EFFECT OF TRANSCATHETER AORTIC VALVE MATERIALS ON THROMBOGENESIS

A Thesis
Presented to
The Academic Faculty

by

Priya Jayadev Nair

In Partial Fulfillment
of the Requirements for the Degree
Bachelor of Science in the
School of Biomedical Engineering

Georgia Institute of Technology

EFFECT OF TRANSCATHETER AORTIC VALVE MATERIALS ON THROMBOGENESIS

Approved by:

Dr. Ajit Yoganathan, Advisor
School of Biomedical Engineering
Georgia Institute of Technology

Dr. Lakshmi Dasi
School of Biomedical Engineering
Georgia Institute of Technology

Date approved: April 30, 2020

ACKNOWLEDGEMENTS

I wish to thank my faculty advisor and research mentor, Dr. Ajit Yoganathan, for his guidance. For helping me learn and grow as a researcher, I would like to thank the members of the Cardiovascular Fluid Mechanics Lab at Georgia Tech, particularly Dr. Vahid Sadri and Md. Tausif Salim. I would also like to acknowledge the contributions of my fellow undergraduate researchers, Daniel Schwaner and Mugdha Apte.

TABLE OF CONTENTS

ABSTRACT

Transcatheter aortic valve replacement (TAVR) is a minimally invasive technique introduced to treat patients with aortic stenosis who are at high surgical risk. Despite its advantages over the traditional open-heart surgery technique, several complications including thrombosis are being increasingly reported. Much of the research so far has focused on the impact that fluid dynamics has on thrombosis. This study explores the contribution of the different foreign materials in the TAV to the initiation of thrombus formation. A steady flow loop with minimized volume was developed such that multiple experiments could be conducted with blood from a single human donor. Three conditions - control, stent-with-skirt, and whole valve - were tested using the flow loop at two different anticoagulant: reversing agent ratios (8:1 and 6:1). Serum collected from blood samples drawn at the start and end of the experiment were used to measure concentrations of D-dimer and thrombin anti-thrombin (TAT), biomarkers of thrombosis. Higher D-dimer and TAT concentrations in the stent-with-skirt compared to the valve in the 8:1 condition suggest that, over time, exposure to the stent with the skirt can shift the blood to a more prothrombotic state. Lack of significant differences between groups in the 6:1 condition indicates that when blood is already in a significantly high prothrombotic state, different materials of the TAV do not have significantly different effects on thrombus formation. The results of this study help to better understand the process of initiation of thrombus formation in TAV from a foreign materials perspective.

INTRODUCTION

Aortic stenosis is the most common heart valve disease worldwide and occurs in about 2-4% of patients over the age of 75[1,2]. The narrowing of the aortic valve (that regulates blood flow from the left ventricle to the aorta) prevents the valve from opening fully, thereby reducing or blocking blood flow to the aorta. Progressive calcification of the valve is often the cause of stenosis in patients over the age of 65, but aortic stenosis can also be observed in younger patients who are born with valvular defects (such as bicuspid aortic valves) or in patients who develop rheumatic disease[1]. The traditional approach to treating aortic stenosis has been open heart surgery to replace the stenosed valve with a mechanical or bioprosthetic valve. While effective, this approach is very invasive and involves a long procedure time and recovery time. Considering the nature of the procedure, it is not usually an option for patients at high surgical risk. To address this gap, a minimally invasive approach called transcatheter aortic valve replacement (TAVR) was introduced to treat patients with aortic stenosis. In TAVR, a compressed replacement valve is inserted through a catheter and expanded in the aortic sinus, thereby taking over the function of the native valve. TAVRs typically require shorter procedure time and are considered an effective treatment for patients with aortic stenosis who are deemed to be at intermediate or greater surgical risk and cannot undergo open heart surgery. Since the success of this procedure within the high-risk population, substantial effort has gone into expanding TAVR to younger, lower surgical risk patients.

However, evidence collected since the procedure was introduced has revealed that TAVR can lead to several complications such as paravalvular leak, prosthesis-patient mismatch and thrombosis. Thrombosis in TAVR is a major concern because the formation of blood clots within the prosthetic valve can restrict the normal leaflet motion (reported in 10 – 15% of patients with

TAVs[3,4]), rendering the TAV ineffective. The presence of clots on the leaflets can be detected using imaging modalities such as computed tomography (CT), as shown in Figure 1[5], often recognized due to increase in leaflet thickness known as hypo-attenuated leaflet thickening (HALT).

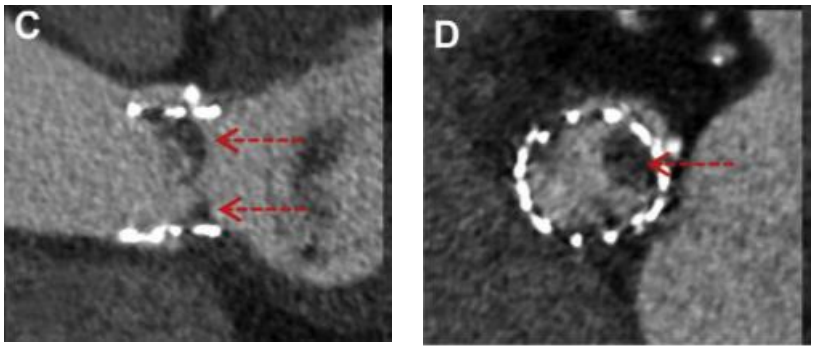


Figure 1. Post-TAVR CT in an 89-year-old male with a SAPIEN 3 prosthesis one year after procedure. Red arrows show presence of leaflet thrombosis.

Explanted TAVs have also aided in confirming the occurrence of leaflet thrombosis after TAVR, as seen in Figure 2[6].

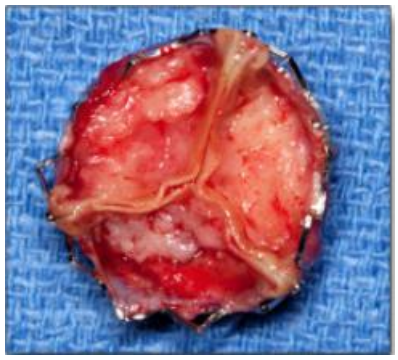


Figure 2. Explanted TAV in which thrombosis was confirmed by visual inspection and pathological analysis.

Reports indicate that the rate of leaflet thrombosis in surgical bioprostheses is between 0.8% and 4.0%[3,7] , whereas the reported rates in TAVs range from 4.5% to 40%[3,4,8-11]. Besides restricted leaflet motion, thrombus formation can also result in degeneration of the TAVR and reduced

valve durability in the long term. Additionally, blood clots formed at the site of implantation can break into micro-emboli, which may migrate into the cerebral circulation[12] and cause transient ischemic attacks. The implications of thrombosis in TAVs are severe, but several questions surrounding the initiation of thrombus formation remain unanswered.

Significant advances have been made in understanding the mechanism of thrombus mass accumulation from a hemodynamics perspective; the impact of flow stasis in the TAV neo-sinus has been studied in great detail. Midha et al.'s study[13] evaluated factors that contribute to TAV thrombosis through a combined approach of in vitro testing and retrospective postprocedural 4DCT data analysis of patients who had undergone TAVR. Using 3D reconstruction, they determined thrombus volume in these patients. They identified the neo-sinus region (between the native and TAV leaflets) to be a critical region of flow stasis and long blood residence time with high occurrence of thrombosis in this region. They also identified specific characteristics such as percentage of expansion and height of deployment in different valve types as factors that can have an impact on neo-sinus volume and therefore contribute to the risk of thrombosis. SAPIEN 3s without thrombus were under-expanded on average by a further 10% by diameter than those with thrombus (P<0.001), as shown in Figure 3. However, no such relationship existed among CoreValve/Evolut Rs.

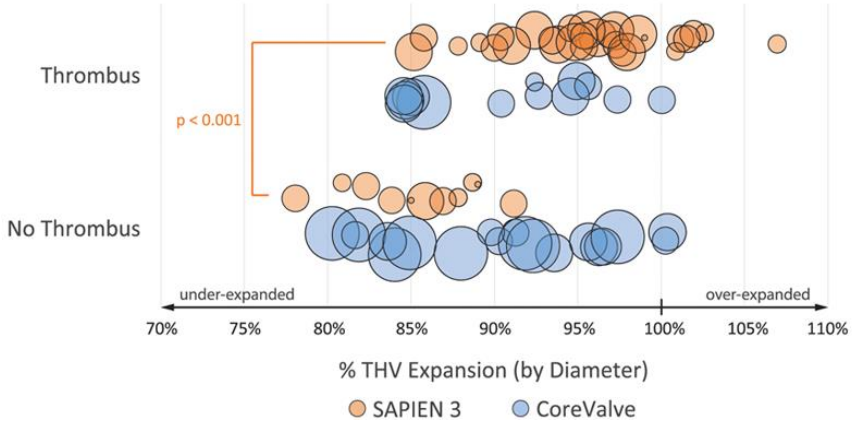


Figure 3. THV expansion versus presence of thrombus. SAPIEN 3 valve with leaflet thrombosis were, on average, 10% more expanded than those without. No such relation was observed in CoreValve/ Evolut R with or without thrombus.

No significant difference occurred in mean implant depth between valves with and without thrombus (−1.4±5.6 mm versus −3.1±2.7 mm, P=0.11). However, in patients with thrombus, implant depth did appear to influence clot volume. In CoreValve/Evolut Rs with thrombus, the thrombus volume increased with THV implant depth (R^2=0.6956, P<0.001), shown in Figure 4[13]. However, this trend did not exist with SAPIEN 3.

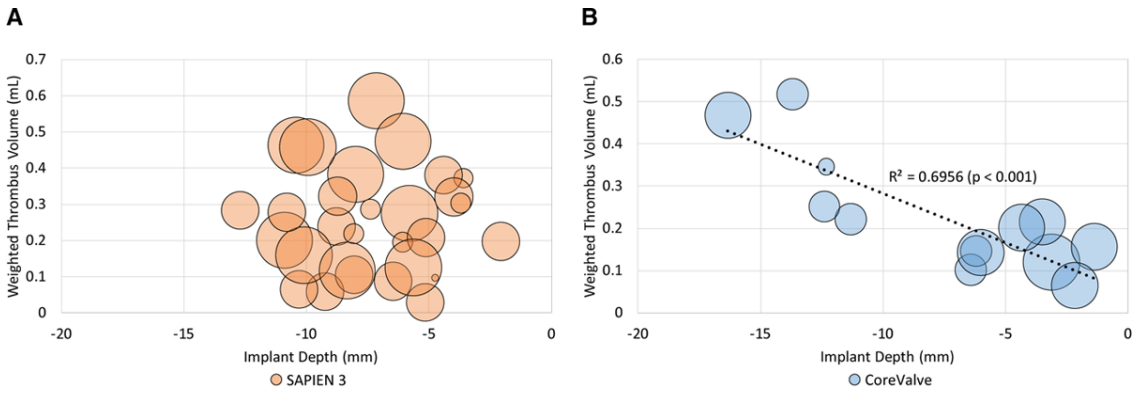


Figure 4. THV implant depth versus thrombus volume. Fig. 4A shows no relation between implant depth and thrombus volume in SAPIEN 3 valves. Fig 4B shows an increase in thrombus volume with increase in deployment depth of CoreValve/ Evolut R.

This study emphasized the significance of the neo-sinus fluid dynamics in thrombus mass accumulation.

Trusty et al. also established a strong positive correlation between neo-sinus flow stasis and thrombus volume (TV)[14]. Using data from patients with a 26 mm Edwards SAPIEN valve, thrombus volume for each neo-sinus was determined through segmentation. Patient-specific models replicating geometry and deployment characteristics were used for *in vitro* studies in which a dye was injected immediately downstream of the TAV and washout time was calculated. The study found no differences between the left, right and non-neo-sinuses, but found positive

correlation between TV and washout time, shown in Figure 5. Positive correlations were also found between TAV stent height with TV, as well as TAV cusp angle with TV and washout time.

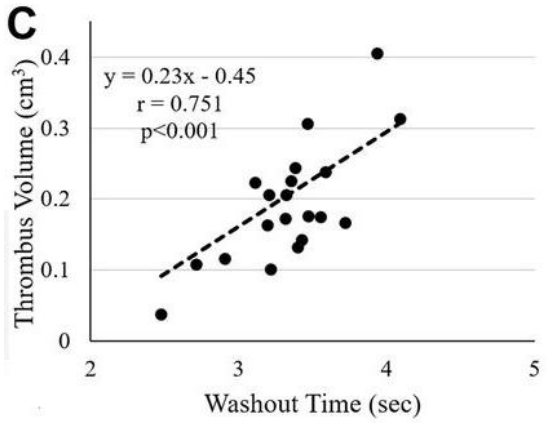


Figure 5. Thrombus volume versus washout time. A strong, statistically significant ($p<0.001$) positive correlation exists between thrombus volume in the neosinus and washout time.

The study, along with one by Sadri et al. that found a similar correlation between flow stasis and TV across multiple TAVs[15], established the importance of flow stasis as a contributor to thrombus formation in TAVs. However, according to the study conducted by Trusty et al.[14], the occurrence of flow stasis only accounts for 60% of the variability observed with TV. This indicates that there are other factors at play that need to be considered.

According to the Virchow's triad, there are three potential contributors to thrombosis – flow stasis of blood flow, presence of foreign material, and hypercoagulability. While the hemodynamics surrounding TAVs, has been studied in detail, the contribution of other aspects of the Virchow's triad, namely foreign materials, has received little attention. TAVs currently on the market typically have three foreign material components: a cobalt-chromium stent, a polyethylene terephthalate (PET) skirt, and leaflets made of bovine/ porcine pericardium. This

study explores the initiation of thrombus formation from the perspective of each of these foreign materials present in TAVs.

MATERIALS AND METHODS

Experimental Setup

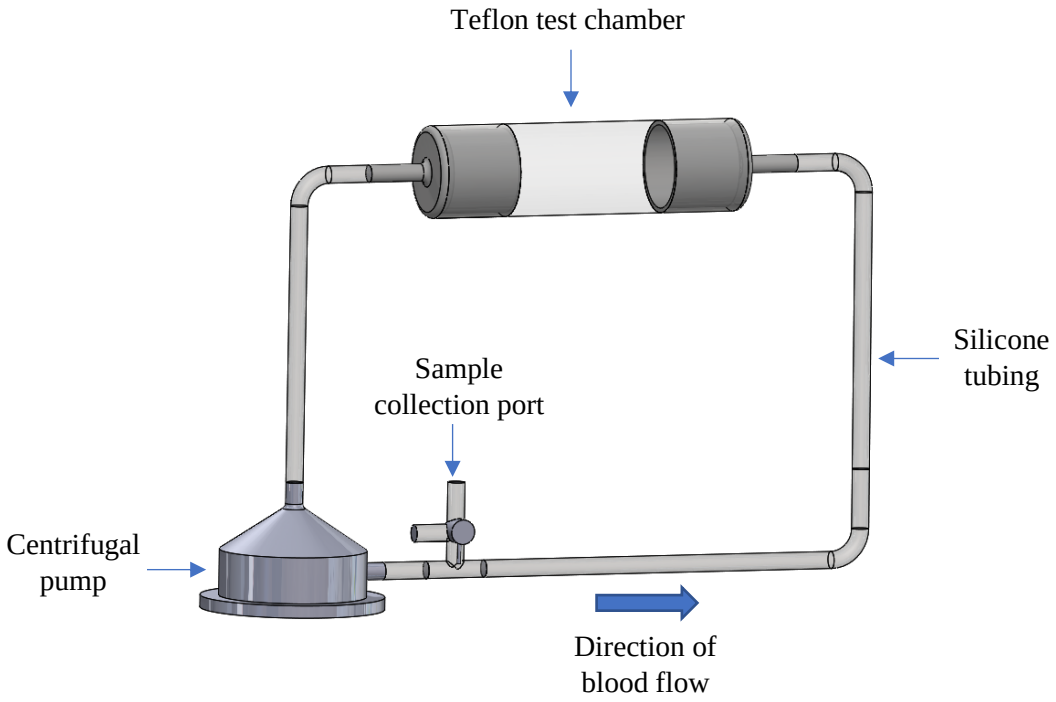


Figure 6. Experimental setup showing the different components of the flow loop.

The steady flow loop system consisted of the PediMag centrifugal pump (Thoratec, Pleasanton, CA), 23 mm ID biocompatible Teflon test chamber, and 23 mm ID machined PTFE (polytetrafluoroethylene) connectors on either side of the test chamber to connect the chamber to the pump via 6.35 mm ID silicone tubing (VWR, Bridgeport, NJ). The tubing fit snugly on the pump head and PTFE pieces, and did not cause any leaks. Since the Teflon test chamber and the PTFE connectors had the same inner dimensions, they were held together by biocompatible Teflon tape to provide a semi-permanent connection. The connections were then wrapped in

electrical tape to make them secure even in the case of axial forces. Clamps were used at each of the connections to provide additional strength to the joints. The total volume of the flow loop was approximately 55 mL.

Experimental Approach

This study consisted of 3 setups: control (empty test chamber), stent with skirt, and the whole valve (stent, skirt and leaflets), shown in Figure 7.

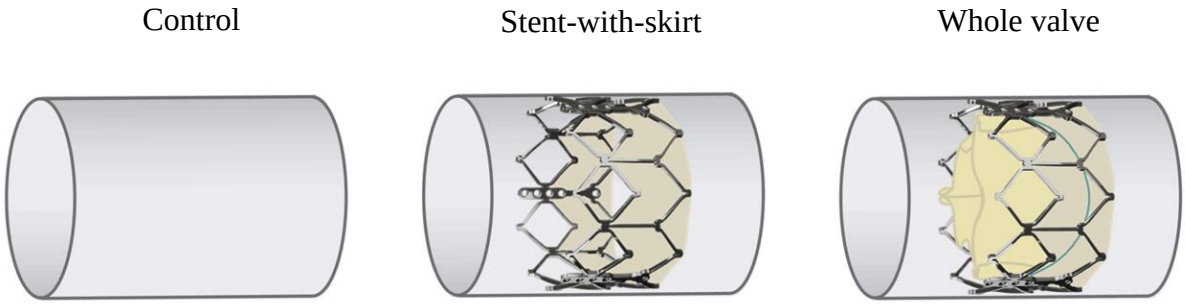


Figure 7. Three experimental conditions tested – control (left), stent-with-skirt (center), whole valve (right). Teflon test chamber was left empty for the control. For the stent-with-skirt, 23 mm Edwards SAPIEN XT valve was inserted after the pericardial leaflets were removed. 23 mm Edwards SAPIEN XT valve as is was inserted in the chamber for the whole valve.

For experimental conditions requiring a stent or stent frame, a 23 mm SAPIEN XT valve (Medtronic, Minneapolis, MN) was used and inserted into the test chamber. The loop was filled with anticoagulated blood and the pump was set to rotate at 1000 rpm (flow rate of approximately 0.8 L/min). Calcium chloride (reversing agent) was injected into the system through a port in the tube just before the inlet of the pump, to allow for optimal mixing with the blood before entering the test chamber.

In the initial experiments, the steady flow loop was run for 1 hour, and 1 mL blood samples were collected at 2 time points (0 hour and 1 hour) during this duration. After obtaining preliminary

results, the duration of the experiment was increased so future experiments had a run time of 2 hours. 1 mL blood samples were collected at two timepoints: 0 hour and 2 hours. After each run, the loop was drained and rinsed with saline before the next run.

Blood collection and reconstitution

These studies were approved by the Institutional Review Board for human research by the Georgia Institute of Technology (IRB no. H17314).

A reversible anticoagulant system was developed and the optimal ratio of anticoagulant to reversing agent was determined through bench tests, where the optimal ratio is defined as the ratio at which thrombus formation is enabled but there is no gross thrombosis in the flow loop. Sodium citrate (Sigma, St. Louis, MO) was used as the anticoagulant, and calcium chloride (Sigma, St. Louis, MO) was used to reconstitute the blood.

According to industry standards, blood was collected by venipuncture into 60 mL syringes containing 3.2% sodium citrate solution in a blood: citrate volumetric ratio of 9:1 to prevent clotting. 180 mL of whole blood was drawn from the donor in a single sitting. Reversing agent, 100 mM calcium chloride was added to the flowing blood at a citrate: Ca^{2+} molar ratio of 8:1. After obtaining preliminary results, static human blood experiments with citrate: Ca^{2+} ratios in a range from 2:1 to 8:1 in both microcentrifuge tubes and glass (agonist) vials were performed to refine the titration parameters. To further validate the citrate: Ca^{2+} ratio in a shear-mediated system, steady flow experiments using pig blood were conducted for 30 minutes with citrate: Ca^{2+} ratios in a range of 2:1 to 6:1. Through these experiments, the optimal ratio was identified to be a citrate: Ca^{2+} ratio of 6:1. This 6:1 ratio was used for experiments thereafter.

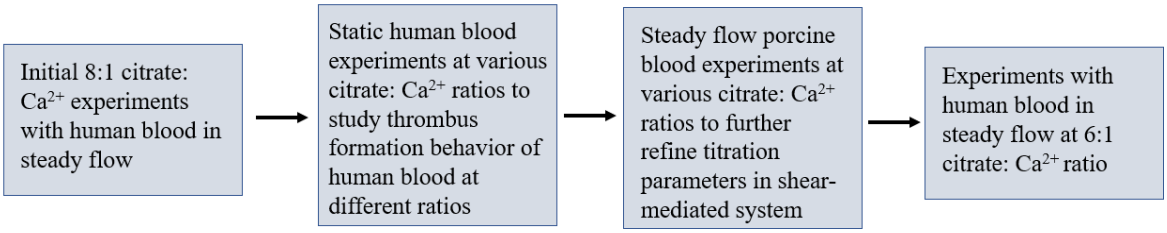


Figure 8. Sequence of experiments to determine optimal ratio of anticoagulant to reversing agent.

The anticoagulant – reversing agent combination of heparin and protamine was not used because past studies have shown that the system is too sensitive. It can also interact with blood components in many different ways, unlike the current system which only uses the calcium chelating mechanism[16].

Sample preparation and ELISA Assay

Collected blood samples were stored at 4°C until the end of the experiment, returned to room temperature and then centrifuged at 2000G for 10 minutes. Serum was separated from the samples and then preserved for later analysis at -80°C. ELISA kits (Abcam, Cambridge, UK) were used to measure concentrations of D-dimer and thrombin anti-thrombin (TAT), known biomarkers of thrombogenicity. D-dimer concentration was measured in 6 initial steady flow experiments that used human blood at the 8:1 citrate: Ca^{2+} ratio (n=6). 2 additional experiments were conducted under the same experimental conditions, and TAT concentration was measured in the samples from these experiments as well as the previous 6 experiments (n = 8). In subsequent experiments (n=7) using human blood at the 6:1 citrate: Ca^{2+} ratio, only the TAT assay was conducted.

Statistical Analysis

Data is presented as the mean ± standard error of the mean. The Shapiro-Wilk test was used to assess the normality of each dataset. Paired sample t-test was used to compare concentrations of D-dimer and TAT between timepoints. Two-tailed unpaired t-test was used to compare D-dimer and TAT concentrations between groups. P-value of less than 0.05 was considered statistically significant. Extreme outlier data points, determined based on the interquartile range, were excluded from statistical analyses. All statistical analysis was carried out using IBM SPSS Statistics software.

RESULTS

Steady flow human blood experiments with 8:1 citrate: Ca^{2+} ratio and 1-hour run time

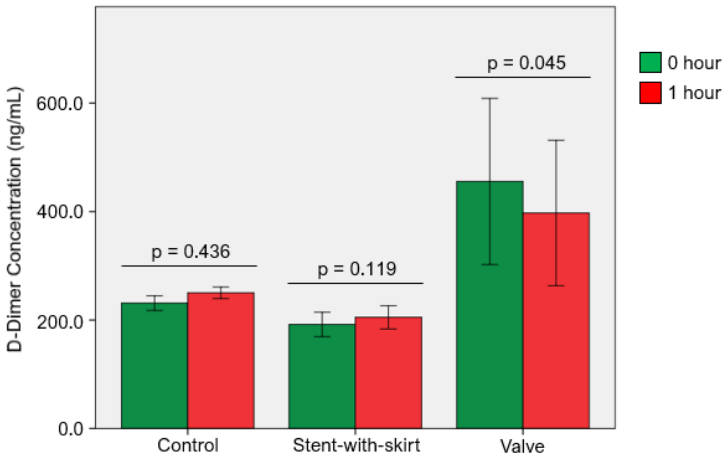


Figure 9. Concentration of D-dimer in human blood under steady flow over 1-hour duration (n=6).

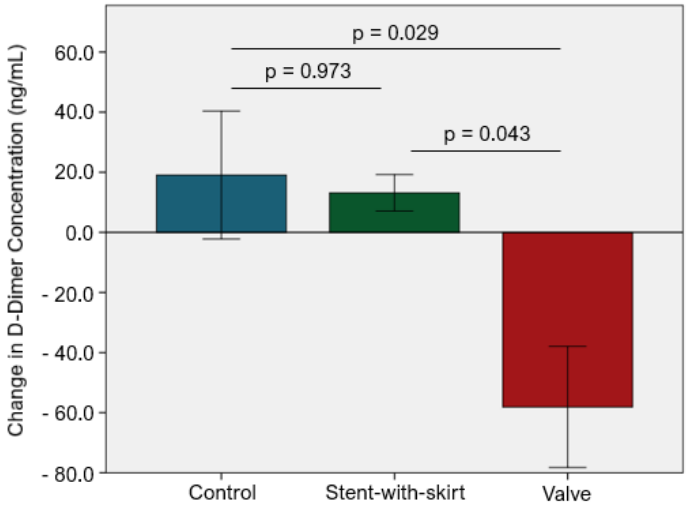


Figure 10. Change in D-dimer concentration in human blood under steady flow over time (n=6).

While the stent-with-skirt condition did not have a significant change in D-dimer concentration either over time or when compared to the control, a significantly higher D-dimer concentration was observed in comparison to the valve (P = 0.043).

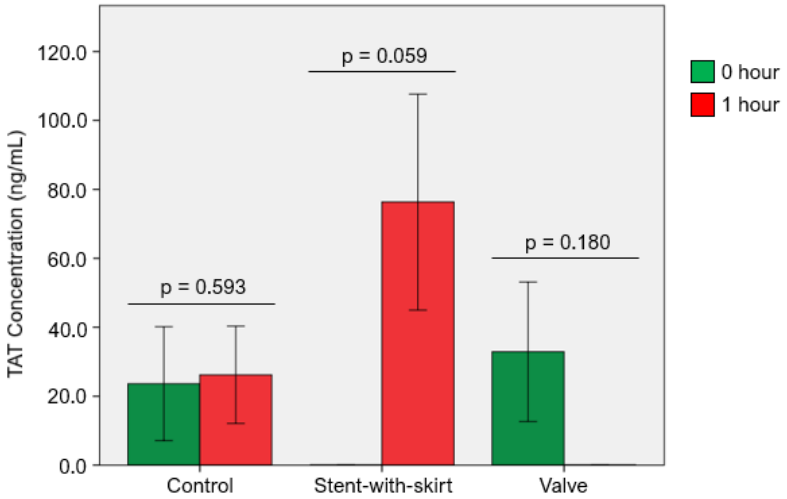


Figure 11. TAT concentration in human blood under steady flow over 1-hour duration (n=8).

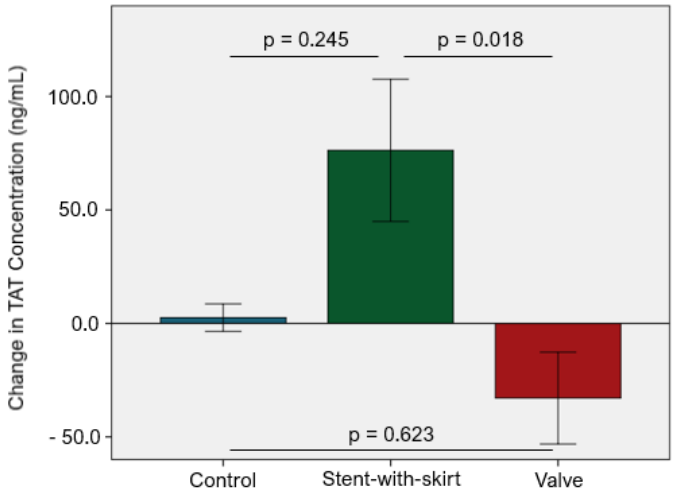


Figure 12. Change in TAT concentration in human blood under steady flow over time (n=8).

No significant changes were observed in any of the groups over the duration of the experiment; however, a significantly higher TAT concentration was observed in the stent-with-skirt setup when compared to the valve (P = 0.018). Upon examination of the stent and valve after each run, no clots were observed.

Since changes in D-dimer and TAT during the 1 hour of the experiment was minimal and therefore not significant, alterations were made to the experimental approach. Run time was increased from 1 hour to 2 hours, and new citrate: Ca^{2+} ratios were explored to simulate a more hypercoagulable condition.

Refining citrate: Ca^{2+} ratio

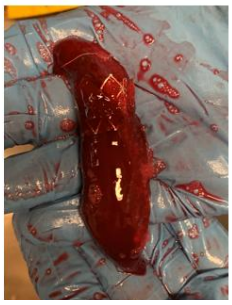
Static experiments with human blood

Static experiments were performed with human blood in various molar ratios of citrate: Ca^{2+}, ranging from 1:1 to 8:1, in both microcentrifuge tubes and glass (agonist) vials. Static experiments were conducted since they required minimal blood volume as opposed to steady flow experiments that would require enough volume to fill the loop. These experiments were

used to get a preliminary understanding of behavior of human blood at different citrate: Ca^{2+} ratios. The results showed that 6:1 would be the optimal ratio to use. It was the highest ratio at which clots were not observed to form even after 4 hours. At ratios even slightly higher, such as at 5:1, gross thrombosis was observed within a time well under the experimental duration, thereby rendering them unusable.

Steady flow experiments with porcine blood

To further test the titration parameters in a shear-mediated system, steady flow experiments were conducted with porcine blood in the presence of an agonist biomaterial (stent-with-skirt). Porcine blood was used for these trial experiments due to limited access to human blood and the cost associated with it. Gross thrombosis was observed in the flow loop at 2:1 and 4:1 ratios. Formation of clot on the stent was observed in the 5:1 and 6:1 condition. Quantitative measurements such as concentration of D-dimer or TAT in the porcine blood was not possible since ELISA assay kits specific to porcine blood are not available.



(A) Citrate: Ca^{2+} = 2:1

(B) Citrate: Ca^{2+} = 4:1

(C) Citrate: Ca^{2+} = 5:1

(D) Citrate: Ca^{2+} = 6:1

Figure 13. Effect of citrate: Ca^{2+} ratio on thrombosis in the presence of stent-with-skirt using porcine blood under steady flow. Gross thrombosis throughout the flow loop is observed in A and B. Thrombus formation around the stent is observed in C and D.

Based on the results from the static human blood experiments and qualitative results from the steady flow porcine blood experiments, the 6:1 citrate: Ca^{2+} was used moving forward.

Steady flow human blood experiments with 6:1 citrate: Ca^{2+} ratio and 2-hour run time

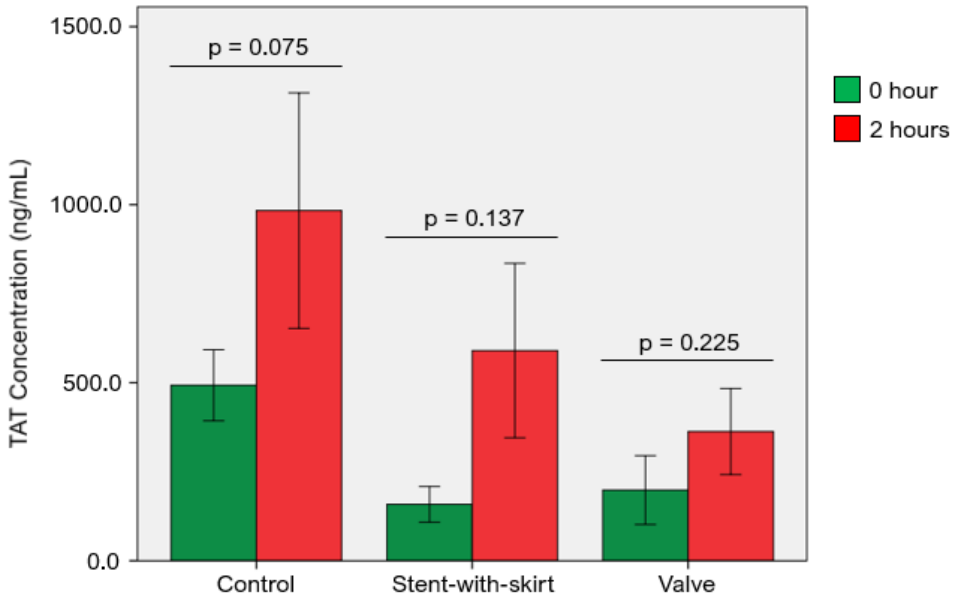


Figure 14. TAT concentration in human blood under steady flow over 2-hour duration (n=7).

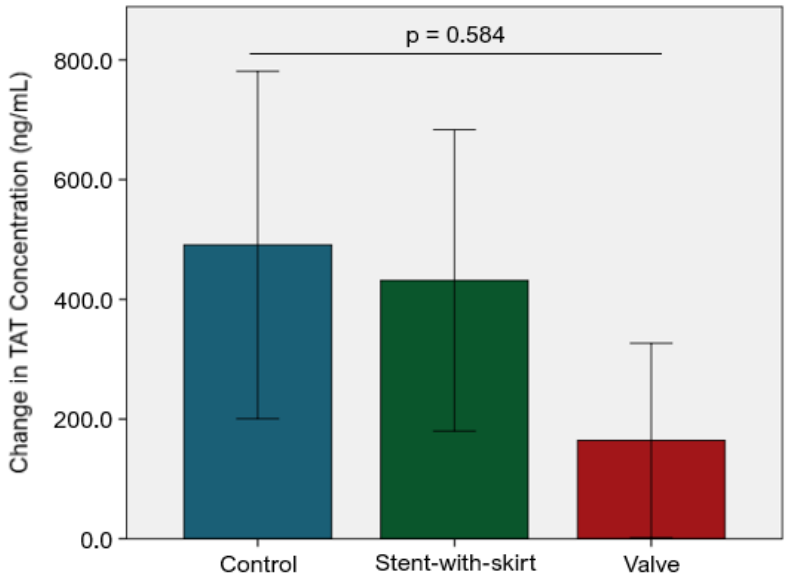


Figure 15. Change in TAT concentration in human blood under steady flow over time (n=7).

No significant change in TAT concentration was observed in any group over time. Additionally, the change in TAT concentration over the duration of the experiment was not significantly different between the control, stent-with-skirt and valve groups. A small clot was observed in one experiment performed in the stent-with-skirt condition, shown in Figure 15. Thrombus formation was not observed in any other experiments.

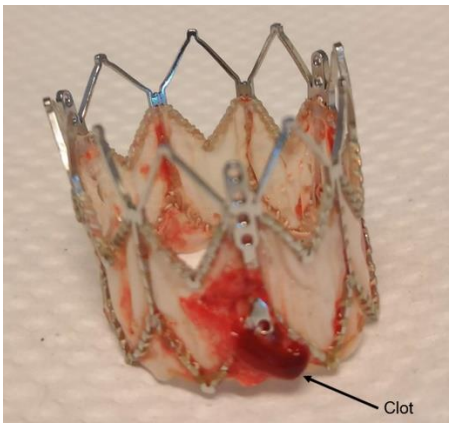


Figure 16. Formation of clot with stent-with-skirt using human blood at 6:1 citrate: Ca^{2+} in steady flow for 2 hours.

No significant correlation was found between the complete blood count (CBC) and change in D-dimer and TAT concentration.

DISCUSSION

This study provides insight to the process of thrombus formation in TAVs from a foreign materials perspective that has been left relatively unexplored. By comparing thrombus formation under steady flow between stents with skirts and whole valves, the study sought to understand how each foreign material in the TAV contributes to thrombogenesis.

The significantly higher D-dimer and TAT concentrations observed in the stent-with-skirt condition when compared to the valve in 8:1 citrate: Ca^{2+} ratio indicate that the stent with the skirt is more thrombogenic than the whole valve. It implies that exposure of blood to the stent with the skirt for abnormally long periods of time can cause the blood to shift to a more prothrombotic state over time. This is supported by the observation that thrombus formation occurs readily and relatively quickly in the stent-with-skirt experimental condition under steady flow using porcine blood. It can be hypothesized that the lower D-dimer and TAT concentration in the whole valve compared to the stent-with skirt is due to the pericardial leaflets in the whole valve reducing the interaction of blood with the stent and skirt under steady flow. By pushing up against the stent, the leaflets have a protective effect against exposure to the foreign materials.

In human blood with the higher citrate: Ca^{2+} ratio of 6:1 i.e. a hypercoagulable state, changes in TAT concentration within the groups over time and between groups was not significant. These results suggest that after the blood reaches a sufficiently high prothrombotic state, the different foreign materials in the TAV do not have significantly different effects on thrombus formation.

Overall, this study was successful in establishing a protocol to study the effect of TAV materials on thrombogenesis using blood from a single human donor, which is unprecedented. The results of the study also bring us a step closer towards understanding the mechanism of initiation of thrombus formation in TAV from a foreign materials perspective that can have implications on future TAV design. While the results of the study are useful to develop a preliminary understanding, further investigation is necessary to elucidate specifics of thrombus formation. This study had several limitations, the primary one being the use of steady flow which does not capture the fluid dynamics typically experienced by TAVs during pulsatile blood flow in the heart. Future studies will focus on implementing a similar study in a pulsatile flow loop. In addition, access to only one centrifugal pump allowed for testing of only one experimental condition at a time, thereby generating a time lag between experiments. To minimize the impact of the time lag on the results, the order in which the experimental conditions were tested was randomized for each donor. Lastly, a SAPIEN XT (second generation) valve was used instead of the most recently available SAPIEN 3 (third generation valve). However, since the valve differ by stent design and presence of an outer skirt, not the materials used, both valves can be expected to have the same effect on thrombus formation.

REFERENCES

1. Supino PG, Borer JS, Preibisz J, Bornstein A. The epidemiology of valvular heart disease: a growing public health problem. *Heart Fail Clin.* 2006;2(4):379-393.
2. Pibarot P, Dumesnil JG. Prosthetic heart valves: selection of the optimal prosthesis and long-term management. *Circulation.* 2009;119(7):1034-1048.
3. Chakravarty T, Søndergaard L, Friedman J, et al. Subclinical leaflet thrombosis in surgical and transcatheter bioprosthetic aortic valves: an observational study. *The Lancet.* 2017;389(10087):2383-2392.
4. Makkar RR, Fontana G, Jilaihawi H, et al. Possible subclinical leaflet thrombosis in bioprosthetic aortic valves. *New England Journal of Medicine.* 2015;373(21):2015-2024.
5. Ruile P, Minners J, Breitbart P, et al. Medium-Term Follow-Up of Early Leaflet Thrombosis After Transcatheter Aortic Valve Replacement. *JACC: Cardiovascular Interventions.* 2018;11(12):1164.

6. Pislaru SV, Nkomo VT, Sandhu GS. Assessment of Prosthetic Valve Function After TAVR. *JACC: Cardiovascular Imaging.* 2016;9(2):193-206.
7. Egbe AC, Pislaru SV, Pellikka PA, et al. Bioprosthetic valve thrombosis versus structural failure: clinical and echocardiographic predictors. *Journal of the American College of Cardiology.* 2015;66(21):2285-2294.
8. Hansson NC, Grove EL, Andersen HR, et al. Transcatheter aortic valve thrombosis: incidence, predisposing factors, and clinical implications. *Journal of the American College of Cardiology.* 2016;68(19):2059-2069.
9. Del Trigo M, Muñoz-Garcia AJ, Wijeysundera HC, et al. Incidence, timing, and predictors of valve hemodynamic deterioration after transcatheter aortic valve replacement: multicenter registry. *Journal of the American College of Cardiology.* 2016;67(6):644-655.
10. Schirmer SH, Mahfoud F, Fries P, Scheller B. Thrombosis of TAVI prosthesis—cause for concern or innocent bystander? A comment and review of currently available data. In: Springer; 2017.
11. Yanagisawa R, Hayashida K, Yamada Y, et al. Incidence, predictors, and mid-term outcomes of possible leaflet thrombosis after TAVR. *JACC: Cardiovascular Imaging.* 2017;10(1):1-11.
12. Al-Atassi T, Lam K, Forgie M, et al. *Cerebral Microembolization After Bioprosthetic Aortic Valve Replacement Comparison of Warfarin Plus Aspirin Versus Aspirin Only.* Vol 1262012.
13. Midha PA, Raghav V, Sharma R, et al. The fluid mechanics of transcatheter heart valve leaflet thrombosis in the neosinus. *Circulation.* 2017;136(17):1598-1609.
14. Trusty P, Sadri V, Madukauwa-David ID, et al. *Neo-sinus Flow Stasis Correlates with Thrombus Volume in Patients post-TAVR.* Vol 32019.
15. Sadri V, Trusty P, Bhat SS, et al. NEO-SINUS FLOW STASIS CORRELATES WITH LEAFLET THROMBOSIS ACROSS MULTIPLE TRANSCATHETER AORTIC VALVES. *Journal of the American College of Cardiology.* 2020;75(11 Supplement 1):1104.
16. Fallon AM. *The Development of a Novel in vitro Flow System to Evaluate Platelet Activation and Procoagulant Potential Induced by Bileaflet Mechanical Heart Valve Leakage Jets*: Chemical and Biomolecular Engineering, Georgia Institute of Technology; 2006.