FATIGUE OF HUMAN RED BLOOD CELLS IN HEALTH AND DISEASE

Ming Dao[1]*, Yuhao Qiang[1], E Du[2] and Subra Suresh[1,3]

[1]*Massachusetts Institute of Technology, Cambridge, MA, USA,* [2]*Florida Atlantic University, Boca Raton, FL, USA,*
[3]*Nanyang Technological University, Singapore*
** Presenting Author email: mingdao@mit.edu*

Abstract

Human red blood cells (RBCs) are responsible for delivering oxygen to the organs and tissues from the lungs. During its lifespan, an RBC needs to squeeze through the smallest openings (i.e., smallest capillaries and splenic interendothelial slits) in the human body many times, and go through repeated hypoxia-normoxia cycles. Using our established microfluidic platform, we have shown that both mechanical fatigue and hypoxia-normoxia fatigue (through hypoxia-normoxia cycles) may cause significant mechanical degradation of RBCs. The results are compared between healthy RBCs and sickle cell disease (SCD) RBCs, and provide underlying mechanisms for a much shorter lifespan of SCD RBCs.

1. Introduction

Human red blood cells (RBCs) are well known for their striking resilience and deformability in blood circulation. An RBC's distinct membrane structure provides its intrinsic deformability for traversing through the narrowest microvascular pathways such as small capillaries and splenic interendothelial slits. RBCs continuously undertake fluctuating stresses and strains as well as hypoxia-normoxia cycles in circulation. Will the mechanical fatigue or normoxia-hypoxia cycles cause mechanical degradation? Using our established microfluidic assays [1-5] for testing RBC mechanical degradation caused by mechanical fatigue [4] or due to hypoxia-normoxia cycles [3,5], we systematically studied both healthy RBCs and diseased RBCs from sickle cell disease (SCD) patients. These recent studies build upon extensive work on the mechanical and rheological properties of RBCs in health and disease, e.g. [6-9].

2. Results

Figure 1 compares the decreases in RBC deformability, evaluated by the maximum RBC stretch ratio λ^*_{max} as a function of accumulated time under two levels of maximum electrodeformation force. The relative degradation in cell deformability due to cyclic loading at t = 30 mins is 11.9 ± 0.01% for 1.2 V electrodeformation load and increases to 21.7 ± 0.02% for 2 V electrodeformation load [4]. At the same accumulated loading time, cyclic loading causes a faster deformability degradation than static loading. Our results also suggest that SCD RBCs degrade faster than healthy RBCs due to accumulated fatigue damage.

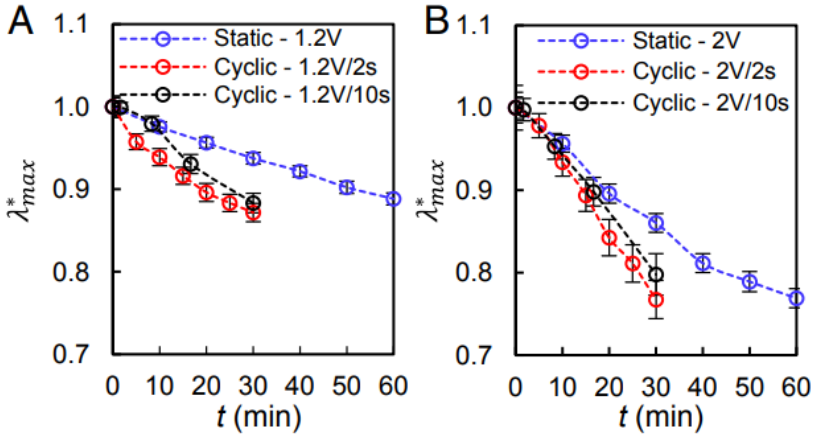

Fig.1 – Reduction of maximum relative deformation of RBCs as a function of accumulated loading time under static loading and cyclic loading with a rectangular waveform: Normalized maximum stretch ratio λ^*_{max} of healthy cells under (A) 1.2 V static loading (n = 35, blue circles), 1.2 V−2 s cyclic loading (n = 58, red circles), and 1.2 V−10 s cyclic loading (n = 49, black circles) and (B) 2.0 V static loading (n = 27, blue circles), 2.0 V−2 s cyclic loading (n = 20, red circles), and 2.0 V−10 s cyclic loading (n = 40, black circles). Figure taken from [4].

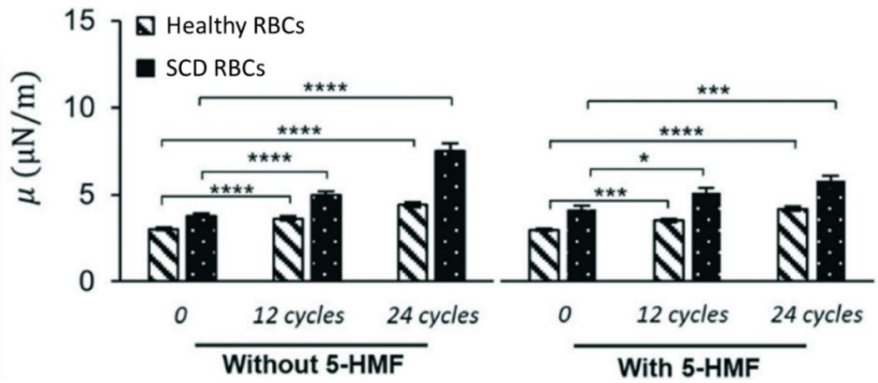

Fig.2 –Comparison of changes in shear modulus μ of healthy and SCD RBCs under hypoxia–normoxia (120–30 s) cycles, with and without treatment of the anti-sickling compound 5-HMF. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.001$. Reproduced from [5] with permission from the Royal Society of Chemistry.

Figure 2 shows changes in shear modulus μ of healthy RBCs and SCD RBCs with increasing hypoxia-normoxia cycles [5]. Both healthy RBCs and SCD RBCs degrade mechanically (i.e. losing deformability) with increasing hypoxia cycles, while SCD RBCs degrade much faster than healthy RBCs. Moreover, treatment of the anti-sickling compound 5-HMF can significantly slow down mechanical degradation.

3. Conclusions

We have established an *in-vitro* microfluidic platform capable of inducing controlled mechanical fatigue and hypoxia-normoxia fatigue. Our results show that both types of fatigue loading cause accumulated mechanical degradation with increasing loading cycles for healthy RBCs and SCD RBCs, while SCD RBCs exhibit faster mechanical degradation. These results provide possible underlying mechanisms for a much shorter average lifespan of SCD RBCs than that of healthy RBCs.

Acknowledgments

MD & YQ acknowledge support by National Institutes of Health under Grant No. R01HL158102 and R01HL154150. ED acknowledges support by National Science Foundation under Grant No. 1635312 and 1941655. S.S. acknowledges Nanyang Technological University, Singapore, for support through the Distinguished University Professorship.

References

[1] E Du, M. Dao, S. Suresh, "Quantitative Biomechanics of Healthy and Diseased Human Red Blood Cells using Dielectrophoresis in a Microfluidic System," *Extreme Mech. Lett.*, **1** (2014) 35-41.

[2] E Du, M. Diez-Silva, G.J. Kato, M. Dao, S. Suresh, "Kinetics of sickle cell biorheology and implications for painful vasoocclusive crisis," *Proc. Natl. Acad. Sci. USA*, **112** (2015) 1422-1427.

[3] E. Du, M. Dao, "Faster Sickling Kinetics and Sickle Cell Shape Evolution during Repeated Deoxygenation and Oxygenation Cycles," *Exp. Mech.*, **59** (2019) 319-325.

[4] Y. Qiang, J. Liu, M. Dao, S. Suresh, E. Du, "Mechanical fatigue of human red blood cells," *Proc. Natl. Acad. Sci. USA*, **116** (2019) 19828-19834.

[5] Y. Qiang, J. Liu, M. Dao, E Du, "In Vitro Assay for Single-cell Characterization of Impaired Deformability in Red Blood Cells under Recurrent Episodes of Hypoxia," *Lab Chip*, **21** (2021) 3458-3470.

[6] M. Dao, C. T. Lim, S. Suresh, "Mechanics of the human red blood cell deformed by optical tweezers," *J. Mech. Phys. Solids*, **51** (2003) 2259-2280.

[7] M. Diez-Silva, M. Dao, J. Han, C.-T. Lim, S. Suresh, "Shape and Biomechanical Characteristics of Human Red Blood Cells in Health and Disease," *MRS Bulletin*, **35** (2010) 382-388.

[8] I.V. Pivkin, Z. Peng, G.E. Karniadakis, P.A. Buffet, M. Dao, S. Suresh, "Biomechanics of red blood cells in human spleen and consequences for physiology and disease," *Proc. Natl. Acad. Sci. USA*, 113 (2016) 7804-7809.

[9] S. Cai1, H. Li1, F. Zheng1, F. Kong, M. Dao, G.E. Karniadakis, S. Suresh, "Artificial intelligence velocimetry and microaneurysm-on-a-chip for three-dimensional analysis of blood flow in physiology and disease," *Proc. Natl. Acad. Sci. USA*, **118** (2021) e2100697118.