FROM FLOW TO ADHESION: MONITORING ERYTHROCYTE ADHESION IN VASCULAR MODELS

Under normal physiological conditions, red blood cell (RBC) adhesion to endothelial cells (ECs) remains negligible, allowing for smooth capillary transit and efficient oxygen delivery. While healthy individuals maintain low-level adherence, pathological changes can cause adhesion to increase dramatically. These changes, ranging from phosphatidylserine (PS) exposure to cytoskeletal remodelling and surface receptor activation, enable RBCs to bind to endothelial ligands such as thrombospondin-1, laminin, ICAM-1, and CD36. Studying these interactions provides insight into how red cell properties contribute to vascular dysfunction and opens up avenues for identifying therapeutic targets in a range of diseases (see references).

The adhesion of RBCs to ECs presents a powerful model for investigating the interface between vascular biology, hematology, and disease pathogenesis. Pathological increases in RBC-endothelial adhesion are critical in various blood disorders. This phenomenon is particularly significant in inherited hemoglobinopathies such as thalassemia and sickle cell disease, as well as in acquired conditions like malaria. Diabetes mellitus also demonstrates elevated RBC adhesion, where hyperglycemia modifies red blood cell proteins and promotes binding through advanced glycation end products, leading to diabetic vascular complications (see references). Endothelial–RBC adhesion assays using the **Flow & Patch** system offer a flexible experimental platform to model various pathological conditions in vitro. By manipulating variables such as shear stress, temperature, cytokine exposure, or redox state, researchers can simulate disease-relevant microenvironments that mimic those found in sickle cell disease, thalassemia, diabetes mellitus, or malaria. This system also allows for controlled testing of pharmacological agents, such as antioxidants, channel modulators, or adhesion-blocking antibodies, that may reduce or induce adhesion and improve or disrupt vascular flow. Co-culture models using primary endothelial cells or endothelial-like cell lines (e.g., HUVECs) can further enable exploration of dynamic signaling between RBCs and the endothelium, including EC activation, barrier integrity, and nitric oxide bioavailability (see references).

Beyond basic mechanistic studies, RBC–EC adhesion assays using the **BioExp Flow & Patch** system allows high-throughput screening and quantitative image analysis, especially with the integration of microfluidic platform and machine learning- or AI-based image recognition. These tools enhance reproducibility and allow for detailed phenotyping of RBC behavior under varying conditions, including altered hydration, ion channel modulation, or genetic manipulation. Moreover, combining adhesion measurements with complementary assays, such as deformability testing, flow cytometry, or patch-clamp electrophysiology, can provide a comprehensive view of how mechanical and molecular factors regulate RBC function in both health and disease. The system can be further enhanced by incorporating additional AI capabilities. As such, RBC-endothelial adhesion remains a highly promising and versatile research area for exploring the vascular complications of systemic disease and for evaluating targeted therapeutic interventions. For more information or specific inquiries, please feel free to contact us using the details below.

Keywords: *Erythrocyte adhesion, Endothelial cells, Oxidative stress, Sickle cell disease, Diabetes mellitus, Microcirculation, RBC-EC adhesion, Pathological adhesion, Hemoglobinopathies.*

Advantages of the “Flow & Patch” chamber

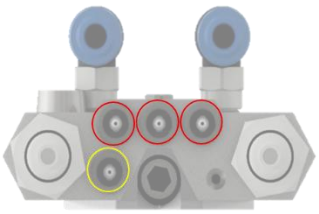
- Fast analysis with a low-volume blood sample (finger prick)
- Non-invasive, time and cost effective
- Customizable experiment setup
- One platform for all: Experiment and evaluation under one software
- Direct chemical addition via flow system without pre-incubation (physiologically relevant)
- Real-time AI evaluation with user feedback, interactive image review and correction
- Continuous AI learning and improvement

How to use the “Flow & Patch”?

The **“Flow & Patch”** system offers a user-friendly platform for quantifying RBC-EC adhesion through precise AI detection of stained RBCs on EC monolayers under physiologically relevant but also customizable flow conditions. By cultivating EC monolayers on microscope slides and exposing them to blood samples under physiological or pathological shear stress conditions, **Flow & Patch** enables assessment of RBC adhesion dynamics.

After flow exposure, slides are stained with standard cell stains (such as Diff-Quick, see references) and imaged using AI-based analysis, which quantifies adherent RBCs and calculates RBC to EC ratios. This approach eliminates complex labelling protocols, and allows for testing of various experimental conditions while closely replicating in-vivo microvascular environments. With automated counting capabilities and adaptive AI-learning, the system provides accurate, reproducible measurements for both research applications and clinical assessment of pathological RBC adhesion.

The chamber is equipped **with three inlets as seen marked red on the figure and an outlet marked in yellow**, enabling parallel testing of up to two different substances on the same cell monolayer, while one channel is reserved for cell media/buffer. Test solutions may include agents or buffers that simulate pathological conditions such as high glucose solutions, calcium ionophores, nitric oxide donors and/or antioxidants, offering experimental flexibility. Measurements can also be performed using whole blood or autologous patient plasma, for patient-specific conditions. The system also features a precision thermostat, allowing measurements under physiological conditions (37 °C) with a temperature stability of ± 0.2 °C . If desired, various temperatures can be tested to simulate pathological environments and study their effects on RBC adhesion on the vascular endothelium.



1. Required Materials

- Flow&Patch chamber
- Microscope Camera
- Microscope Slides (EC monolayer)
- Blood Sample*
- Cell media/ Buffer/ Test substance**

*** Recommended Haematocrit value <5%,**

**** May vary depending on the specific experimental conditions**

2. Blood Sample Preparation

Blood Collection and Haematocrit Check

Blood is drawn according to standard laboratory procedures (capillary, venous, or arterial). The haematocrit value is determined (i.e. using a Hct centrifuge) to ensure precise dilution.

Plasma Removal and Resuspension (if working with plasma free blood)

Blood is washed at least twice to remove plasma. After centrifugation at 1000g for 5 minutes, the plasma is carefully removed. The process is then repeated. Washed cells are gently resuspended in PBS (without albumin) at RT°C. The PBS volume is adjusted based on the desired haematocrit. The user can skip this step if using whole blood. Recommended hematocrit: <5% HCT for optimal results and reduce crowding.

3. EC Monolayer Preparation

- Culture endothelial cells using standard protocols in endothelial growth medium until reaching confluency. Avoid overgrowth to prevent contact inhibition–induced apoptosis.
- Prepare a microscope slide by coating it with a suitable extracellular matrix protein (i.e., fibronectin, collagen, or Matrigel).
- Seed cells onto the coated slide and continue cultivation under optimal conditions.
- Allow cells to form a uniform monolayer; once this is achieved, the slide is ready for use.

4. Flow & Patch Setup

- Install the software for **"RBC-EC adhesion"** and ensure all devices are properly connected.
- Place the flow chamber base (without the top cover) onto the microscope stage.
- Launch the RBC-EC adhesion software to begin the setup process.
- Position the microscope slide onto the base of the flow chamber.
- Depending on the experimental setup, either fill a solution chamber with the blood sample, or simply apply a drop of the blood sample (e.g. 20 µL) to the left side of the slide. Then carefully cover the slide with the top part of the flow chamber and lock the top and bottom parts together to ensure leak-proof sealing.

5. Step-by-Step measurement

i. Heating

The software will run a temperature check. If the internal system temperature is at **37±0.2°C**, next step can be started. User is free to skip the heating step if they are using temperature as a trial parameter.

ii. Position Camera

Ensure that the appropriate connection for docking the camera to the microscope is available (BRESSER MikroCam SP 5.0 microscope camera has a C-mount). Attach the camera to the

microscope. If necessary, adjust the camera slightly (adjust the screws forwards or backwards) to ensure horizontal alignment with the flow chamber (tested applying a short flushing process).

iii. **Place slide inside the chamber**

Open the flow chamber and insert the slides in the desired orientation. Before closing the flow chamber, ensure that the seal is positioned correctly. Place the flow chamber back in the holder.

iv. **Fill Channels**

User can utilize this step to ensure the contents of all three medium reservoirs, and will then use the pumps manually to fill the active channels and displace any air bubbles.

v. **Prewash**

A priming flow of 0.25 Pa will be initiated by the software to condition the EC monolayer.

vi. **Blood wash**

The channel containing the test solution, whether buffer, chemical compound, or whole blood (for high-volume setups), is opened. A customizable flow will be applied to allow RBCs to pass over the EC monolayer for a user-defined duration.

vii. **Waiting**

A brief pause will be introduced to promote initial cell–surface interactions. While not intended as a full sedimentation step, this short waiting period is recommended to support early adhesion dynamics. Duration will be customizable and may be skipped if not required by the user protocol. Alternatively, the user can skip this step and use continuous flow conditions.

viii. **Remove non-adherent Cells**

Non-adherent RBCs will be gently removed using a controlled wash. Flow rate and duration are fully adjustable to ensure effective removal while preserving adhered cells.

ix. **Manual Staining and Capture**

Upon completion of the wash step, the microscope slide must be carefully removed from the chamber and stained using a standard staining solution such as Diff-Quik. Once staining is completed, the slide will be reinserted into the chamber for image capturing.

x. **Cleaning**

In the final step, the system will once again active the flow to wash and clean the slide and the chamber. For this step, preferably a reservoir with 70% ethanol is chosen.

Images may be acquired with an external imaging system, if it offers comparable or higher resolution. These can be uploaded into the software for further analysis.

Note: Use of a lower-resolution camera is strongly discouraged, as it may compromise image quality and lead to inaccurate or misleading analysis.

6. Measurement evaluation

- Images taken are used for measurement evaluation. User has the ability to upload multiple pictures for analysis for comparative reasons.
- The results will be given back in the forms below after the AI software labels and evaluates each image:
  - ❖ Graph: Cell count for EC and RBC
  - ❖ Cell count of EC and RBC in list form with count of adhered RBCs of chosen image.
  - ❖ A log of recorded images with time stamps, and both cell counts with adhered RBC/EC ratio
- All images can be seen on the evaluation page via a toggle button.
- The results can be exported in the file formats **pdf, csv.**

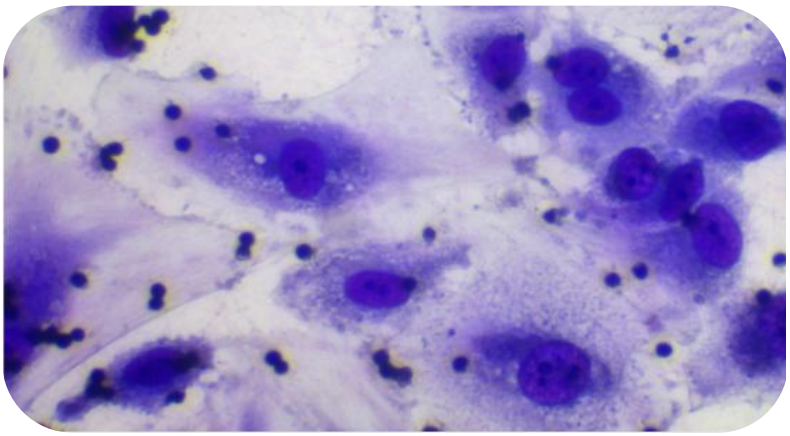


Fig. 1: *Coronary artery endothelial cells were cultured to confluence on fibronectin-coated microscope slides. The monolayers were perfused with washed human red blood cells (RBCs) at a shear stress of 2 Pa for 5 minutes, followed by a 15-minute static incubation to allow for adhesion. Non-adherent RBCs were then removed by applying a wash step at 0.25 Pa. After the wash, the slide was removed from the flow chamber and stained using the Diff-Quik™ stain kit according to the manufacturer's instructions.*

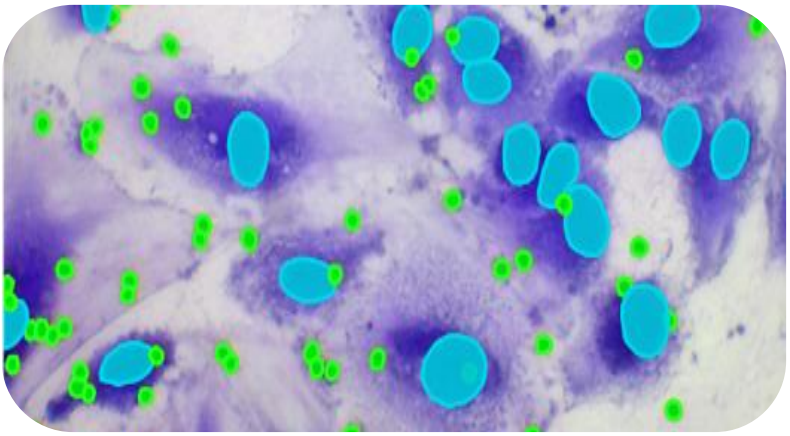


Fig. 2: *AI-recognition of the EC nucleus (cyan) and RBCs (green).*

Unlock More with Flow & Patch

Beyond its standard deformability assessment capabilities, the "Flow & Patch" system offers additional functionalities that can be tailored to specific research needs. The AI-based evaluation platform is continuously evolving and can be further customized or enhanced upon customer request.

Furthermore, specialized test protocols such as those for investigating sickle cell disease, diabetes and haemolytic anaemia including the identification of RBC subpopulations are available upon inquiry. If you're interested in expanding the scope of your research, feel free to contact us to explore the full potential of the system.

Ready to Get Started?

Your device is designed for precision, reliability, and ease of use. With a successful trial run, you're ready to explore its full potential in your research or clinical workflow.

For further assistance, troubleshooting, or advanced applications, our support team is here to help.

Contact us:

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References

1. Guizouarn, H., & Barshtein, G. (2022). Editorial: Red Blood Cell Vascular Adhesion and Deformability, volume II. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.849608
2. Baskurt, O. K., & Meiselman, H. J. (2023). Blood rheology and hemodynamics. *Seminars in Thrombosis and Hemostasis*, *50*(06), 902–915. https://doi.org/10.1055/s-0043-1777802
3. Ramot, Y., Koshkaryev, A., Goldfarb, A., Yedgar, S., & Barshtein, G. (2008). Phenylhydrazine as a partial model for β-thalassaemia red blood cell hemodynamic properties. *British Journal of Haematology*, *140*(6), 692–700. https://doi.org/10.1111/j.1365-2141.2007.06976.x
4. Kucukal, E., Ilich, A., Key, N. S., Little, J. A., & Gurkan, U. A. (2018). Red blood cell adhesion to heme-activated endothelial cells reflects clinical phenotype in sickle cell disease. *American Journal of Hematology*, *93*(8), 1050–1060. https://doi.org/10.1002/ajh.25159
5. Mohandas, N., & An, X. (2012). Malaria and human red blood cells. *Medical Microbiology and Immunology*, *201*(4), 593–598. https://doi.org/10.1007/s00430-012-0272-z
6. Wang, Y., Yang, P., Yan, Z., Liu, Z., Ma, Q., Zhang, Z., Wang, Y., & Su, Y. (2021). The Relationship between Erythrocytes and Diabetes Mellitus. *Journal of Diabetes Research*, *2021*, 1–9. https://doi.org/10.1155/2021/6656062
7. Dalimot, J. J., Klei, T. R. L., Beuger, B. M., Dikmen, Z., Bouwman, S. a. M., Mombo-Ngoma, G., Zoleko-Manego, R., Ndzebe-Ndoumba, W. F., Egée, S., Kuijpers, T. W., Grobusch, M. P., & Van Bruggen, R. (2022). Malaria-associated adhesion molecule activation facilitates the destruction of uninfected red blood cells. Blood Advances, 6(21), 5798–5810. https://doi.org/10.1182/bloodadvances.2021006171
8. Barshtein, G., Zelig, O., Gural, A., Arbell, D., & Yedgar, S. (2021). Determination of red blood cell adhesion to vascular endothelial cells: A critical role for blood plasma. *Colloids and Surfaces B Biointerfaces*, *210*, 112226. https://doi.org/10.1016/j.colsurfb.2021.112226
9. Artmann, G. M., Li, A., Ziemer, J., Schneider, G., & Sahm, U. (1996). A photometric method to analyze induced erythrocyte shape changes. Biorheology, 33(3), 251–265. https://doi.org/10.1016/0006-355X(96)00020-0 **[foundational concept]**
10. Artmann, Gerhard Michael, et al. (2025). *The Molecular Origin of Body Temperature in Homeothermic Species*, doi:10.1101/2024.09.10.612206.
11. Abay, A., Simionato, G., Chachanidze, R., Bogdanova, A., Hertz, L., Bianchi, P., Van Den Akker, E., Von Lindern, M., Leonetti, M., Minetti, G., Wagner, C., & Kaestner, L. (2019). Glutaraldehyde – a subtle tool in the investigation of healthy and pathologic red blood cells. Frontiers in Physiology, 10. https://doi.org/10.3389/fphys.2019.00514
12. Hyperthermia increases intercellular adhesion molecule-1 expression and lymphocyte adhesion to endothelial cells - PubMed. (n.d.). Retrieved June 12, 2024, from https://pubmed.ncbi.nlm.nih.gov/7914035/

13. Asaro, R. J., Profumo, E., Buttari, B., & Cabrales, P. (2023). The Double-Edged Sword of Erythrocytes in Health and Disease via Their Adhesiveness. International Journal of Molecular Sciences 2023, Vol. 24, Page 10382, 24(12), 10382. https://doi.org/10.3390/IJMS241210382
14. Akkaya, B., Kucukal, E., Little, J. A., & Gurkan, U. A. (2019). Mercury leads to abnormal red blood cell adhesion to laminin mediated by membrane sulfatides. Biochimica et Biophysica Acta (BBA) - Biomembranes, 1861(6), 1162–1171. https://doi.org/10.1016/J.BBAMEM.2019.03.008
15. Oberleithner, H., Wälte, M., & Kusche-Vihrog, K. (2015). Sodium renders endothelial cells sticky for red blood cells. Frontiers in Physiology, 6(JUN). https://doi.org/10.3389/FPHYS.2015.00188
16. Guizouarn, H., & Barshtein, G. (2020). Editorial: Red Blood Cell Vascular Adhesion and Deformability. Frontiers in Physiology, 11, 549871. https://doi.org/10.3389/FPHYS.2020.00657/BIBTEX
17. McMahon, T. J. (2019). Red Blood Cell Deformability, Vasoactive Mediators, and Adhesion. Frontiers in Physiology, 10, 489551. https://doi.org/10.3389/FPHYS.2019.01417/BIBTEX
18. Yang, Y., Koo, S., Lin, C. S., & Neu, B. (2010). Specific Binding of Red Blood Cells to Endothelial Cells Is Regulated by Nonadsorbing Macromolecules. The Journal of Biological Chemistry, 285(52), 40489. https://doi.org/10.1074/JBC.M110.116608
19. Yedgar, S., Kaul, D. K., & Barshtein, G. (2008). RBC Adhesion to Vascular Endothelial Cells: More Potent than RBC Aggregation in Inducing Circulatory Disorders. Microcirculation, 15(7), 581–583. https://doi.org/10.1080/10739680802105482
20. Wautier, J. L., & Wautier, M. P. (2013). Molecular basis of erythrocyte adhesion to endothelial cells in diseases. Clinical Hemorheology and Microcirculation, 53(1–2), 11–21. https://doi.org/10.3233/CH-2012-1572
21. Tombak, A. (2017). Red Blood Cells and Relation to Thrombosis. Transfusion Medicine and Scientific Developments. https://doi.org/10.5772/INTECHOPEN.69551
22. Wandersee, N. J., Punzalan, R. C., Rettig, M. P., Kennedy, M. D., Pajewski, N. M., Sabina, R. L., Paul Scott, J., Low, P. S., & Hillery, C. A. (2005). Erythrocyte adhesion is modified by alterations in cellular tonicity and volume. British Journal of Haematology, 131(3), 366–377. https://doi.org/10.1111/J.1365-2141.2005.05767.X
23. Mohandas, N., Hochmuth, R. M., & Spaeth, E. E. (1974). Adhesion of Red Cells to Foreign Surfaces in the Presence of Flow*. In J. BIOMED. MATER. RES (Vol. 8).
24. Kaul, D. K., Koshkaryev, A., Artmann, G., Barshtein, G., & Yedgar, S. (2008). Additive effect of red blood cell rigidity and adherence to endothelial cells in inducing vascular resistance. Am J Physiol Heart Circ Phys-lol, 295, 1788–1793. https://doi.org/10.1152/ajpheart.253.2008.-To
25. Nguyen, D. B. (2010). Phosphatidylserine exposure in red blood cells: A suggestion for the active role of red blood cells in blood clot formation.