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Scriptwriter Name: Poornima G

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**Title: Thrombus Profiling Assay: A Microfluidics-Based Platform for
Comprehensively Characterizing Biomechanical Thrombogenesis**

Authors and Affiliations:

Fiyinfoluwa Ola-Daniel*, Misbahud Din*, Atreyee Biswas*, Yunfeng Chen¹

Department of Biochemistry and Molecular Biology and Department of Pathology,
University of Texas Medical Branch

*These authors contributed equally

Corresponding Authors:

Yunfeng Chen yunfchen@utmb.edu

Email Addresses for All Authors:

Fiyinfoluwa Ola-Daniel	faoladan@utmb.edu
Misbahud Din	misbah@bs.qau.edu.pk
Atreyee Biswas	atbiswas@utmb.edu
Yunfeng Chen	yunfchen@utmb.edu

Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 25

Number of Shots: 55 (8 SC)

Introduction

Videographer: *Obtain headshots for all authors available at the filming location.*

INTRODUCTION:

Authors: The questions will not be displayed in the final video

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Yunfeng Chen:** We have developed a new method that can adequately capture the biomechanical attributes of thrombosis and detect prothrombotic abnormalities in humans.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What research gap are you addressing with your protocol?~~

- 1.2. **Atreyee Biswas:** Currently, no bioassay is available to evaluate the prothrombotic tendency of human patients and our work attempts to fill this gap.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Fiyinfoluwa Ola-Daniel:** By detecting the biomechanical activity of blood samples, our assay can comprehensively evaluate the prothrombotic attributes of human subjects.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How will your findings advance research in your field?~~

- 1.4. **Yunfeng Chen:** Our assay can be developed into a clinical test for detecting prothrombotic tendency in patients, and it can also be used to screen next-generation antithrombotic drugs.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Authors: Please let us know who delivered this statement after the shoot

Videographer: *Obtain headshots for all authors available at the filming location.*

Ethics Title Card

This research has been approved by the Human Research Ethics Committee at The University of Texas Medical Branch

Protocol

2. Microfluidic Device Preparation

Demonstrator: Atreyee Biswas

~~2.1. To begin, design a master mold to contain rectangular channels that are 200 micrometers wide and 50 micrometers high, incorporating a site of 80 percent luminal narrowing [1]. Include dedicated inlets and outlets on each channel for tubing connections [2].~~

~~2.1.1. WIDE: Talent at a workstation sketching or working in design software to lay out rectangular microchannels with the specified dimensions and an 80 percent narrowing site.~~ **Videographer's NOTE: 2.1.1 & 2.1.2 - was told to skip**

~~2.1.2. Close-up of the design showing labeled inlet and outlet ports intended for tubing connections.~~

2.2. To begin, use a silicon wafer to fabricate an SU-8 photoresist master mold via standard photolithography based on the design [1] and secure the master mold at the bottom of a 15-centimeter Petri dish with tape [2].

2.2.1. Talent handling a silicon wafer and preparing the SU-8 photoresist master mold using standard photolithography workflow.

2.2.2. Close-up of talent taping the completed master mold flat at the bottom of a 15-centimeter Petri dish.

2.3. Prepare the polydimethylsiloxane or PDMS mixture by mixing the prepolymer base and curing agent from the silicone elastomer kit at a 10 to 1 weight ratio [1]. Pour the PDMS mixture onto the master mold [2]. Now, place the plate containing the PDMS mixture in a vacuum desiccator [3-TXT].

2.3.1. Close-up of talent measuring and combining prepolymer base and curing agent to achieve a 10 to 1 weight ratio.

2.3.2. Close-up of talent pouring the mixed PDMS onto the master mold in the Petri dish.

2.3.3. Talent placing the plate with the PDMS mixture into a vacuum desiccator and closing the lid. **TXT: Degas the mixture**

2.4. Thermally cure the PDMS mixture by placing it in an incubator set to 75 degrees Celsius

[1]. Then, remove the sample from the incubator [2] and carefully cut out the cured PDMS from the master mold [3] and section the cured PDMS into individual chip units [4].

2.4.1. Talent placing the plate into an incubator and setting the temperature to 75 degrees Celsius. **Videographer's NOTE: 2.4.1 - broken up into two shots**

Added shot: 2.4.2.1 - Talent removes petri dish

2.4.2. Close-up of talent peeling and cutting the cured PDMS away from the master mold.

2.4.3. Close-up of talent sectioning the PDMS slab into separate, individual chip units.

2.5. Using a probe needle, punch holes at the designated locations to create the inlet and outlet [1].

2.5.1. Close-up of talent punching holes through the PDMS chip.

2.6. In a chemical hood, use a 75 percent ethanol-wetted task wipe to clean the glass slides and dry them [1]. Treat the glass slides with a high-frequency generator for 30 seconds [2] and treat the PDMS chips with a high-frequency generator for 20 seconds [3]. Align each chip with a glass slide and gently press the chip onto the glass slide surface to bind it [4]

2.6.1. Close-up of talent wiping the full surface of a glass slide with the ethanol-wetted task wipe. **Videographer's NOTE: 2.6.1, 2.6.2, 2.6.3, & 2.6.4 – Combined into one shot**

2.6.2. Talent operating a high-frequency generator inside a chemical hood to treat a glass slide.

2.6.3. Close-up of talent placing a PDMS chip within the high-frequency generator.

2.6.4. Close-up of talent gently pressing across the chip surface to ensure full contact and binding to the glass slide.

2.7. Now, thermally bond the devices by placing them in an incubator set to 150 degrees Celsius for 15 minutes [1]. After bonding, store the devices at room temperature under dust-free conditions [2].

2.7.1. Talent placing the assembled devices into an incubator and setting the temperature to 150 degrees Celsius for 15 minutes.

2.7.2. Talent placing the finished devices into a clean, dust-free storage area at room temperature.

2.8. Then, using a pincer, remove the plastic part of the probe needles [1] and bend the metal tubes to 90 degrees to create connectors for the microfluidic devices [2].

2.8.1. Close-up of talent gripping a probe needle with a pincer and pulling off the plastic component.

2.8.2. Close-up of talent bending the exposed metal tube to a 90-degree angle and placing the bent tube with other prepared connectors.

3. Fluorescent Sensor Purification

Demonstrators: Misbahud Din, Fiyinfoluwa Ola-Daniel

3.1. After preparing the required reagents, dilute the fibrinogen and the antibody in their respective buffers [1 and 2].

3.1.1. Talent adding buffer to a tube. **Videographer's NOTE:** 3.1.1 covers buffer prep, protein dilution, buffer addition, dye reaction- One shot, but just use what is appropriate

3.1.2. TEXT ON 3.1.1's BACKGROUND:

Step	Fibrinogen–Alexa Fluor 405	Antibody–Alexa Fluor 555
Buffer preparation	0.1 M NaHCO ₃ , pH 8.5	0.1 M NaHCO ₃ , pH 8.5
Protein dilution	1 mg/mL in PBS	1 mg/mL in amine-free buffer
Buffer addition	Add 1/10 th volume bicarbonate buffer	Add 1/10 th volume bicarbonate buffer
Dye reaction	1 mL fibrinogen + 1 mg Alexa Fluor 405 NHS-ester	100 µL antibody + 100 µg Alexa Fluor 555 NHS-ester
Incubation	1 h, RT, rotator, dark	1 h, RT, rotator, dark
Measurement	A ₂₈₀ and A ₄₀₅	A ₂₈₀ and A ₅₅₅

3.2. Take the mixtures in purification columns and centrifuge at 1,100 *g* for 2 minutes to remove the storage buffer [1].

3.2.1. Talent placing the purification column into a centrifuge and setting it to 1,100 g for 2 minutes.

3.3. After discarding the flow-through, load the reaction solution onto the purification column [1]. Mount the column onto a compatible collection vial [2] and centrifuge at 1,100 g for 5 minutes to harvest the required mix [3].

3.3.1. Close-up of talent pipetting the reaction solution onto the purification column.

Videographer's NOTE: 3.3.1 & 3.3.2 - One Shot

3.3.2. Close-up of talent placing the column onto a compatible collection vial and securing the setup.

3.3.3. Talent placing the set-up in a centrifuge.

3.4. Using a spectrophotometer, measure the absorbance for the protein and the dye signal [1]. Calculate the protein concentration and the fluorescence-to-protein molar ratio [2]. Store the dyes at 4 degrees Celsius in the dark [3].

3.4.1. Talent placing the sample in the spectrophotometer

3.4.2. Show the spectrophotometer interface set to measure absorbance.

3.4.3. Talent placing the vials in a fridge.

4. Setting Up the Thrombus Profiling Assay

Demonstrator: Fiyinfoluwa Ola-Daniel

4.1. Add heparin to the Tyrode buffer to a final concentration of 0.32 units per milliliter per 10 milliliters of blood to be collected [1] and prepare the syringe by aspirating 500 microliters of Tyrode buffer at pH 7.4 [2].

4.1.1. Talent adding heparin to the Tyrode buffer at the bench. **Videographer's NOTE: 4.1.1 & 4.1.2- One shot**

4.1.2. Close-up of talent aspirating 500 microliters of the heparin-containing Tyrode buffer into a syringe.

4.2. Collect the blood via venipuncture slowly into the heparin-containing syringe, minimizing platelet activation [1].

- 4.2.1. Show blood inside the heparin syringe. **Authors:** We need not show drawing blood from a human here. We can simply show the blood inside the tube
- 4.3. Transfer the collected blood into a 15-milliliter centrifuge tube and keep it at 37 degrees Celsius [1].
 - 4.3.1. ~~Close up of talent transferring blood from the syringe into a 15 milliliter centrifuge tube.~~ **Videographer's NOTE:** 4.3.1 - told to skip
 - 4.3.2. Close-up of the tube placed in an incubator set to 37 degrees Celsius.
- 4.4. Dilute von Willebrand factor monomer to 2 micrograms per milliliter in PBS [1]. Pre-coat the microfluidic devices with the diluted von Willebrand factor monomer [2] and incubate them for 1 hour at room temperature [3].
 - 4.4.1. Talent preparing the diluted von Willebrand factor monomer solution at the bench. **Videographer's NOTE:** 4.4.2 & 4.4.3 - One shot
 - 4.4.2. Close-up of talent adding the von Willebrand factor monomer solution into the microfluidic devices.
 - 4.4.3. Close-up of coated microfluidic devices placed on the bench for incubation at room temperature.
- 4.5. Now incubate the blood sample with fluorescent sensor Set 1 or Set 2 for 10 minutes at room temperature [1].
 - 4.5.1. Talent adding the selected fluorescent sensor set to the blood sample and gently mixing.
- 4.6. Next, connect a microfluidic device with inlet and outlet connectors and tubing [1]. Connect the other end of the inlet connector to tubing containing PBS [2] and then connect the other end of the outlet connector to a syringe [3].
 - 4.6.1. Talent assembling the microfluidic device with inlet and outlet connectors and attached tubing.
 - 4.6.2. Close-up of inlet tubing connected to a tube containing PBS.
 - 4.6.3. Close-up of outlet tubing connected securely to a syringe.
- 4.7. Mount the syringe onto a syringe pump [1] and the microfluidic device onto an inverted microscope [2]. Manually maneuver the microscope stage to locate the site of stenosis [3].

- 4.7.1. Talent securing the syringe onto the syringe pump.
- 4.7.2. Talent placing the microfluidic device onto the stage of an inverted microscope.
- 4.7.3. Talent adjusting the stage to identify the site of stenosis.

- 4.8. Fill the microfluidic channel and tubing with PBS using the syringe pump at a flow rate of 0.5 milliliters per minute [1]. Inspect the device to ensure no air bubbles are present around the site of stenosis [2].
 - 4.8.1. Close-up of syringe pump operating at 0.5 milliliters per minute to fill the channel with buffer.
 - 4.8.2. Talent viewing through the eyepiece.

- 4.9. Connect the inlet tubing to the blood sample [1] and perfuse the blood sample through the microfluidic channel using the syringe pump at a flow rate of 0.018 milliliters per minute [2].
 - 4.9.1. Close-up of talent connecting the inlet tubing to the blood sample tube.
 - 4.9.2. Close-up of syringe pump set to 0.018 milliliters per minute as blood enters the microfluidic channel.

5. Data Acquisition and Analysis

Demonstrators: Atreyee Biswas, Yunfeng Chen

- 5.1. Set the exposure time and gain for each fluorescence channel [1]. Perform real-time multicolor fluorescence imaging by alternating among the 4 channels and exciting one fluorophore at a time [2]. Adjust the focus plane to the thrombus [3] and record fluorescence signals at the site of stenosis for 15 to 30 minutes [4].
 - 5.1.1. SCREEN: 5.1.1.mp4.
 - 5.1.2. SCREEN: 5.1.2.mp4.
 - 5.1.3. SCREEN: 5.1.3-5.1.4.mp4 00:00-00:15.
 - 5.1.4. SCREEN: 5.1.3-5.1.4.mp4 00:20-00:35.

- 5.2. For data analysis, open the data file using ImageJ version 1.53 [1].
 - 5.2.1. SCREEN: 5.2.1.mp4.

5.3. Use the SZ22-FITC (*S-Zee-22-fit-see*) channel to identify the contour of the thrombus [1]. Then, using **Polygon selections**, roughly select the area of the thrombus [2].

5.3.1. SCREEN: 5.3.1.mp4.

5.3.2. SCREEN: 5.3.2.mp4. 00:04-00:17

5.4. For each channel, eliminate background signal by selecting **Image**, choosing **Adjust**, and then selecting **Threshold** [1]. Finally, measure the area and average signal intensity within the thrombus by selecting **Analyze** and choosing **Measure** [2].

5.4.1. SCREEN: 5.4.1.mp4.

5.4.2. SCREEN: 5.4.2.mp4.

Results

6. Results

6.1. Representative snapshots showed thrombi formed in healthy blood within the stenotic channel, with platelets, fibrinogen, von Willebrand factor, and P-selectin visualized using Sensor Set 1 [1], and platelets, phosphatidylserine, E-positive integrin alpha IIb beta 3, and activated integrin alpha IIb beta 3 visualized using Sensor Set 2 [2].

6.1.1. LAB MEDIA: Figure 4A. *Video editor: Sequentially highlight images.*

6.1.2. LAB MEDIA: Figure 4B. *Video editor: Sequentially highlight images.*

6.2. A single fluorescence channel image was processed by selecting the thrombus area, removing background signal, and measuring the signal area and average brightness to enable quantitative analysis [1].

6.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight C.*

6.3. Continuous analysis of fluorescence signal intensity over time generated a signal-versus-time curve for platelet accumulation during thrombus formation [1].

6.3.1. LAB MEDIA: Figure 6.

6.4. For each time point, total signal intensities were obtained for 4 biomarkers in Sensor Set 1 and 4 biomarkers in Sensor Set 2 [1].

6.4.1. LAB MEDIA: Figure 7A. *Video editor: Highlight Sensor Set 1 and Sensor Set 2.*

6.5. Thrombus size was calculated and a 7-dimensional thrombus profile was generated [1].

6.5.1. LAB MEDIA: Figure 7B.

1. Silicon

Pronunciation link: <https://www.merriam-webster.com/dictionary/silicon>

IPA: /'sɪlɪˌkɑːn/, /'sɪlɪkən/

Phonetic Spelling: sih·luh·kahn, sih·luh·kuhn

2. SU-8

Pronunciation link: No confirmed link found

- IPA: /'es'ju: ɛɪt/
Phonetic Spelling: es·yoo·eight
3. Photoresist
Pronunciation link: <https://www.merriam-webster.com/dictionary/photoresist>
IPA: /'foʊtoʊrɪˌzɪst/
Phonetic Spelling: foh·toh·ri·zist
 4. Photolithography
Pronunciation link: <https://www.merriam-webster.com/dictionary/photolithography>
IPA: /ˌfoʊtoʊ.lɪ'θɑːɡrəfi/
Phonetic Spelling: foh·toh·lih·thaa·gruh·fee
 5. Polydimethylsiloxane
Pronunciation link: <https://www.merriam-webster.com/dictionary/polydimethylsiloxane>
IPA: /ˌpɒliˌdaɪˌmɛθɪl.sɪ'ləksɛn/
Phonetic Spelling: pah·lee·dai·meh·thil·sih·lok·sayn
 6. Prepolymer
Pronunciation link: <https://www.merriam-webster.com/dictionary/prepolymer>
IPA: /ˌpriː'pɑːlɪmə/
Phonetic Spelling: pree·pah·luh·murr
 7. Desiccator
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccator>
IPA: /'dɛsɪˌkɛtər/
Phonetic Spelling: deh·sih·kay·turr
 8. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /'ɪŋkjəˌbeɪtər/
Phonetic Spelling: ing·kyuh·bay·turr
 9. Probe
Pronunciation link: <https://www.merriam-webster.com/dictionary/probe>
IPA: /proʊb/
Phonetic Spelling: proh·b
 10. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'ɛθəˌnɔːl/, /'ɛθəˌnɑːl/
Phonetic Spelling: eh·thuh·naal, eh·thuh·nawl
 11. Microfluidic
Pronunciation link: <https://www.merriam-webster.com/dictionary/microfluidic>
IPA: /ˌmaɪkroʊfluˈɪdɪk/
Phonetic Spelling: my·kroh·floo·ih·dik
 12. Fluorescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>
IPA: /flʊ'resənt/, /flɔː'resənt/
Phonetic Spelling: floo·reh·suhnt
 13. Fibrinogen
Pronunciation link: <https://www.merriam-webster.com/dictionary/fibrinogen>

- IPA: /faɪˈbrɪnəjən/
Phonetic Spelling: fye·brin·uh·jin
14. Alexa Fluor
Pronunciation link: No confirmed link found
IPA: /əˈlɛksə flɔr/
Phonetic Spelling: uh·lek·suh·floor
15. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsɛntrɪˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
16. Spectrophotometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>
IPA: /ˌspɛktroʊfoʊˈtɑːmɪtər/
Phonetic Spelling: spek·troh·foh·taa·mih·turr
17. Heparin
Pronunciation link: <https://www.merriam-webster.com/dictionary/heparin>
IPA: /ˈhɛpərɪn/
Phonetic Spelling: heh·puh·rin
18. Tyrode buffer
Pronunciation link: No confirmed link found
IPA: /taɪˈroʊd ˈbʌfər/
Phonetic Spelling: ty·roh·d·buff·urr
19. Venipuncture
Pronunciation link: <https://www.merriam-webster.com/dictionary/venipuncture>
IPA: /ˈvɛnɪˌpʌŋktʃər/
Phonetic Spelling: veh·nuh·puhngk·churr
20. von Willebrand factor
Pronunciation link: <https://www.merriam-webster.com/dictionary/von%20Willebrand%20factor>
IPA: /vɒn ˈvɪləˌbrænd ˈfæktər/
Phonetic Spelling: vawn·vih·luh·braand·fak·turr
21. Phosphatidylserine
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphatidylserine>
IPA: /ˌfɒsfəˈtɪdɪlˈsɪrɪn/
Phonetic Spelling: faas·fuh·ty·dil·see·reen
22. Integrin
Pronunciation link: <https://www.merriam-webster.com/dictionary/integrin>
IPA: /ˈɪntɪgrɪn/
Phonetic Spelling: in·tih·grin
23. Thrombus
Pronunciation link: <https://www.merriam-webster.com/dictionary/thrombus>
IPA: /ˈθrɑːmbəs/
Phonetic Spelling: thraam·buhs
24. Fluorophore
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorophore>

IPA: /'florə,fə:r/

Phonetic Spelling: floo·ruh·for

25. Stenosis

Pronunciation link: <https://www.merriam-webster.com/dictionary/stenosis>

IPA: /stə'noʊsɪs/

Phonetic Spelling: stuh·noh·sis

26. ImageJ

Pronunciation link: No confirmed link found

IPA: /'ɪmɪdʒ 'dʒeɪ/

Phonetic Spelling: ih·mij·jay

27. Biomarker

Pronunciation link: <https://www.merriam-webster.com/dictionary/biomarker>

IPA: /'baɪoʊ ,mɑːrkə/

Phonetic Spelling: bye·oh·maar·kurr