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REVIEW

Erythrocytes and their role as health indicator: Using structure in a patient-orientated precision medicine approach

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ABSTRACT

The relevance of erythrocyte light microscopy analysis (a well-known haematological method) is under the spotlight, however there is a place for innovative electron microscopy, (together with biochemical markers) in a pathology laboratory. Inflammation is a key indicator of the health status and erythrocytes are extremely sensitive to oxidative stress or cytokine upregulation, which typically accompany systemic inflammation in most diseases. They are probably the most adaptable cells, and due to their short lifespan, may form a vital indicator of health, and could play a central part in tracking disease and treatment. As the NIH is proposing a precision medicine approach and because individualised medicine should form an essential part in diagnosis and treatment, biophysical combined with biochemical analysis of erythrocytes may be a novel method to track the inflammatory status before and after treatment. This will allow a fully individualised patient orientated precision medicine approach, where one-medication-regime-fits-all is no longer appropriate.

1. Introduction

Most diseases involve inflammatory pathophysiology in one or more vascular systems, and erythrocytes (RBCs) can be seen as key cellular indicators of the general well-being of an individual [1]. During inflammation, RBCs show both biochemical, as well as biophysical alterations. Biochemical changes are seen in the molecular arrangement of particularly the membranes of these cells, whilst biophysical changes are noted when looking at the general structural arrangement and RBC shape, including cell elasticity/rigidity — translating to structural cell deformability and changes in rheology. RBC shape changes in disease are not a new phenomenon, and for decades haematologists have used the RBC shape, as viewed under the light microscope, to classify conditions and diagnose patients. In a very insightful editorial in Haematologica, with the title: *Should clinical hematologists put their microscopes on eBay*? and a follow-up comment, the authors argue the usefulness, as well as the premise that clinical haematologists should abandon their microscopes [2,3]. They lament this thought, specifically because diagnostics in malignant haematology are changing towards a more high-tech approach and greater specialisation, and the authors note that the significance of morphology, as part of this process is changing with it. In the current paper we argue that we need a more comprehensive use of morphology, perhaps not as it has been used traditionally, where haematologists use only light microscopy, but where they are willing to use a more high-tech electron microscopy, together with flow cytometry and confocal microscopy, to closely look at the biophysical and biochemical functions of RBCs to both determine the severity of the inflammatory profile of the disease and to follow the treatment regime progress of patients.

This review therefore aims to provide a structured overview of RBC morphology, with special reference to the biochemical and biophysical arrangements of the RBC membrane and how it is changed in the presence of inflammation, in order to raise awareness of the usefulness of this cell type as a health indicator of a patient that may help in disease management. The presence of these RBCs with aberrant morphology is typically seen in most of the RBCs present in a blood sample of patients diagnosed with systemic inflammatory profile, like type II diabetes, thromboembolic stroke, rheumatoid arthritis, Parkinson's and Alzheimer's disease as well as conditions like hereditary haemochromatosis and hyerpferritineamia [1,4–9].

1.1. Why is the RBC (and particularly the membrane) such a good cellular indicator of healthiness of an individual?

Biochemical membrane changes, as a result of oxidative stress and upregulation of inflammatory molecules, cause biophysical shape changes, and determining the cause of these biochemical changes will ultimately address the cause and treatment of disease. RBCs are probably the most adaptable cells in our bodies, and due to their short life span, may form a vital indicator of health. We hope that our insights will stimulate new, important questions regarding the use of morphology in an environment where a greater specialisation is prevalent. Importantly, the NIH is now driving the process where precision medicine and

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Table 1
Structural components of the erythrocyte membrane (for a comprehensive review see [14]).

Phospholipid bilayer	
Choline, containing phosphatidylcholine and sphingomyelin mostly in the outer layer and amine-containing phospholipids like phosphatidylethanolamine and phosphatidylserine (PS) mostly in the inner layer;	[15–22]
Sphingomyelins and phosphatidylcholines constitute more than 50% of membrane phospholipids; Sphingomyelin is the most abundant sphingolipid; sphingolipids constitute a class of structural lipids with ceramide as the hydrophobic backbone;	
Membrane cholesterol is unesterified and lies between the two layers of the lipid bilayer.	
Transmembrane proteins	
The phospholipid bilayer is embedded with transmembrane proteins, and the maintenance of the asymmetric distribution of phospholipids across the plasma membrane is a prerequisite for the survival of RBCs.	[23]
Glycophorin-A (GPA), GPB, GPC, and GPD and GPG constitute a group of transmembrane proteins in RBCs.	
Peripheral proteins that play a key role in the structure of the RBC cytoskeleton	
Spectrin: (bands 1 and 2) — the most abundant membrane protein consists of two chains, α and β wound around each other. They are flexible rods with actin-binding sites at each end and line the intracellular side of the plasma membrane).	[24–27], [28], [27,29–37]
Globular actin: is formed by filaments that bind weakly to the tail end of both α and β spectrins.	
Protein 4.1, adducin, tropomyosin, tropomodulin, and dematin; are the principal proteins at the spectrin–actin junction. Particularly protein band 4.1 interacts with spectrin and short actin filaments to form the RBC membrane skeleton and regulates membrane physical properties of mechanical stability and deformability by stabilizing spectrin–actin interaction.	
Protein 4.2 is a cytoskeleton protein found in red blood cells. Erythrocyte membrane protein band 4.2 is an ATP-binding protein which may regulate the association of protein 3 with ankyrin.	
Ankyrin: spectrin is coupled to the inner surface of the RBC membrane, primarily through association with ankyrin, and the transmembrane protein bands 3 and 4.1.	
Human erythrocyte p55 is a peripheral membrane protein that contains three distinct domains in its primary structure: an N-terminal domain, an SH3 motif, and a C-terminal guanylate kinase domain. p55 is associated, in precise proportions, with the protein 4.1-glycophorin-C complex, linking the skeleton and the membrane.	
Spectrin–actin cytoskeleton network is anchored to the phospholipid bilayer and ankyrin proteins. The interaction of ankyrin and spectrin yields the major anchor between the membrane skeleton and the lipid bilayer is important for RBC deformability and stability.	
Integral proteins	
Band 3 is an abundant RBC integral membrane protein and it regulates the structure and function of the RBCs. It facilitates anion transport via the RBC membrane and it is an important binding site for cytoskeletal and other RBC proteins.	[38–41]
Band 3 is a multi-spanning ion transport channel or trans-membrane protein, and the band 3 tetramers tether the bilayer to the skeleton via an interaction between its cytoplasmic domain and ankyrin, which is associated with spectrin.	
Three integral types of proteins facilitate the transmembrane passage as well as the structural arrangement of lipids in biomembranes: flippases, floppases and scramblases.	
Solvent resistant domains	
Stomatin (a cytoplasmically oriented monotopic integral membrane protein stomatin)	[42–45]
Flotillins 1 and 2 (constitutively associate with cholesterol-enriched lipid microdomains). Stomatin, a major lipid-raft component of erythrocytes whilst proteins flotillin-1 and flotillin-2 are lipid-raft marker proteins. They are encased within sphingolipid and cholesterol-rich segments of the lipid layer. These protein segments are anchored onto an acetylcholinesterase layer by glycosylphosphatidyl inositol (GPI).	
Aquaporins	
Aquaporins. These are also water channels called that help maintain osmotic balance, calcium pumps and their regulators like calmodulin.	[46]
Glycoproteins	
Sialylated glycoproteins of the RBC membrane are responsible for a negatively charged surface, which creates a repulsive electrostatic potential between cells.	[47,48]
CD47 (integrin-associated glycoprotein) functions as a marker of self on RBCs.	[49–51]

particularly individualised medicine should form an essential part in the diagnosis and treatment of patients [10]. Therefore, we need to adopt novel and creative applications, particularly where morphology is concerned. Here we argue that adaptive ultra-morphology, (where we combine biophysical (e.g. rheology), as well as biochemical readouts), should form an irreplaceable part of science and medicine. In the following paragraphs, we review RBC shape with special reference to the biochemical and biophysical natures of the membrane, as well as mention briefly some of the most important techniques available.

2. RBC shape and the biochemical indicators of the RBC membrane

RBCs are well known for their remarkable ability to change their shape in response to an external force and to pass through the narrowest blood capillaries and splenic sinuses [11]. Cell deformability is postulated to be a major determinant of impaired perfusion, increase of blood viscosity and occlusion in microvessels [11]. The foundation of laboratory haematologic diagnosis is the complete blood count and review of the peripheral smear and the diagnostic assessment includes the assessment of RBC shape, size, colour, inclusions, and arrangement [12]. Central to the shape changes visible under the light microscope are the RBC-affiliated molecules in the RBC membranes and their ability to change during pathology. RBC membranes are made up of various areas, particularly the lipid bilayer, the actin–spectrin cortex and the integral and peripheral proteins [13]. Table 1 lists, and Fig. 1 visually illustrates the positioning of these membrane components.

Structural alterations to the lipids, as well as band 3 and spectrin cause RBC structural shape changes [1,52]. In particular, three integral proteins facilitate the transmembrane passage, as well as the structural arrangement of lipids in biomembranes: flippases, floppases and scramblases [53]. Under normal conditions, the neutral phosphatidylcholine and sphingomyelin are mostly found on the outside, and the charged phosphatidylserine (PS), phosphatidylinositol and phosphatidylethanolamine, are found mostly on the inner membrane leaflet. Scramblase typically facilitates the flip-flop of lipids in a non-selective fashion from the inner, as well as the outer leaflet [54] — this process is a bidirectional process, down a concentration gradient. In the presence of calcium, scramblase behaves like a channel for lipids, allowing them to diffuse from one monolayer to the other, according solely to the concentration gradient. Flippase typically pumps the amino-containing phospholipids from the outer to the inner leaflet. Floppase typically controls the reverse transfer of choline-containing phospholipids (e.g. phosphatidylserine or PS, sphingolipid (SL) and cholesterol against concentration gradients) from the inner leaflet to the outer leaflet. Disruption of the membrane leaflet phospholipid symmetry happens where PS is externalised, resulting in RBC membrane vesicle