some uncertainty remains about whether a microscopically observed interaction accurately represents the original interaction, because of the in situ nature of this study.

Conclusion

Two types of interaction between thrombus and stent-retriever are observed after thrombectomy for acute ischemic stroke: adhesive and mechanical. The predominant type of interaction is adhesive, which correlates strongly with a denser thrombus surface. A better understanding of stent–thrombus interaction could lead to improved efficacy of mechanical thrombectomy devices.

Appendix

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Disclosures

Erasmus MC received funds from Stryker® for consultations by Diederik Dippel, Aad van der Lugt, Bart Emmer, and from Bracco Imaging® for consultations by Diederik Dippel. Amsterdam Medical Centre received funds from Stryker® for consultations by Charles Majoie, Yvo Roos, and Olvert Berkhemer. Maastricht University Medical Centre received funds from Stryker® for consultations by Wim van Zwam, Bart Emmer received funds from consultations for DEKRA b.v.

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SUPPLEMENTAL MATERIALS

Table S1: Surface vs interaction using SEM. Adhesive interaction was more frequently observed at interaction sites with a dense surface (38 of 58), as compared to adhesive interaction at interaction sites with a porous filamentous surface (6 of 21) (p=0.011).

	Dense	Porous Filamentous	Total
Adhesive	38	6	44
Mechanical	20	15	35
Total	58	21	79

Table S2: Thrombus Characteristics. The presence of Zahn lines, cholesterol crystals, nuclear fragmentation is given per thrombus and determines thrombus age. In our study most thrombi were classified as lytic according to the criteria by Rittersma et al[1].

Thrombus	Thrombus content histology				
	Erythrocyte content (%)	Nuclear fragmentation	NETs	Zahn lines	Cholesterol crystals
1	55	-	+	-	-
2	88	+	-	-	-
3	93	+	+	-	-
4	87	+	+	-	-
5	95	+	+	-	-
6	73	+	-	+	-
7	47	+	+	+	-

Figure S1: Stent 1. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Both mechanical (C, D) and adhesive interactions (G) are observed using SEM at a magnification factor of 40, 60 and 230 respectively. E and F, magnifications of the small blue and red box in (C and D) revealing both porous filamentous and dense surfaces, at a magnification factor of 430 and 450, respectively. Histological staining using HE (H), RF (I) and Okajima (J) show thrombus containing both fibrin and erythrocyte rich areas. Fibrin is pink in (H), red in (I) and purple in (J).

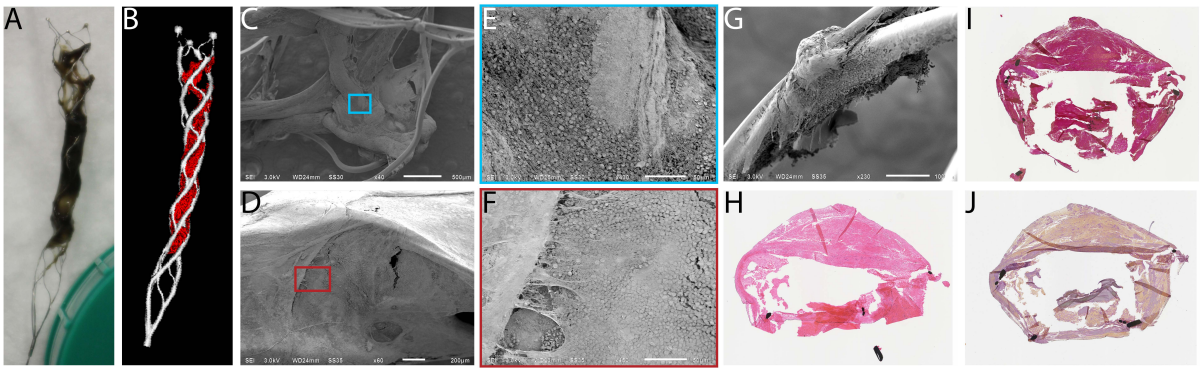


Figure S2: Stent 2. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Predominantly mechanical interaction is observed using SEM (C, D), at a magnification factor of 30 and 130, respectively. E and F, magnifications of the small blue and red box in (C and D), revealing that the thrombus surface is mainly dense (E) but spots of porous filamentous surface were also observed (F), at a magnification factor of 370 and 1100, respectively. Histological staining using HE (G), RF (H) and Okajima (I) shows thrombus containing mostly erythrocytes. Fibrin is pink in (G), red in (H) and purple in (I).

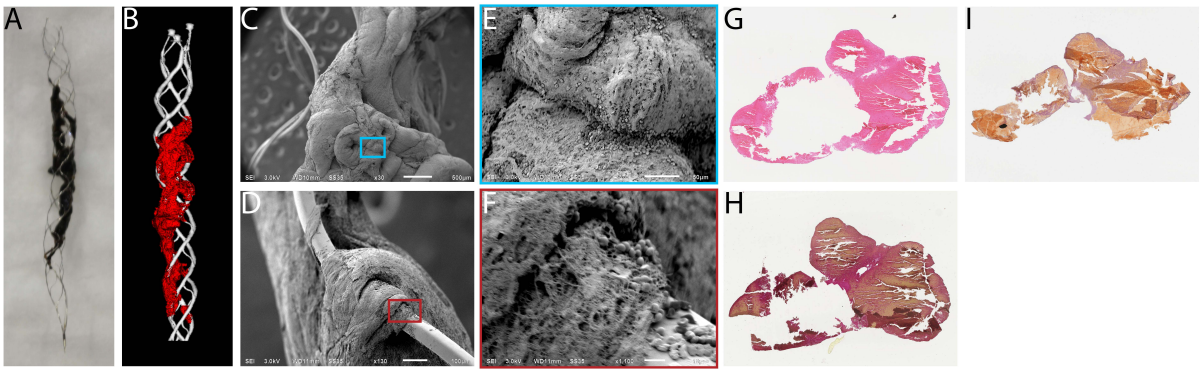


Figure S3: Stent 3. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Both Adhesive (C) and mechanical interactions (D) are observed using SEM, at a magnification factor of 120 and 100, respectively. E, F and G, magnifications of the small blue, yellow and red box in (C and D), revealing that the thrombus surface is mainly dense (E, F, G), at a magnification factor of 650, 800 and 650, respectively. Histological staining using HE (H), RF (I) and Okajima (J) show thrombus containing mostly erythrocytes. Fibrin is pink in (H), red in (I) and purple in (J).

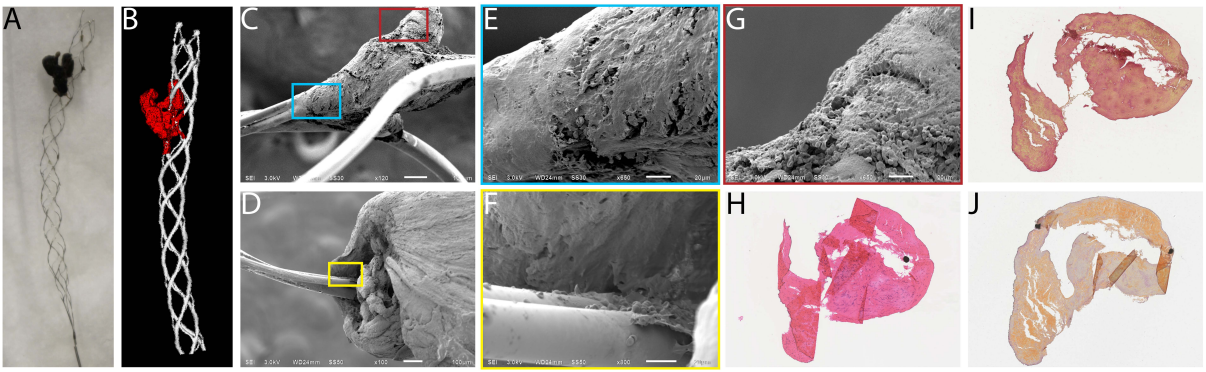


Figure S4: Stent 4. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Both mechanical (C) and adhesive interactions (D) are observed using SEM, at a magnification factor of 75 and 65, respectively. E and F, magnifications of the small blue and red box in (C and D), revealing that the thrombus surface is mainly porous filamentous (E, F), at a magnification factor of 850 and 400, respectively. Histological staining using HE (G), RF (H) and Okajima (I) show thrombus containing mostly erythrocytes. Fibrin is pink in (G), red in (H) and purple in (I).

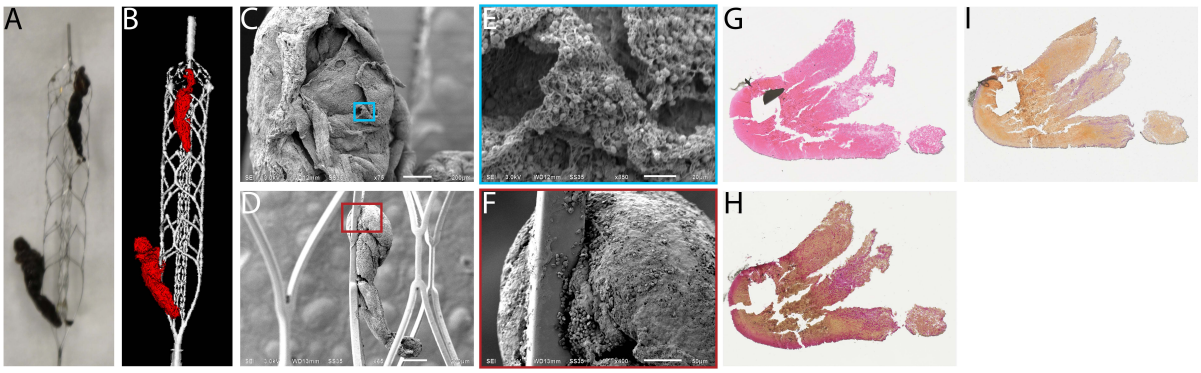


Figure S5: Stent 5. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Predominantly adhesive interaction is observed using SEM (C, E), at a magnification factor of 27 and 430, respectively. E and F, magnifications of the small blue and red box in (C and D), revealing that the thrombus surface is mainly dense (D and E) but spots of porous filamentous surface were also observed (F), at a magnification factor of 150, 430 and 750, respectively. Histological staining using HE (G), RF (H) and Okajima (I) show thrombus containing mostly fibrin in these shown sections. Fibrin is pink in (G), red in (H) and purple in (I).

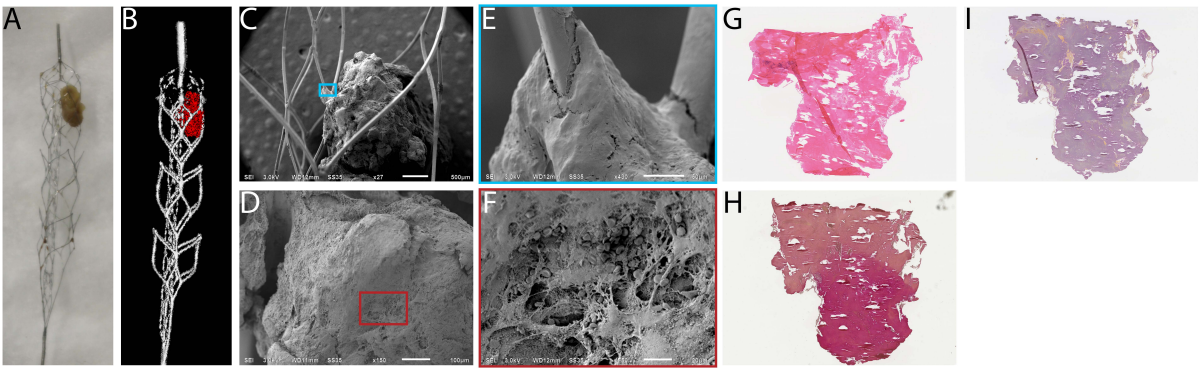


Figure S6: Stent 6. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Predominantly adhesive interaction is observed using SEM (C, D), at a magnification factor of 27 and 350, respectively. E and F, magnifications of the small blue and red box in (C and D), revealing that the thrombus surface is mainly dense (F) but spots of porous filamentous surface were also observed (E), at a magnification factor of 1100 and 600, respectively. Histological staining using HE (G), RF (H) and Okajima (I) show thrombus containing both fibrin and erythrocyte rich areas. Fibrin is pink in (G), red in (H) and purple in (I).

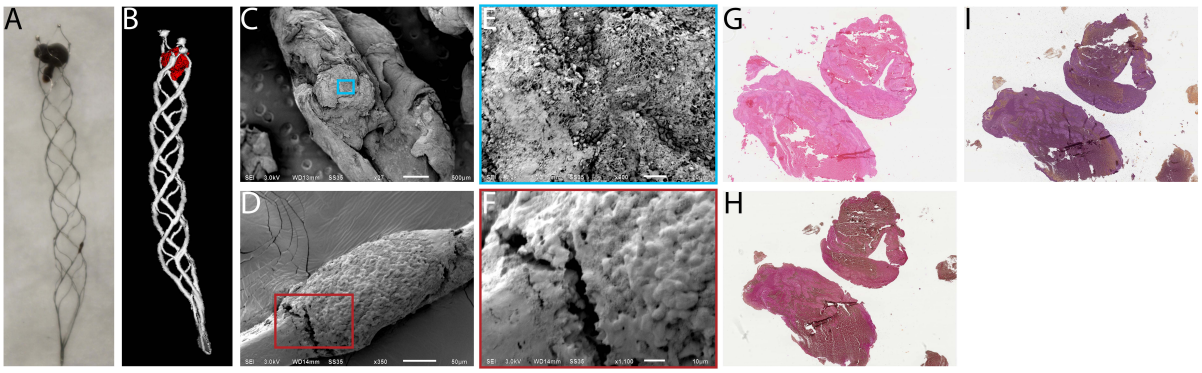
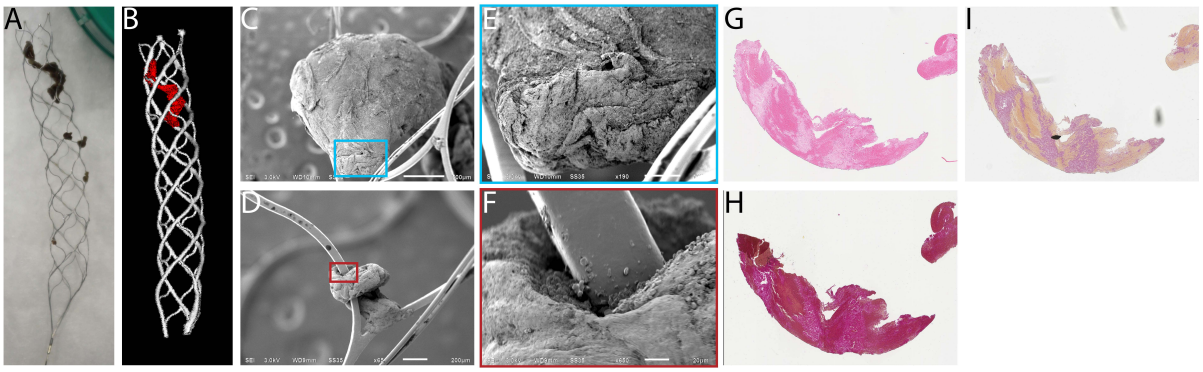


Figure S7: Stent 7. Thrombus entrapped in stent photographed (A) and visualized using micro-CT (B). Predominantly mechanical interaction is observed using SEM (C, D), at a magnification factor of 43 and 65, respectively. E and F, magnifications of the small blue and red box in (C and D), reveal both porous filamentous (E) and dense surfaces (F), at a magnification factor of 190 and 650, respectively. Histological staining using HE (G), RF (H) and Okajima (I) show thrombus containing both fibrin and erythrocyte rich areas. Fibrin is pink in (G), red in (H) and purple in (I).



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