The Clinical Relevance of In Vitro Blood Loop Testing for Fibrin Sheath and Thrombus Formation

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In vitro blood loop testing has been used to analyze the effectiveness of a variety of drugs and devices, including thrombolytic agents and indwelling vascular catheter materials.[1, 2] While investigators commonly admonish against extrapolating in vitro blood loop results to human clinical outcomes, to date there is limited information regarding the correlation of such *in vitro* results to living systems.[3, 4]

The present pilot study sought to investigate whether the apparently diminished thrombogenicity (based on in vitro blood loop testing) of a certain intravenous catheter material, BioFlo® (AngioDynamics, Inc., Latham, NY) was sustained *in vivo*, using a living sheep model. Another commonly used catheter material, ChronoFlex® C (AdvanSource Biomaterials, Wilmington, MA) was used as a control.

This study was reviewed and approved by the Institutional Animal Care and Use Committee. All work was performed at Clemson University under an IACUC approved protocol. Animal care and use at Clemson is USDA registered, OLAW assured and AAALAC International accredited. All work adhered to the AWA/USDA standards and The Guide for the Care and Use of Animals.

METHOD

Following anesthesia, intubation and maintenance of normal vital signs, the internal jugular vein of the animal was exposed. The study catheter was then inserted under direct vision according to the manufacturer's directions for use.

At predetermined intervals, the catheter was withdrawn and a 2 cm segment cut and photographed under 45X magnification (Figures 1 and 2); the catheter segment was then preserved in formaldehyde solution for subsequent electron microscopy (Figures 3, 4 and 5). Special care was taken to prevent a squeegee effect whereby fibrin or thrombus could have been scraped off during catheter withdrawal: specifically, a 1-2 mm axial incision was made through the vein wall beginning at the insertion site and extending distally. Following withdrawal of the catheter, the incision was clamped so as to achieve hemostasis without impeding flow through the vessel. The incision was subsequently unclamped prior to each withdrawal and re-clamped immediately following cutting of the exposed catheter segment.

The entire procedure was repeated precisely with the control catheter in the contralateral internal jugular vessel (Figures 6-9).

RESULTS

Figure 1 demonstrates substantial fibrin sheath formation on the study material, after 3 minutes of dwell time. The scanning electron micrograph (SEM) (Figure 3) of the 3 minute study segment shows both fibrin strands and the early aggregation of formed elements of the blood.

Figure 2 demonstrates progressive accumulation of fibrin with overlying formation of thrombus on the study material, after 30 minutes of dwell time. The scanning electron

micrograph (Figure 4, 5) confirms the heaping of formed elements upon a more dense and circumferential fibrin sheath.

The control material appears to have performed similarly if not better at 3 minutes, as seen both with 45X magnification (Figure 6) and SEM (Figure 7). Large areas of catheter material are devoid of fibrin deposition at this time.

At 30 minutes, the control material demonstrates increased fibrin deposition which appears less circumferential and less dense than the fibrin on the study material (Figures 8, 9). Moreover, unlike the BioFlo material, entrapped formed elements of the blood (e.g., red cells, white cells and platelets) are not evident.

DISCUSSION

The study material had previously demonstrated reduced thrombus formation when compared with a competitive polyurethane material in a blood loop model, suggesting its possible clinical advantage in living systems. Regrettably, dwell times from that study were not published and only low magnification photographs from a light microscope were provided.

It is possible that at dwell times under 3 minutes the study material does retard fibrin and clot formation when compared with other catheter materials. However, the clinical relevance of such a finding is unlikely. Indwelling vascular catheters are generally intended to reside within bloods vessel for days or, sometimes, months.

The present study shows that in a living system, the study material rapidly acquires a fibrin sheath which over thirty minutes ensnares formed elements of the blood. The ultimate result is thrombus formation on the external surface of the catheter.

The control material, ChronoFlex C, performed at least as well--with respect to fibrin sheath and thrombus formation--and arguably better: appearing in the earliest stages to

have retarded fibrin sheath formation and in the later stages to have forestalled the aggregation of cellular elements.

CONCLUSION

This pilot study demonstrates the hazard of extrapolating *in vitro* blood loop testing of intravenous catheter material to living systems, at least when such testing is performed over clinically significant periods of time. Specifically, in the living sheep system, BioFlo® did not appear to retard fibrin sheath or thrombus formation.

This finding underscores the wisdom of the general requirements of the British Standard, BS EN ISO 1993-4:2009, which states: "It follows from the above that devices… whose intended use is *in vivo* (e.g. implants) should be tested *in vivo* in an animal model simulating as closely as possible conditions of clinical use."[5]

The finding that ChronoFlex® C qualitatively outperformed BioFlo® with respect to both fibrin sheath and thrombus formation was unexpected. As this outcome may have important clinical implications with respect to device-related bloodstream infections, further study—including human outcome trials—would appear to be in order.[6]

DISCLAIMER

Amparo Medical Technologies, Inc. avows no financial interest in either AngioDynamics, Inc. or AdvanSource Biomaterials, Inc.

Amparo Medical Technologies, Inc. formulates/manufactures hydrocolloid for would care ostomy and device attachment. Hydrocolloid dressing is available in a variety of sizes, private labeled per customer specifications under the Amparo brand. Amparo custom formulates and can provide master rolls or die cut parts. Device attachment applications include negative pressure, catheter securement and our hitack hydrocolloid that will keep a device attached for two weeks.

BioFlo

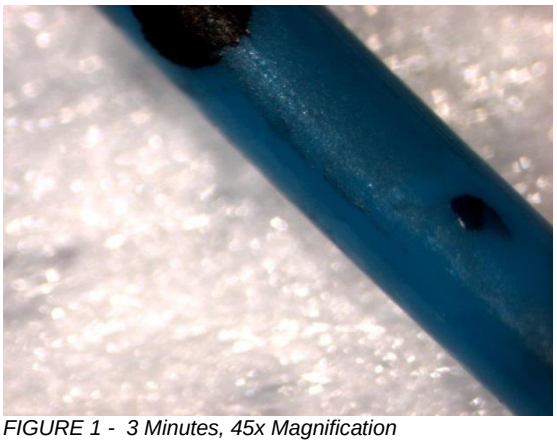

FIGURE 1 - 3 Minutes, 45x Magnification

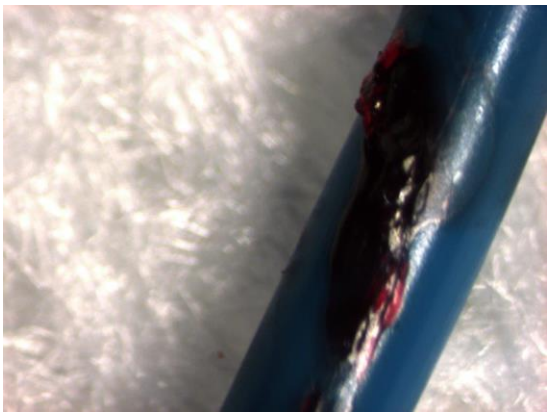

FIGURE 2 - 30 Minutes, 45x Magnification

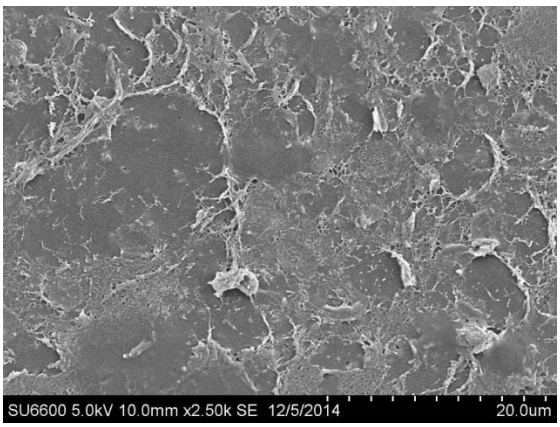

FIGURE 3 - 3 Minutes, Scanning Electron Microscope

ChronoFlex C

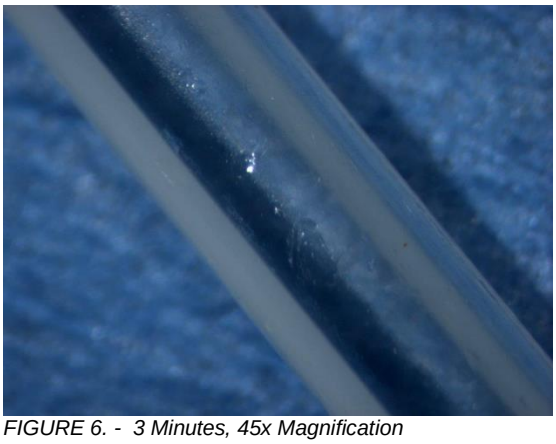

FIGURE 6. - 3 Minutes, 45x Magnification

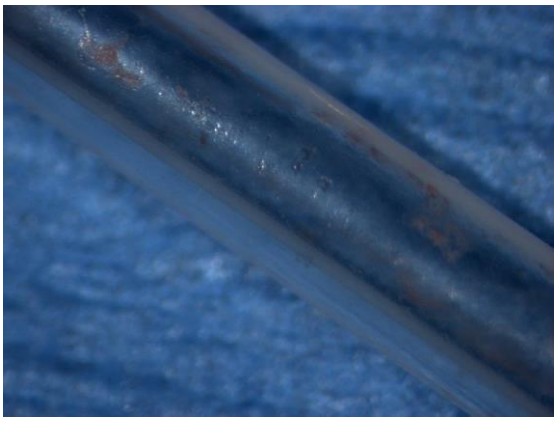

FIGURE 7 - 30 Minutes, 45x Magnification

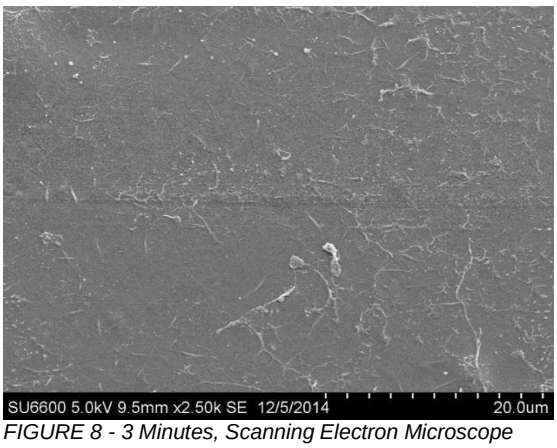

FIGURE 8 - 3 Minutes, Scanning Electron Microscope

BioFlo

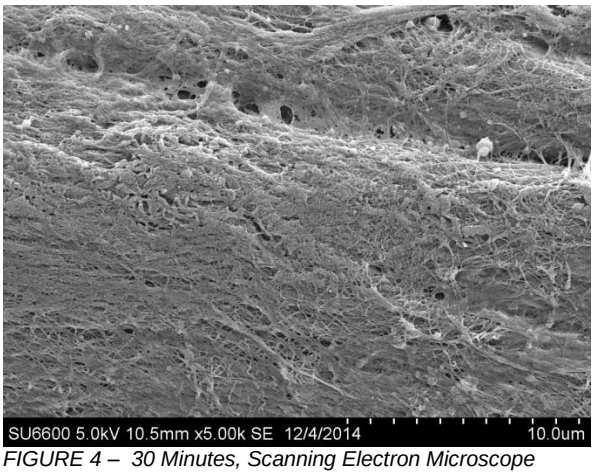

FIGURE 4 – 30 Minutes, Scanning Electron Microscope

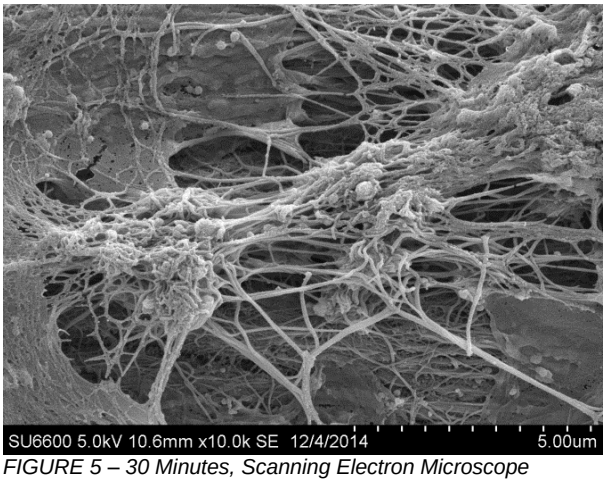

FIGURE 5 – 30 Minutes, Scanning Electron Microscope

ChronoFlex C

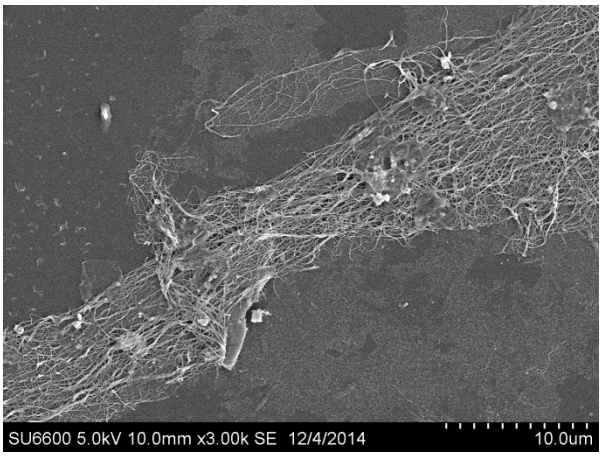

FIGURE 9 - 30 Minutes, Scanning Electron Microscope

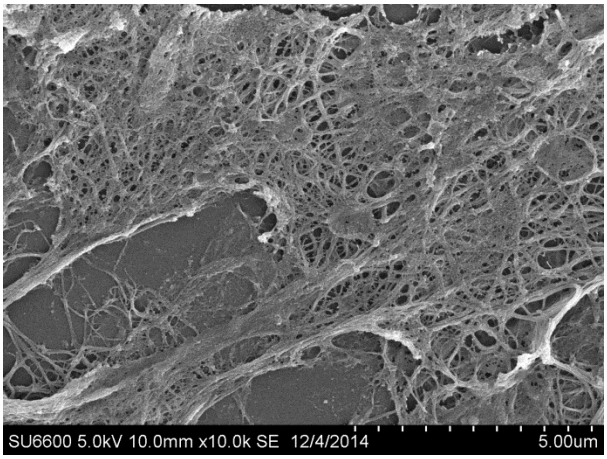

FIGURE 10 – 30 Minutes, Scanning Electron Microscope

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