A QUANTITATIVE ANALYSIS OF RED BLOOD CELL AGGREGATION FROM BOVINE BLOOD

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Abstract. Red blood cell (RBC) aggregation is an important component of whole blood viscosity and the major cause of non-Newtonian blood flow properties. Red cell aggregation has been shown to play a role in viscosity at low shear rates and deformability at high shear rates. At the moment there are two co-existing "models" for RBC aggregation but this phenomenon is not fully understood yet. Samples from peripheral bovine blood were prepared by using May-Grüwald Giemsa stain. With the aid of a microscope we obtained many photos of the aggregates. In this paper, we used erythrocyte planar images of clusters formed in cow blood to compute the fractal dimension of the aggregates by means of HarFA, a harmonic and fractal image analyzer.

Key words: RBC aggregation, bovine blood, HarFA soft.

INTRODUCTION

RBC aggregation is an important component of whole blood viscosity and is the major cause of non-Newtonian blood flow properties. Viscometric measurements proved that apparent blood viscosity rises with decreasing shear rates. Red cell aggregation has been shown to play a role in the viscosity at low shear rates and deformability at high shear rates. At low shear rates the cells layers are composed of aggregated cells, but at higher shear rates, the aggregates degrade to form thinner layers of oriented cells. Axial migration of red blood cells together with plasma sleeving represents a phase separation, and this process may be considered a self-organisation one and may be treated as a problem of non-linear dynamics and chaos. Although considerable data is now available regarding the physiological and clinical importance of this phenomenon, the specific mechanism involved in RBC aggregation has still to be elucidated. The process of RBC aggregation can be considered the result of a balance between aggregating and disaggregating forces. At present there are two co-existing "models" for RBC aggregation [2, 9]:

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1) Bridging Model hypothesises that the aggregation occurs when binding forces, due to the absorption of macromolecules onto adjacent cell surfaces, exceed disaggregation forces due to the electrostatic repulsion, membrane strain and mechanical shearing;

2) Depletion Model proposes a preferential exclusion of macromolecules from RBC surface, thereby generating an osmotic gradient, a flow of fluid away from the intercellular gap, and a movement of adjacent cells.

Many researchers are paying great attention to the clinic research of blood viscosity as red blood cell aggregation may be a very useful parameter of hemorheology from the point of view of pathology and diagnosis. In [5] Gudmundsson *et al*. demonstrated that blood viscosity and red cell aggregation were significantly higher in rheumatoid arthritis than in controls. Singh and Kumaravel [11] showed that jaundice may be associated with changes in the aggregation process and deformability of erythrocytes.

Comparative animal studies showed wide variations of whole blood and erythrocyte aggregation among different mammalian species. Data gathered by Popel et al. [8] shows that athletic species exhibit a consistently higher degree of red blood cell aggregation than their sedentary counterparts. For example, many authors [2, 3] reported high values for horse RBCs aggregation. Both traditional mechanical and mathematical methods proved to be insufficient in describing the aggregation process.

Over the last years, fractal geometry has been applied with great success to many different physical, chemical and biological systems. Fractal geometry is the geometry of nature; it deals with irregular, complex but self-similar structures or natural phenomena. Many physiological systems have been found to be both spatial and temporal fractals. Men-Zhen Kang and co-workers [6] found that RBC aggregation shows fractal characteristics by analysing the aggregation images. Their research presents the time dependence of Information Dimension for RBC in human blood samples. A CCA model was used to reveal the relationship between the fractal dimension and the binding energy. In order to investigate the properties of aggregates, in [4], Bozhokin used the fractal analysis method. In other papers we also used the fractal analysis to study the aggregation of RBC for human and horse blood [7, 10]. In this paper we used fractal analysis to study the aggregation of erythrocytes in bovine blood.

MATERIALS AND METHOD

Samples from peripheral bovine blood were prepared by using May-Grüwald Giemsa stain. With the aid of a microscope we obtained many photos of normal cells and aggregates. Then we modified the photos in order to apply the fractal analysis. We used erythrocyte planar images of the clusters formed from erythrocytes in cow blood to compute the fractal dimension of the aggregates by means of

HarFA software. In HarFA we used a modification of the traditional Box Counting Method. Three fractal dimensions can be obtained with this modification: the first characterizes properties of black plane DB, the second the black-white border of black object DBW (this information is the most interesting) and the third the properties of white background DW. The fractal dimension is the slope of the straight line "Black&White" [12].

RESULTS AND DISCUSSION

Fig. 1 shows RBCs morphology under static conditions for bovine blood when the RBCs are not aggregated and Fig. 2 shows aggregated erythrocytes.

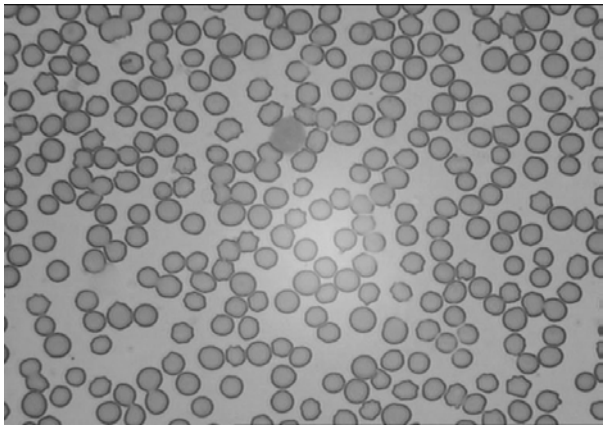

Fig. 1. The erythrocytes from healthy cow blood.

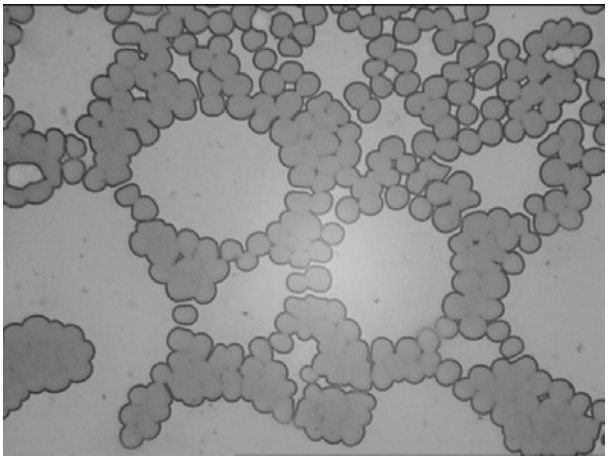

Fig. 2. The aggregated erythrocytes from cow blood.

The fractal dimension for a normal blood sample is presented in Fig. 3 and for aggregated erythrocytes in Fig. 2 and Fig. 4.

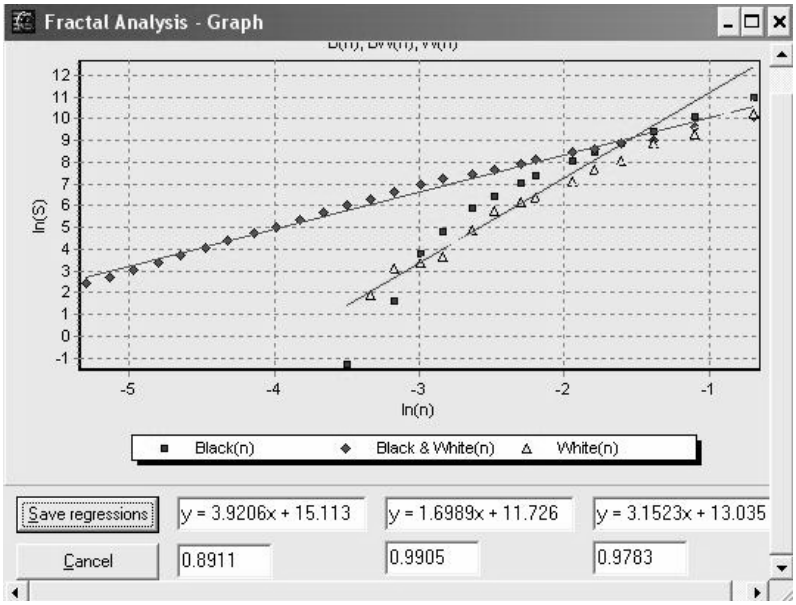

Fig. 3. Fractal dimension for normal blood sample.

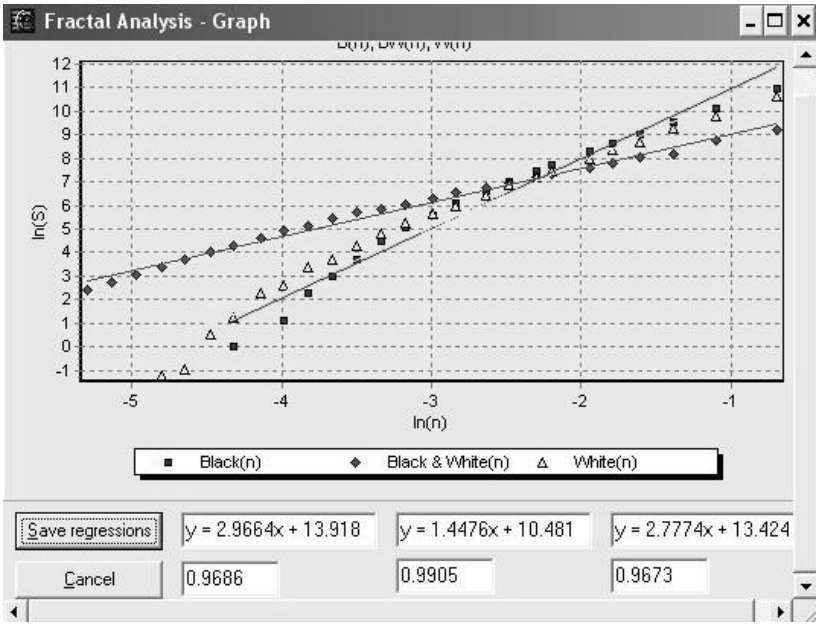

Fig. 4. Fractal dimension for aggregated cells.

CONCLUSION

To our knowledge, there is not any available data to compare with our results on erythrocytes aggregation for animals using fractal analysis.

For the fractal dimension of healthy bovine blood we obtained a value of 1.7; for aggregated red blood cells, meaning a case of a disease, we obtained a fractal dimension of 1.45, in accordance with Kang and co-workers [6] who showed that fractal dimension decreased during RBC aggregation.

We consider that RBCs aggregation is a reversible dynamic process determined by a nonlinear phenomenon not yet understood. If for this process the laws of thermodynamics are valid, then the Newtonian aspects of blood are related to the linear phenomena and the non-Newtonian behaviour is related to the nonlinear ones (self organisation). This is the reason why we consider that fractal analysis which contains both experimental and theoretical aspects is a convenient and efficient method to analyze the aggregation of erythrocytes.

Despite the fact that RBCs aggregation is one of the major factors defining the rheologic properties of blood in the capillaries, there is not yet any adequate method to study the aggregation of erythrocytes *in vivo*.

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