Physiological and pathophysiological significance of erythrocyte senescence, density and deformability: Important but unnoticed trinity

Toru Maruyama1,*, Mitsuhiro Fukata1, Takehiko Fujino2

1Department of Hematology, Oncology and Cardiovascular Medicine, Kyushu University Hospital, Fukuoka, 812-8582 Japan
2Institute of Rheological Function of Foods, Co. Ltd., Hisayama, 811-2501 Japan

Received: 5 May 2020 / Accepted: 7 July 2020
© Japanese Society of Biorheology 2020

Abstract Erythrocytes are the most abundant cells and acting as carrier, deliverer and sensor of oxygen. Therefore, human erythrocyte behavior is a fundamental health indicator. Lifespan of circulating erythrocytes is about 120 days, and hence erythrocyte population shows distribution of aging. The physicochemical property of hemoglobin (Hb) influences the density and the deformability of erythrocytes. Senescent erythrocytes are dense, shrunk, less deformable and finally removed from circulation by several mechanisms such as phagocytosis and eryptosis. Earlier removal leads to the short lifespan of less deformable erythrocytes. Herein, anemic and cardiometabolic diseases are presented in order to consider the relationship between the age-dependent erythrocyte density and deformability. The main cause of impaired deformability in sickle cell disease is the presence of dense cells characterized by cellular dehydration and polymerization of sickle Hb, that in hereditary hemolytic diseases is cellular geometry, and that in iron deficiency anemia is an increased susceptibility of lighter erythrocytes to the oxidative stress. Diabetic erythrocytes show seemingly normal density and reduced deformability under the enhanced oxidative stress. This article addresses that distribution profiles of both erythrocyte density and deformability are important for better understanding of the encapsulated Hb interacting membrane of erythrocytes showing individual aging.

Keywords deformability, density, erythrocyte, senescence

1. Introduction
Circulating human erythrocytes are acting as carrier, deliverer and sensor of oxygen. Normal lifespan of these most abundant cells in the body is about 120 days. Healthy human erythrocytes have biconcave disc-like shape with diameter of about 6–8 μm, whereas the minimum diameter of microvascular bed is 3–4 μm. Therefore, circulating erythrocytes have to deform to pass narrow capillaries, i.e., one of the most important rheological functions of erythrocytes is the deformability to maintain microcirculation all over the body. After the maturation in the bone marrow, all circulating erythrocytes show different age, and important age-dependent characteristics are their internal density and deformability. Erythrocytes encapsulating hemoglobin (Hb) show individual density, and the erythrocyte density is highly dependent on the concentration and characteristics of Hb. This article aims to review the relationship between the erythrocyte aging, density and deformability by including our laboratory findings. Because the interdependency of this trinity is not yet fully understood but a core problem of erythrocyte rheology and relevant clinically to consider the pathophysiology of some common diseases showing microcirculatory disturbance.

2. Senescence of Erythrocytes

Although the entire body of erythrocyte aging is not yet clear, age-dependent alterations are known with respect to erythrocyte morphology, biochemistry and physiology. The main morphological change in erythrocyte aging is a decrease in cell size and volume. Aged erythrocytes are dense associated with an increase in the cellular hemoglobin concentration and hence separated by density-gradient fractionation [1]. Age-dependent biochemical changes include an increase in glycated hemoglobin and tyrosine-phosphorylation levels of band 3 protein and a decrease in creatine content and many antioxidative enzymatic activities [2–4]. Biophysical properties of erythrocyte aging
include reduced oxygen delivery, intracellular Ca\(^{2+}\) accumulation and augmented K\(^+\) efflux based on the reduced internal Ca\(^{2+}\) handling activity [5, 6]. Internal Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) in silent young erythrocytes of 20–50 nM increases transiently by mechanical stimuli [7] and slowly according to senescence to saturating levels of about 1 \(\mu\)M [8]. This threshold [Ca\(^{2+}\)]\(_i\) activates Ca\(^{2+}\)-dependent K\(^+\) channels (Gardos channels) [9]. The maximum Gardos channel activation corresponds to full dehydration (loss of about 50% cell water) and shrinkage (loss of about 40% cell volume). However, the overall relationship of morphological, biochemical and physiological events according to aging remains to be elucidated.

3. Clearance of Erythrocytes

All circulating erythrocytes show different age, senescent erythrocytes are removed after 120 days, and early erythrocyte clearance is associated with short lifespan. Main mechanism of aged erythrocytes clearance is phagocytosis by macrophages in spleen, liver and reticuloendothelial system. These macrophages recognize the aged erythrocytes characterized by phosphatidylserine (PS) exposure at the outer membrane. PS surface exposure as a result of membrane scrambling is termed ‘eat me’ signal and playing a role of tagging erythrocytes for removal [10]. Macrophages engulf and degrade the ‘tagged’ erythrocytes. However, the other mechanisms of erythrocyte removal have been clarified, i.e., 1) suicidal death termed eryptosis [11], 2) phagocytosis by angiogenic endothelial cells (endothelial erythropagocytosis) [12], and 3) intravascular hemolysis releasing cell-free Hb, which lowers the availability of nitric oxide (NO) [13]. Phagocytosis of less deformable erythrocytes by endothelial cells is promoted by angiogenic growth factor. Suicidal eryptosis is triggered by abnormal shear stress or oxidative stress and characterized by cell shrinkage, membrane blebbing and scrambling, PS externalization and internal Ca\(^{2+}\) accumulation. Eryptosis removes defective erythrocytes before intravascular hemolysis, which is important for defective erythrocytes to escape intravascular hemolysis and to prevent the hemolysis-induced organ damages.

4. Erythrocyte Density and Senescence

The erythrocyte volume decreases according to cell aging, because erythrocytes slowly dehydrate and become denser and shrunk over the entire lifespan. Therefore, erythrocyte density gradient fractionation reflects heterogeneity of erythrocyte population with different ages, i.e., aged (denser) cells are small, rich in Hb, less in K\(^-\) and dehydrated relative to young (light) cells [14]. Overall erythrocyte density is estimated by mean corpuscular hemoglobin concentration (MCHC), but the density-separated erythrocyte distribution profile is important to know the cellular aging process and the pathophysiology of many diseases. Although erythrocyte separation by density gradient using isotonic Percoll solution is standard [15], density separation using phthalate ester mixture with differential specific gravity (\(\rho\)) yields the erythrocyte density distribution curve [16]. Phthalate ester mixture is prepared by mixing methyl phthalate (\(\rho\) 1.189) and di-\(n\)-butyl phthalate (\(\rho\) 1.042), and the mixture encompasses the physiologically relevant erythrocyte density ranging from 1.062 to 1.138 with increment of 0.004. Glass capillaries filled with peripheral blood and separating phthalate ester mixture are centrifuged simultaneously for 14 minutes at 12,000 \(\times\) g. Then, capillaries are aligned in the order of phthalate ester \(\rho\) (Fig. 1A). Proportion of packed erythrocytes (\%) passing phthalate ester is plotted as a function of phthalate ester \(\rho\), and the erythrocyte density distribution is fitted by sigmoidal curve (Fig. 1B). This curve fitting yields two parameters, i.e., 1) the phthalate ester \(\rho\) allowing just half erythrocytes passage corresponding to the median erythrocyte density (MED) and 2) the maximum tangential slope representing the \(\rho\) distribution width. Moreover, this curve detects the pathologically dense cells defined as \(\rho\) > 1.120 g/cm\(^3\) influencing greatly the entire erythrocytes deformability [17]. Aside from the density gradient separation techniques, a new technique using magnetic levitation coupled with portable imaging analysis is reported to estimate the density distribution of erythrocytes. The smartphone-based device of this technique has advantages in portability, usability and cost-effectiveness and is applied to the diagnosis of sickle cell disease [18].

5. Erythrocyte Deformability and Senescence

Circulating erythrocytes have to deform within capillaries, recover the deformation after passing capillaries, and keep their morphology till next entering into capillaries. This cyclic shaping and reshaping are the fundamental erythrocyte function. Bending deformation of erythrocytes within microchannel is visualized by high-speed video camera, showing parachute-, slipper-, or bullet-like configuration [19]. Biconcave disc configuration is favorable to keep gas exchange efficacy and deforming flexibility. The concept of erythrocyte deformability was first described by Burton (1972) [20] but has no physical unit. Evaluation of deformability depends on the measurement technique with its own sensitivity and reproducibility. The advantage and disadvantage of representative tests evaluating deformability are listed in Table 1 [21]. In brief, centrifugal methods to elongate suspended erythrocytes are popular as diffraction ektacytometry [22] or automated rheoscope [23]. Optical tweezer enables stretching erythrocyte deformation [24]. Micropipette technique and atomic force microscopy allow
the selective measurement of a single cell membrane viscoelasticity [25]. Observation of erythrocyte behavior using microfluidic device is clinically relevant to explore how erythrocytes behave in vivo microvasculature. However, this method highly depends on the microfluidic devices. Among them, automated rheoscope is a promising tool to estimate deformability distribution [23]. Because the deformability distribution is more informative than the average of deformability in order to distinguish the small fraction of dense cells (ρ > 1.120 g/cm³) impairing the whole deformability from the homogeneously small reduction of the entire deformability. The erythrocyte deformability is a primary determinant in microcirculation and influenced by geometry, internal density and membrane properties [26]. Less deformable erythrocytes cause disturbed microcirculation, less oxygen delivery and tissue hypoxia. Aged erythrocytes are dehydrated and dense. Therefore, this erythrocyte subpopulation obtained by density gradient fractionation shows poor deformability [14, 27]. The following anemic or cardiometabolic diseases are representative disorders impairing deformability and enhancing eryptosis [28] while showing different erythrocyte density distribution profiles.

6. Hematological Diseases Showing Anemia

6.1. Sickle Cell Disease

Sickle cell disease (SCD) is an autosomal recessive hemoglobinopathy, i.e., a molecular disease caused by a point mutation in the β-globin gene generating the characteristic sickle Hb (HbS; βGlu6Val). Erythrocytes are shaped like sickle in hypoxic condition (Fig. 2). Erythrocyte sickling impairs the deformability profoundly. This impairment is not due to the characteristic cell morphology but due to an abnormal increase in the internal density caused by cellular dehydration and polymerization of HbS [29]. Considering the deformability as a primary determinant of microcirculation, erythrocyte sickling induces systemic microvascular obstruction. Increased rigidity and reduced deformability in sickle erythrocytes are associated either with removal by splenic macrophages or intravascular hemolysis leading to sickle cell anemia. In addition, intravascular hemolysis increases cell-free HbS, and free heme reduces NO availability, elevating vascular tonus. The constricted vessels further promote microvascular occlusion. The drugs for SCD approved by the US Food and Drug Administration are hydroxyurea (HU) and hydroxycarbamide. HU increases fetal hemoglobin (HbF), attenuates
Table 1  Main methodologies exploring the erythrocyte deformability.

| Method                  | Principle                                                                 | Advantages                                                                                     | Disadvantages                                                                 |
|-------------------------|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| Ektacytometry           | Laser diffraction of erythrocyte elongation caused by constant rotational shear stress (0.3 to 30 Pa). Ektacytometry estimates Elongation Index (EI) defined as $EI = (a - b)/(a + b)$ as a function of shear stress. | This technique requires no training and experience. Stable technique to evaluate EI under the wide range of shear stress by changing cuvette rotation speed (rpm). Osmotic gradient ektacytometry enables to estimate osmotic fragility and surface-to-volume ratio. | Population analysis. Elongation requires relatively high shear stress, which lowers intra-assay and inter-device reproducibility. |
| Optical tweezer          | Evaluation of erythrocyte stretching by two silica microbeads attached to the opposed ends of the living cell after trapping erythrocyte by three-dimensional gradient of electromagnetic energy generated by highly focused laser beam. | Superior sensitivity relative to micropipette. Optical tweezer evaluates the mechanical behavior of the optically trapped whole erythrocyte (shear modulus under the stretching). Measurement of the displacement of attached beads under the stretching with known magnitude along a single axis of the targeted erythrocyte. | Single cell information. Expensive. Controversial physiological relevancy of cell stretching as deformability. |
| Micropipette aspiration | Erythrocyte surface is aspirated by micropipette mouse, and elastic modulus of erythrocyte tongue is evaluated. Erythrocyte entry time or pressure is estimated by the whole erythrocyte entry into the micropipette. | Purely local membrane stiffness, elasticity and strength are evaluated. Low cost. Automated micropipette aspiration assay is now available with lower operator skill requirement and easier sample processing. | Original micropipette manipulation assay requires operator skill training and experience. Single cell information. |
| Atomic force microscopy (AFM) | Scanning and depressing the cellular surface by a specialized needle (cantilever) and reconstructing the 3D topography. | Force spectroscopy evaluates local membrane depression with cantilever and imaging mode reconstructs surface topography. AFM can analyze unfixed living erythrocytes in their buffer with minimal sample preparation. | Cantilever manipulation requires training and experience. Expensive. Single erythrocyte targeted. |
| Microfluidic devices    | Deformability is evaluated by erythrocyte passage time, retained pressure or flow rate during continuous filtration through the microchannel or micropore. | Practical, low cost, clinically and physiologically relevant and easy for analysis. Microfluidic device is under the further improvement to mimic in vivo microcirculation. Direct microscopic observation of deformation in erythrocytes passing through microchannel is possible depending on the device. | Data reliability and reproducibility are completely dependent on the microfluidic devices such as filter, microchannel, and microchannel array chip. Therefore, technical standardization is required to overcome the inter-device variability. Population analysis of whole erythrocytes with different deformability. |
| Automated rheoscope     | The first generation rheoscope estimates microscopic elongation of erythrocytes in the countering transparent cone-and-plate chamber. Automated rheoscope quantifies distribution of erythrocyte elongation under the controllable rotating shear stress. | High throughput information about erythrocyte elongation distribution. Excellent sensitivity and reproducibility. Elongation distribution pattern is important to distinguish the presence of erythrocytes group with highly impaired deformability from the condition of homogeneously impaired deformability. | Complexity of image analysis and data processing. Sophisticated but expensive. Poor estimation of erythrocytes overlapping and outside of the focused layer. |

(modified from reference [21])
intravascular hemolysis, improves NO availability and restores the deformability.

Severe deformability impairment and its improvement by HU in SCD have been reported so far by some rheological techniques such as ektacytometry [30], micropipette [31], filtration method [32], and laser optical tweezer [33]. Sickle erythrocytes show a wide range of density distribution under the oxygenated and deoxygenated cycle, and dense cell fraction impairs the entire deformability [17]. Internal density depends highly on the physicochemical properties of HbS, i.e., inhomogeneous increase in sickle erythrocyte density caused by HbS polymerization is a major cause of profound impairment of deformability [29]. As demonstrated in Fig. 3, the sigmoidal curve reconstructed by literature [17] investigating sickle erythrocyte density distribution shows high MED (1.100) and low slope (34.6). These indicate that sickle erythrocytes are heterogeneously dense, reflecting various extent of HbS polymerization. The presence of dense (ρ > 1.120 g/cm$^3$) cells is characteristic of SCD [34] impairing severely the entire deformability.

6.2. Nutritional Anemia

Nutritional anemia is mainly caused by a lack of iron, folate and vitamin $B_{12}$, affecting women, children, elderly and correctable by dietary improvement. Iron is essential in all living cells for energy metabolism, electron transport and enzymatic activity. Human body contains about 4 g iron, and 3 g iron is related to hemoglobin synthesis. Iron deficiency anemia (IDA) is highly prevalent worldwide and associated with various adverse health outcomes. The major rheological problem of IDA is poor erythrocyte deformability [35, 36]. This is reflected by the short lifespan of iron-deficient erythrocytes, which further exacerbates anemia. Less deformable erythrocytes are exposed to circulating mechanical stimuli causing further impairment of deformability and are targeted by splenic macrophages. Low oxygen transport capacity, impaired deformability and disturbed microcirculation cause chronic tissue hypoxia in concert. MCHC is an overall index of internal cellular density, and this index is reduced in patients with IDA. Erythrocyte density distribution curve shows low MED (1.078) in our patient with IDA as compared with MED (1.092) in control (Fig. 3). Although denser erythrocytes tend to be less deformable, iron-deficient light erythrocytes show poor deformability [35, 36]. This contradiction is explained by an increased sensitivity to oxidative stress and reduced antioxidative defense mechanisms in the iron-deficient erythrocytes [37, 38]. IDA accelerates auto-oxidation of Hb and subsequent generation of reactive oxygen species (ROS). Persistent exposure to ROS increases membrane rigidity leading to the impaired deformability. Slope of sigmoidal curve in IDA (36.1) is less than that in control (44.2), reflecting the heterogeneity of iron-deficient erythrocyte population (Fig. 3). This finding is compatible to the oxidative and inflammatory environment suppressing the iron-deficient erythropoiesis observed in IDA. Erythrocyte removal targets aged (and hence relatively denser) erythrocytes, which further reduces the erythrocyte density and shortens the erythrocyte lifespan. On the other hands, megaloblastic anemia is mostly caused by the deficiency of folate and/or vitamin $B_{12}$, the former is usually a lack in dietary intake and the latter is almost always malabsorption after the gastrectomy. Macrocystic hyperchromic anemia is a common feature of megaloblastic anemia, but the clinical severity depends on the fraction of hyperchromic cells.
cytosis (HS) is a popular inherited erythrocyte membrane premature clearance of erythrocytes. Hereditary sphero- and associated with poor deformability, short life span and the mutations of genes encoding various membrane proteins.

6.3. Inherited Hemolytic Anemia

Inherited erythrocyte membrane disorders are based on the mutations of genes encoding various membrane proteins and associated with poor deformability, short life span and premature clearance of erythrocytes. Hereditary spherocytosis (HS) is a popular inherited erythrocyte membrane disorder characterized by anemia, jaundice and splenomegaly. Microscopic observation of peripheral smear, flow cytometry and osmotic fragility test are the main screening tests, whereas the electrophoresis of membrane proteins and mutation analyses are the next-step investigation. Affected membrane proteins include ankyrin, band 3 protein, α-spectrin, β-spectrin or protein 4.2. Deformability is deeply impaired in spherocytes showing low surface-to-volume ratio [15]. Abnormal spherocytes are easily trapped and sequestered by spleen, showing short lifespan. Hereditary eliptocytosis (HE) is another inherited erythrocyte disorder caused by the defects of membrane proteins such as protein 4.1 and α-spectrin. These protein defects result in various membrane disorders, i.e., membrane fragmentation and weakened lateral linkage in cytoskeleton leading to reduced membrane surface area and elliptical shape change. Therefore, the main cause of the impaired deformability in HS and HE relies on the erythrocyte geometry, and the disease severity depends on the loss of membrane surface area [41]. On the other hands, erythrocytes index of MCHC in HS is greater than that in controls [42, 43], which is not merely an increase in cellular density as a result of shape change but cellular dehydration caused by augmented K⁺ and water loss [15]. A common feature of HS and HE is the heterogeneity of disease severity, abnormal erythrocyte subpopulation, cellular density, deformability, lifespan and the response to splenectomy [41]. This heterogeneity observed even in the same genotype may depend on the unknown factor regulating erythrocyte membrane structure, and extreme spherocytes are likely removed by spleen before aging. Therefore, hereditary abnormal erythrocyte senescence is a matter of future investigations.

7. Cardiometabolic Diseases

7.1. Diabetes Mellitus

Diabetes mellitus (DM) is a worldwide healthcare burden. A large body of evidence has shown that erythrocyte deformability is impaired in diabetic patients and in diabetic circumstance [19, 44, 45], i.e., ex vivo application of high glucose media or advanced glycation end-product (AGE) impairs the deformability of erythrocytes obtained by healthy volunteers [46, 47]. We also confirmed that erythrocyte filterability is impaired in patients with well-controlled type 2 DM and in streptozotocin (STZ)-induced diabetic rats by our specific filtration system [48, 49]. Such impairment is deeply associated with the development of diabetic complications [50]. This is not surprising, because diabetic complications are based mainly on microcirculatory disturbance, and the erythrocyte deformability is a primary determinant of microcirculation.

Diabetic erythrocytes demonstrate many morphological, biochemical and biophysical abnormalities. Geometry of diabetic erythrocytes is conflicting, i.e., erythrocytes obtained by obese and hyperglycemic ob/ob mice are larger and more biconcave than those in control mice [51]. However, mean corpuscular volume (MCV) and MCHC in STZ-treated diabetic rats did not differ from respective indices in control rats [48]. Clinical diabetes modulates erythrocyte morphology by comorbidities such as dyslipidemia and chronic inflammation causing anemia [52]. Therefore, it is difficult to know the direct correlation between the deformability and morphology in diabetic erythrocytes. We confirmed that the diabetic erythrocyte deformability is negatively
The molecular weight of globin chain and erythrocyte density [53]. However, MED in diabetic group was not different from MED in control group [49]. The lifespan of diabetic erythrocytes is shorter than that of control cells due to accelerated degradation [54, 55]. This early clearance of diabetic erythrocytes is considered to offset an increase in the internal density caused by Hb glycation, resulting in diabetic erythrocyte density distribution superimposed with that in controls [49], i.e., the internal density does not change significantly due to the balanced glycation and early clearance of denser erythrocytes.

Altogether, it is unlikely that erythrocyte geometry and density are the main rheologic factors impairing deformability at least in patients with controlled DM [49]. The remaining determinant of deformability is the membrane property [26]. Erythrocyte membrane contains protein, phospholipid and cholesterol. Hyperglycemia and oxidative stress deteriorate the membrane fluidity and integrity [56]. Cholesterol/phospholipid molar ratio of diabetic erythrocyte membrane is increased [57], indicating that diabetic erythrocyte membrane is rigid. AGE impairs the deformability mediated by inflammatory and oxidative stress [46], and persistent ROS generation causes erythrocyte membrane lipid peroxidation, protein degradation and suppression of Ca$^{2+}$-handling enzymatic activities. Internal Ca$^{2+}$ plays an important role on the erythrocyte membrane protein cross-linking contributing to the erythrocyte shaping [58]. Less deformable erythrocytes subpopulation is easy to suffer the mechanical stress in microvascular perfusion, which in turn elevates [Ca$^{2+}$], leading to further impairment of deformability [59, 60]. Therefore, it is not surprising that impairment of deformability is proportional to HbA1c [49, 61], because HbA1c reduces the availability of NO, which regulates the cellular deformability [62, 63].

7.2 Hypertension and Heart Failure

Essential hypertension is a very common health-care problem worldwide. Hypertension as well as DM acts as atherogenic risk factor and leads to the vascular complications such as stroke and coronary artery disease. Many investigations have reported an increase in the whole blood and plasma viscosities and an elevated hematocrit in hypertensive patients. These rheologic impacts increase the flow resistance in microcirculation and impose the mechanical shear stress on erythrocytes. The impaired erythrocyte deformability is confirmed in hypertensive patients by several rheological methods [64–66]. However, these studies reported no differences of MCHC and MCV in hypertensives from those in controls, implying that reduced deformability in erythrocytes of hypertensives are not due to the increased cellular size or density but likely caused by the rise in [Ca$^{2+}$], and membrane properties altered by mechanical and oxidative stress under the reduced NO availability [65, 66]. These conditions are common in the chronic inflammatory state as cardiometabolic diseases. The clinical importance is that such abnormal erythrocyte rheology sustains high blood pressure and underlies target organ damage. Although evidence for abnormal erythrocyte senescence in patients with essential hypertension is scanty, the erythrocyte creatine in patients with pulmonary hypertension (PH) is greater than that in controls [67], indicating that lifespan in the former is shorter than in the latter. Subclinical hemolysis is observed in patients with SCD and inherited hemolytic diseases, reflecting fragility of affected erythrocytes. Cell-free Hb binds NO quickly and reduces the NO availability profoundly leading to an increase in the pulmonary vascular resistance [68]. Although the main treatment of PH is vasodilating therapy using endothelin receptor antagonists, phosphodiesterase-5 inhibitors and prostacyclin, alternative therapy of NO administration and NO-releasing compounds should be considered to restore the pulmonary vascular endothelial dysfunction in the selected patients with PH associated with subclinical hemolysis.

Chronic heart failure (CHF) is frequently observed in the advanced stage of common cardiac diseases. However, CHF is nowadays a geriatric syndrome associated with dementia and frailty in the aged society. Therefore, hematological and hemorheological abnormalities are common in senile patients with CHF. The prevalence of anemia in CHF patients is about 40%, and the majority of anemic heart failure patients shows normocytic normochromic anemia [69]. This is explained by the inflammatory suppression of erythropoiesis, expanded venous volume (hemodilution) and insufficient action of erythropoietin due to cardio-renal linkage [70]. Since interventional treatment of CHF is not realistic, cardiac rehabilitation is recommended for senile CHF patients. Aerobic training is reported to improve the aerobic capacity and erythrocyte deformability assessed by ektacytometry in patients with anemic heart failure [71]. Left ventricular assist device (LVAD) is required for patients with severe CHF, but this device is associated with hematologic adverse effects of mechanical stress activating platelets and impairing erythrocyte deformability leading to the device thrombosis. Direct interaction of platelets and erythrocytes mediated by FAS ligand-receptor binding enhances procoagulant activity [72]. Therefore, the long-term antiplatelet and/or anticoagulation therapies are prerequisite for LVAD recipients. Subclinical hemolysis speculated by the short lifespan of erythrocytes obtained from LVAD recipients has great impact on the device thrombosis [73] and is a matter of future challenge especially in the situation of limited chances of heart transplantation.

8. Conclusion

Circulating erythrocytes are very sensitive to various stimuli such as exposure to inflammatory cytokines,
mechanical stimuli in microvascular network, and oxidative stress in oxygenation/deoxygenation cycle. It is still challenging to discriminate erythrocyte population showing inhomogeneous age, density and deformability. Erythrocyte senescence is closely linked to an increase in density and a decrease in deformability. Aged erythrocytes are dense, small and less deformable by gradual loss of volume regulation and internal Ca²⁺ homeostasis. However, this principle is variable depending on the specific disease. Main cause of impaired deformability in SCD is the presence of dense cells associated with cellular dehydration and polymerization of HbS, that in HS and HE relates to the cell geometry, that in IDA is an increased susceptibility of light cells to oxidative stress and reduced antioxidative defense mechanisms under oxidative stress impairs oxygen delivery and induces red blood cell aging. Subclinical hemolysis observed in inherited hemolytic diseases reduces NO availability underlying PH. We conclude this article with a future perspective toward better understanding linking density to deformation of erythrocyte showing individual aging.

Conflict of interest The authors have no conflicts of interest to declare.

Acknowledgements The authors would like to thank staff of the Institute of Rheological Function of Foods Co., Ltd., (Hisayama, Japan) for technical assistance. We dedicate this review article to Dr. Nobuhiro Uyesaka (Department of Physiology, Nippon Medical University) to honor his memory.

Funding Sources This work was supported in part by an academic support from SOUSEIKAI Global Clinical Research Center (LTA Medical Corporation, Fukuoka, Japan) and a Grant-In-Aid for Supporting Industry Program (so-called ‘Suppoin’) from the Japanese Ministry of Economy, Trade and Industry (20180830-52).

References

1. Waugh RE, Narla M, Jackson CW, Mueller TJ, Suzuki T, Dale GL. Rheologic properties of senescent erythrocytes: loss of surface area and volume with red blood cell age. Blood. 1992; 79(5): 1351–8.
2. Panzer S, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak R. Glycosylated hemoglobins (GHb): an index of red cell survival. Blood. 1982; 59(6): 1348–50.
3. Ciana A, Minetti G, Balduini C. Phosphotyrosine phosphatases acting on band 3 in human erythrocytes of different age. Bioelectrochemistry. 2004; 62(2): 169–73.
4. Griffiths WJ, Fitzpatrick M. The effects of age on the creatine in red cells. Br J Haematol. 1967; 13(2): 175–80.
5. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Front Physiol. 2014; 5: 84. doi: 10.3389/fphys.2014.00084.
6. Bernhardt I, Nguyen DB, Wesseling MC, Kaestner L. Intracellular Ca²⁺ concentration and phosphatidylinerine exposure in healthy human erythrocytes in dependence on in vivo cell age. Front Physiol. 2020; 10: 1629. doi: 10.3389/fphys.2019.01629.
7. Larsen FL, Katz S, Roufogalis BD, Brooks DE. Physiological shear stresses enhance the Ca²⁺ permeability of human erythrocytes. Nature. 1981; 294(5842): 667–8.
8. Seer RV, Lew VL. I₁ᵥ₂₂ agonist (NS309)-elicited all-or-none dehydration response of human red blood cells is cell-age dependent. Cell Calcium. 2011; 50(5): 444–8.
9. Gardos G. The function of calcium in the potassium permeability of human erythrocytes. Biochim Biophys Acta. 1958; 30(3): 653–4.
10. Segawa K, Nagata S. An apoptotic ‘eat me’ signal: phosphatidylserine exposure. Trends Cell Biol. 2015; 25(11): 639–50.
11. Lang F, Lang KS, Lang PA, Huber SM, Wieder T. Mechanisms and significance of eryptosis. Antioxid Redox Signal. 2006; 8(7–8): 1183–92.
12. Fens MH, Storm G, Pelgrim RC, Ultee A, Byrne AT, Gaillard CA, van Solinge WW, Schifflers RM. Erythropagocytosis by angiogenic endothelial cells is enhanced by loss of erythrocyte deformability. Exp Hematol. 2010; 38(4): 282–91.
13. Gladwin MT, Crawford JJ, Patel RP. The biochemistry of nitric oxide, nitrite, and hemoglobin. Free Radic Biol Med. 2004; 36(6): 707–17.
14. Cohen NS, Ekholm JE, Luthra MG, Hanahan DJ. Biochemical characterization of density-separated human erythrocytes. Biochim Biophys Acta. 1976; 419(2): 229–42.
15. Huisjes R, Makhro A, Lauudet-Planas E, Hertz L, Petkova-Kirova P, Verhagen LP, Pignatelli S, Rab MAE, Schifflers RM, Seiler E, van Solinge WW, Corrons JV, Kaestner L, Maňu-Pereira M, Bogdanova A, van Wijk R. Density, heterogeneity and deformability of red cells as markers of clinical severity in hereditary spherocytosis. Haematologica. 2020; 105(2): 338–47.
16. Danon D, Markovisky V. Determination of density distribution of red cell population. J Lab Clin Med. 1964; 64: 668–74.
17. Rodgers GP, Schechter AN, Naguchi CT. Cell heterogeneity in sickle cell disease. J Lab Clin Med. 1985; 106(1): 30–7.
18. Knowlton SM, Yenilmez B, Amin R, Tasoglu S. Magnetic levitation and analysis of red cell population. J Lab Clin Med. 1964; 64: 668–74.
19. Tsukada K, Sekizuka E, Oshio C, Minamitani H. Direct measurement of erythrocyte deformability in diabetes mellitus with a transparent microchannel capillary model and high-speed video camera system. Microvasc Res. 2001; 61(3): 231–9.
20. Burton AC. Physiology and biophysics of the circulation. Year Book Medical Publishers, 1972.
21. Depond M, Henry B, Buffet P, Ndour PA. Methods to investigate the deformability of RBC during malaria. Front Physiol. 2020; 10: 1613. doi: 10.3389/fphys.2019.01613.
22. Rabai M, Detterich JA, Wenby RB, Hernandez TM, Toth K, Meiselman HJ, Wood JC. Deformability analysis of sickle blood using ektacytometry. Biochimica. 2014; 51(2–3): 159–70.
23. Dobbe JG, Streekstra GJ, Hardeman MR, Ince C, Grimmergen CA, van Wijk R. Measurement of the distribution of red blood cell deformability using an automated rheoscope. Cytometry. 2002; 50(6): 313–25.
24. Lyubin EV, Khokhlova MD, Skryabina MN, Fedyanin AA. Cellular viscoelasticity probed by active rheology in optical tweezers. J Biomed Opt. 2012; 17(10): 101510. doi: 10.1117/1.JBO.17.10.101510.
25. Chen X, Feng L, Jin H, Feng S, Yu Y. Quantification of the erythrocyte deformability using atomic force microscopy. Clin Hemorheol Microcirc. 2009; 43(3): 243–51.
26. Mohandas N, Chasis JA. Red cell deformability, membrane material properties and shape: regulation by transmembrane, skeletal and cytosolic proteins and lipids. Semin Hematol. 1993; 30(3): 171–92.
27. Bransky A, Karin N, Nemirovski Y, Dinnar U. Correlation between erythrocyte deformability and size: a study using a microchannel based cell analyzer. Microvasc Res. 2007; 73(1): 7–13.
28. Jemaà M, Fezai M, Bissinger R, Lang F. Methods employed in cyttofluorometric assessment of eryptosis, the suicidal erythrocyte death. Cell Physiol Biochem. 2017; 43(2): 431–44.

29. Hiruma H, Noguchi CT, Uyesaka N, Hasegawa S, Blanchette-Mackie EJ, Schechter AN, Rodgers GP. Sickle cell rheology is determined by polymer fraction—not cell morphology. Am J Hematol. 1995; 48(1): 19–28.

30. Ballas SK, Connes P; Investigators of the Multicenter Study of Hydroxyurea in Sickle Cell Anemia. Rheological properties of sickle erythrocytes in patients with sickle-cell anemia. Eur J Haematol. 2018; 101(6): 798–803.

31. Athanasios G, Moutzouri A, Kourakli A, Zoumbos N. Effect of hydroxyurea on the deformability of the red blood cell membrane in patients with sickle cell anemia. Clin Hemorheol Microcirc. 2006; 35(1–2): 291–5.

32. Rodgers GP, Dover GJ, Uyesaka N, Noguchi CT, Schechter AN, Nienhuis AW. Augmentation by erythropoietin of the hematocrit in megaloblastic mixed erythrocytes. Am J Clin Pathol. 1976; 66(6): 953–7.

33. Brandão MM, Fontes A, Barjas-Castro ML, Barbosa LC, Costa FF, Cesar CL, Saad ST. Optical tweezers for measuring red blood cell elasticity. Eur J Haematol. 2003; 70(4): 207–11.

34. Kumar AA, Patton MR, Hennek JW, Lee SY, D’Alelio-Spina G, Yang X, Kanter J, Shevkoplyas SS, Brugnara C, Whitesides GM. Oxygenation of the hyperchromic red blood cell subpopulation is associated with decreased red cell deformability. Am J Physiol. 1983; 245(1): C90–6.

35. Yip R, Mohandas N, Clark MR, Jain S, Shohet SB, Dallman PR. Evidence of peroxidative damage to the erythrocyte membrane in iron deficiency. Biochem Biophys Res Commun. 2004; 321(3): 700–6.

36. Shin S, Ku YH, Suh JS, Singh M. Rheological characteristics of erythrocytes incubated in glucose media. Clin Hemorheol Microcirc. 2008; 38(3): 153–61.

37. Saito K, Kogawa Y, Fukuta M, Odashiro K, Maruyama T, Akashi K, Fujino T. Impaired deformability of erythrocytes in diabetic rat and human. J Biorehool. 2011; 25: 18–26.

38. Arita T, Maruyama T, Yokoyama T, Hieda M, Fukuta M, Fujino T, Mawatari S, Akashi K. Impaired deformability and association with density distribution of erythrocytes in patients with type 2 diabetes mellitus under treatment. Clin Hemorheol Microcirc. 2020; 76(1): 73–83.

39. Brown CD, Ghali HS, Zhao Z, Thomas LL, Friedman EA. Association of reduced red blood cell deformability and diabetic nephropathy. Kidney Int. 2005; 67(1): 295–306.

40. Engström KG, Täljedal IB. Increased size and biconavity in red blood cells of obese-hyperglycaemic mice. Biochroemol. 1988; 25(4): 625–38.

41. Arora RD, Dass J, Maydeo S, Arya V, Kotwal J, Bhargava M. Regulation of red blood cell membrane proteins on cellular deformability. J Membr Biol. 2009; 228(2): 109–16.

42. Caimi G, Presti RL. Techniques to evaluate erythrocyte deformability in diabetes mellitus. Acta Diabetol. 2004; 41(3): 99–103.

43. Shin S, Ku YH, Ho JX, Kim YK, Suh JS, Singh M. Progressive impairment of erythrocyte deformability as indicator of microangiopathy in type 2 diabetes mellitus. Clin Hemorheol Microcirc. 2007; 36(3): 253–61.

44. Iwata H, Ukeda H, Maruyama T, Fujino T, Sawamura M. Effect of carbonyl compounds on red blood cells deformability. Biochern Biophys Res Commun. 2004; 321(3): 700–6.

45. Shin S, Ku YH, Suh JS, Singh M. Rheological characteristics of erythrocytes incubated in glucose media. Clin Hemorheol Microcirc. 2008; 38(3): 153–61.

46. Arita T, Maruyama T, Yokoyama T, Hieda M, Fukuta M, Fujino T, Mawatari S, Akashi K. Impaired deformability and association with density distribution of erythrocytes in patients with type 2 diabetes mellitus under treatment. Clin Hemorheol Microcirc. 2020; 76(1): 73–83.

47. Brown CD, Ghali HS, Zhao Z, Thomas LL, Friedman EA. Association of reduced red blood cell deformability and diabetic nephropathy. Kidney Int. 2005; 67(1): 295–306.

48. Engström KG, Täljedal IB. Increased size and biconavity in red blood cells of obese-hyperglycaemic mice. Biochroemol. 1988; 25(4): 625–38.

49. Arora RD, Dass J, Maydeo S, Arya V, Kotwal J, Bhargava M. Regulation of red blood cell membrane proteins on cellular deformability. J Membr Biol. 2009; 228(2): 109–16.

50. Caimi G, Presti RL. Techniques to evaluate erythrocyte deformability in diabetes mellitus. Acta Diabetol. 2004; 41(3): 99–103.
essential hypertension. Clin Hemorheol Microcirc. 2011; 49(1–4): 323–9.
65. Cicco G, Carbonara MC, Stingi GD, Pirrelli A. Cytosolic calcium and hemorheological patterns during arterial hypertension. Clin Hemorheol Microcirc. 2001; 24(1): 25–31.
66. Odashiro K, Saito K, Arita T, Maruyama T, Fujino T, Akashi K. Impaired deformability of circulating erythrocytes obtained from nondiabetic hypertensive patients. Clin Hypertens. 2015; 21: 17. doi: 10.1186/s40885-015-0030-9
67. Fox BD, Okumiya T, Fox LA, Kassirer M, Raviv Y, Kramer MR. Raised erythrocyte creatine in patients with pulmonary arterial hypertension. Respir Med. 2012; 106(4): 594–8.
68. Rother R, Bell L, Hillmen P, Gladwin M. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin. JAMA. 2005; 293(13): 1653–62.
69. Zaharie M, Cârstea D, Cârstea AP, Zaharie SI. Prevalence of erythrocyte changes in patients with heart failure. Curr Health Sci J. 2017; 43(3): 263–8.
70. Clark AL, Cleland JG. Anemia and chronic heart failure. Circulation. 2005; 112(12): 1681–3.
71. Wang JS, Fu TC, Lien HY, Wang CH, Hsu CC, Wu WC, Chien YW, Cherg WJ. Effect of aerobic interval training on erythrocyte rheological and hemodynamic functions in heart failure patients with anemia. Int J Cardiol. 2013; 168(2): 1243–50.
72. Klatt C, Krüger I, Zey S, Krott KJ, Spelleken M, Gowert NS, Oberhuber A, Pfaff L, Lückstädt W, Jurk K, Schaller M, Al-Hasani H, Schrader J, Massberg S, Stark K, Schelzig H, Kelm M, Eilers M. Platelet-RBC interaction mediated by FasL/FasR induces procoagulant activity important for thrombosis. J Clin Invest. 2018; 128(9): 3906–25.
73. Bartoli CR, Zhang D, Kang J, Hennessy-Strahs S, Restle D, Howard J, Redline G, Bermudez C, Athuri P, Acker MA. Clinical and in vitro evidence that subclinical hemolysis contributes to LVAD thrombosis. Ann Thorac Surg. 2018; 105(3): 807–14.