

Fractal Dimensions in Red Blood Cells

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Abstract: We studied the erythrocyte aggregability for different animals using fractal analysis. Red blood cell aggregation is an important component of whole blood viscosity and is the major cause of the non-Newtonian flow properties of blood. To understand the aggregation process many models have been proposed in the literature. Since aggregates formed by aggregation have a fractal structure, mathematical descriptions of their irregular structure can be obtained using fractal geometry.

For this purpose, blood samples were prepared from cows, sheep, rabbits, roosters, horses and humans by diluting 1:200 and mixing for 3 min in adequate reactives. A Turk room, microscope system and computer acquisition system (frame-grabber or video blaster) were used to register and analyse images of cell aggregates. In the case of the blood from cows, sheep, rabbits and roosters no aggregation phenomenon was observed in the microscope slides. However, in the case of horses and humans, erythrocyte aggregates were identified and fractal analysis was carried out by means of a modified box counting method. Higher fractal dimension values were found for horse in comparison to human samples. The results obtained suggest that higher fractal dimensions correspond to higher aggregability, meaning higher complexity of cells' properties of interaction with each other. These results are concordant with literature data. We conclude that horses and humans have more complex structures of blood cells than the other species in this study.

Key Words: Red blood cell aggregation, fractal analysis, HarFA

Introduction

Cell aggregation is the result of promoting and inhibiting factors that can be grouped as biochemical and biophysical. This phenomenon plays a very important role in various processes related to physiological functions.

The most elementary process of particle aggregation in clusters is known as diffusion-limited aggregation (DLA). Particle cluster aggregation can be observed in certain types of electrolytic deposition and in dielectric breakdown. Another growth phenomenon is viscous fingering, which takes place when water is injected at a sufficient velocity into oil impregnating an artificial porous medium made up of cross grooves in a plastic plate. To understand the aggregation process many models have been proposed in the literature. Tang et al. (1) developed a model to predict the strength of

aggregates by accounting for their fractal structure. Since aggregates formed by aggregation have a fractal structure, mathematical descriptions of their irregular structures can be obtained using fractal geometry. In the DLA process used in the description of many growth systems such as the aggregation of protein and polymers, the cluster's fractal dimension is a measure of how the cluster fills the space it occupies. Based on fractal dimensional analysis, the morphological properties such as aggregate porosity or density can be correlated to fractal characteristics, which can be determined using image analysis. The results showed that the fractal structure is in conjunction with the mechanical strength of aggregate formation and rupture during modelling. The authors suggest that this model can be extended to predict the mechanical strength of other types of

aggregate or biological systems. Gonzales (2) developed a computer model for colloidal aggregation. The author showed that the aggregation crossed over from DLA to another type with a higher cluster fractal dimension. Brasil et al. (3) also investigated other properties of aggregates. Cell-to-cell interaction plays a very important role in regulating various processes in normal physiological function.

Anthony van Leeuwenhoek presented the phenomena of aggregation and disaggregation of red blood cells in a letter addressed to the Royal Society on September 25, 1699. J. De Haan communicated the aggregation of horse red blood cells in 1918 and he showed that aggregation for cows and sheep is practically absent. Red blood cell (RBC) aggregation is an important component of whole blood viscosity and is the major cause of the non-Newtonian flow properties of blood (4,5).

At present there are 2 co-existing "models" for RBC aggregation:

1) The bridging model, described by Baskurt et al. (6), hypothesises that aggregation occurs when binding forces due to the adsorption of macromolecules onto adjacent cell surfaces exceed disaggregation forces due to the electrostatic repulsion, membrane strain and mechanical shearing.

2) The depletion model proposes a preferential exclusion of macromolecules from the RBC surface, thereby generating an osmotic gradient, a flow of fluid away from the intercellular gap and a movement of adjacent cells (6).

In recent years, more investigators have been engaged in clinical research on blood viscosity. The aggregation of RBCs may be a more useful parameter of haemorheology from the point of view of pathology and diagnosis. Gudmundsson et al. (7) demonstrated that blood viscosity and red cell aggregation were significantly higher in rheumatoid arthritis patients than in controls. Other studies deal with the aggregation and disaggregation kinetics of lymphocytes and platelets (8). Neelamegham et al. (9) reported the formulation and testing of a mathematical model for the kinetics of homotypic cellular aggregation. This work indicates that aggregation rates strongly depend upon the motility of cells and aggregates, the frequency of cell-to-cell collisions and the strength of intercellular bonds. To compare experimental results with simulation predictions, a video microscopy and image-

processing technique have been used to measure the cell motility parameter and aggregation kinetics of Jurkat cells (a human lymphoblastoid T-cell line).

Traditional mechanical and mathematical methods have proved to be insufficient for describing the aggregation process (10-13). Over the last several years, fractal analysis has been applied with great success to many biological systems. Many physiological systems have been found to be both spatial and temporal fractals (14-16). Kang et al. (17) found by analysing the aggregation images that RBC aggregation shows fractal characteristics. Their research presents the time dependence of the information dimension for RBCs for human blood samples. To investigate the properties of aggregates Bozhokin (18) also used fractal analysis.

Many reports indicated that the cellular deformability and the aggregation characteristics of mammalian RBCs exhibit a wide range among various species (19,20). Therefore, the data reported by Popel et al. (21) showed that athletic species exhibit a consistently higher degree of RBC aggregation than their sedentary counterparts.

The purpose of the present study was to provide more quantitative data on erythrocyte aggregability in horse, cow, sheep, rabbit, rooster and human blood using fractal analysis.

Materials and Methods

In order to determine RBC aggregability, firstly we had to transform an IBM-PC computer into an Image Acquisition System; therefore we designed a frame-grabber and the soft driver necessary to assure the image acquisition taken by a TV camera by means of a microscope. These images are digitised, transformed in a standard format bitmap picture (BMP) and can be compressed and stored or processed. Blood samples were collected from healthy animals (horses, cows, sheep, rabbits, roosters) at the Faculty of Veterinary Medicine of the University of Agronomy and Veterinary Medicine in Iasi. Human blood was obtained from volunteers by venepuncture at the St. Spiridon Hospital in Iasi. Blood samples were collected in test tubes containing EDTA as an anticoagulant (about 4 ml of blood were sampled for every test). We used 5 animals from each species and 10 volunteers. For each subject we used 5 samples.

The blood samples were centrifuged to separate

erythrocytes from plasma (2000 rounds per minute for 10 min) and the latter was then used to dilute the blood to 1:200. The mixture of RBCs in their own plasma was shaken for 3 min by rotating evenly with a radius of 10 cm and 35 rotations per minute. The erythrocyte mixture was then placed in a glass chamber, 0.1 mm in height and 0.04 mm² in surface area. After 5 min the location of aggregates becomes stabilised so that during the following hour the image remains stable. All samples were analysed over 2 h. To determine the fractal dimensions the modified box-counting method (BCM) was used (14). This method is, in our opinion, very easy to use and more accurate and can be applied in cell physiology.

Results

Figures 1 and 2 show the morphology of RBCs and rouleaux under static conditions for human and horse blood, respectively. Horse blood formed long chains of rouleaux linked together to form clusters. Human blood also formed small clusters with rouleaux of RBCs.

The blood images for cows and sheep are presented in Figures 3 and 4, respectively.

Figures 5 and 6 present blood images for rabbits and roosters, respectively.

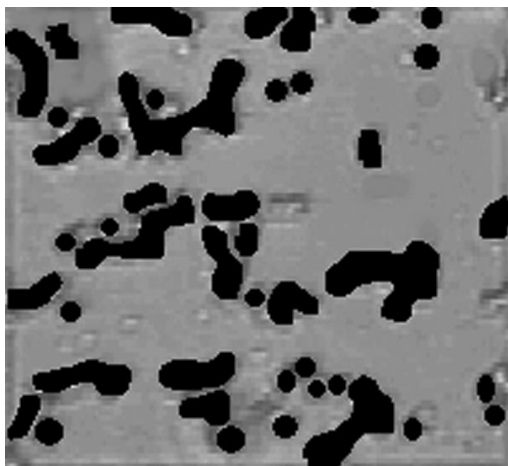


Figure 1. Human blood.

In sheep, cow, rabbit, and rooster blood no aggregation was found. That is the reason that we analysed aggregation images for human and horse blood samples using fractal analysis. HarFA soft (Institute of

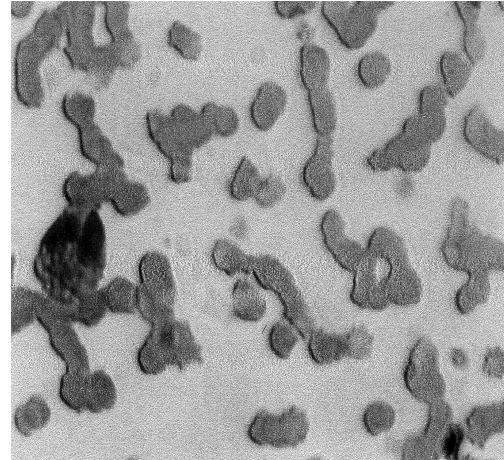


Figure 2. Horse blood.

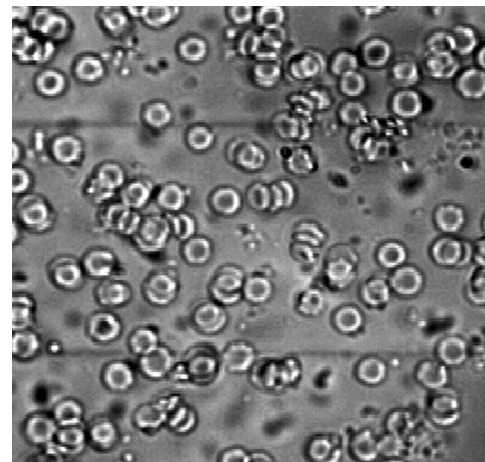


Figure 3. Cow blood.

Physical and Applied Chemistry, Brno University of Technology, Czech Republic) was utilised to study microscopic images of cell aggregates. In HarFA a modification of traditional box counting is used. By this modification one obtains 3 fractal dimensions, which characterise the properties of black plane DB, the properties of white background DW and the black-white border of black object DBW, which is the most interesting information. The fractal dimension is the slope of the straight line "Black&White". The fractal dimension for the human blood sample is presented in Figure 7 and for the horse blood sample in Figure 8. For the human blood sample we found that the fractal dimension was 1.7051 and for the horse blood sample it was 1.8250.

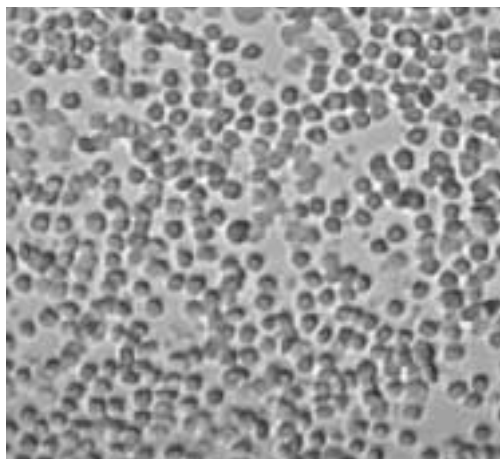


Figure 4. Sheep blood.

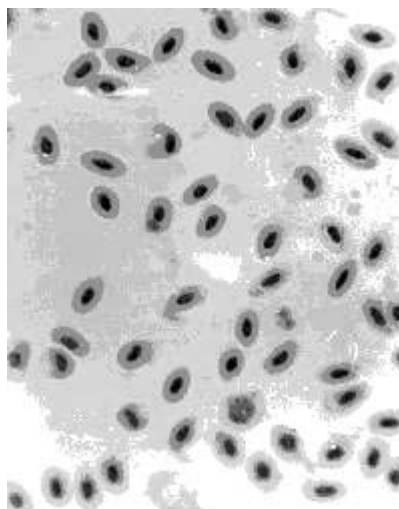


Figure 6. Rooster blood.

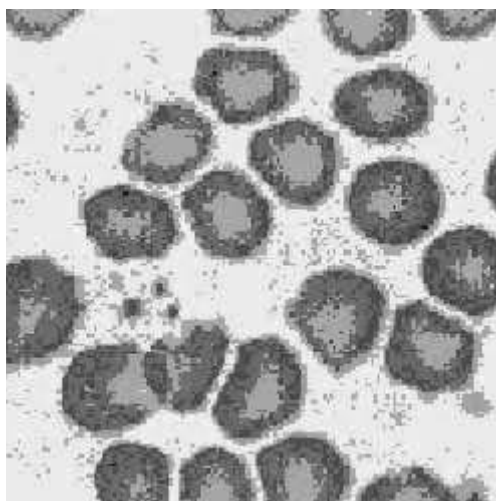


Figure 5. Rabbit blood

Discussion

Using the Smoluchowski equation (9) as a starting point, many authors have developed models to describe aggregation in a variety of biological systems. However, this equation was derived for a dilute suspension where the distribution of spherical rigid particles is homogeneous; that means it may not be useful for RBCs. Barshtein et al. (22) formulated a theoretical model for the spontaneous rouleaux formation for RBCs. The spontaneous rouleaux formation involves both polymerisation, (i.e. interaction between 2 single RBCs) and the addition of a single RBC to the end of an existing rouleau, as well as "condensation" between 2 rouleaux by end-to-end addition. It is now accepted that RBC

aggregation is determined by both suspending medium properties (plasma) and cellular factors. Despite the fact that the relevance of species-specific cellular factors is important in RBC aggregation, the details of these factors remain to be elucidated. The mechanism of rouleaux formation in human red cells is considered very complex but in other animals it is not yet understood. RBC aggregation also affects the fluidity of blood, especially in the low-shear regions of the circulatory system. Generally speaking, haemorheological parameters differ among species, meaning that each species has its own rheological fingerprint. The physiological significance of these variations among mammalian species has not yet been established.

Our results are in good accordance with literature data.

Baskurt et al. (6), using an aggregometer, showed that the aggregation indices in autologous plasma exhibited large differences among animal species. Horse blood has higher aggregability than human blood and the highest aggregability by comparison with the other mammals analysed (the rank order for RBC aggregation was horse > human > rat > rabbit \cong guinea pig). These measurements are in good accordance with our results based on fractal analysis. Baumler et al. (23) also measured RBC aggregation in autologous plasma and in dextran solutions. In agreement with our observations, human and horse RBC form stable rouleaux, whereas bovine RBCs do not aggregate in either plasma or in

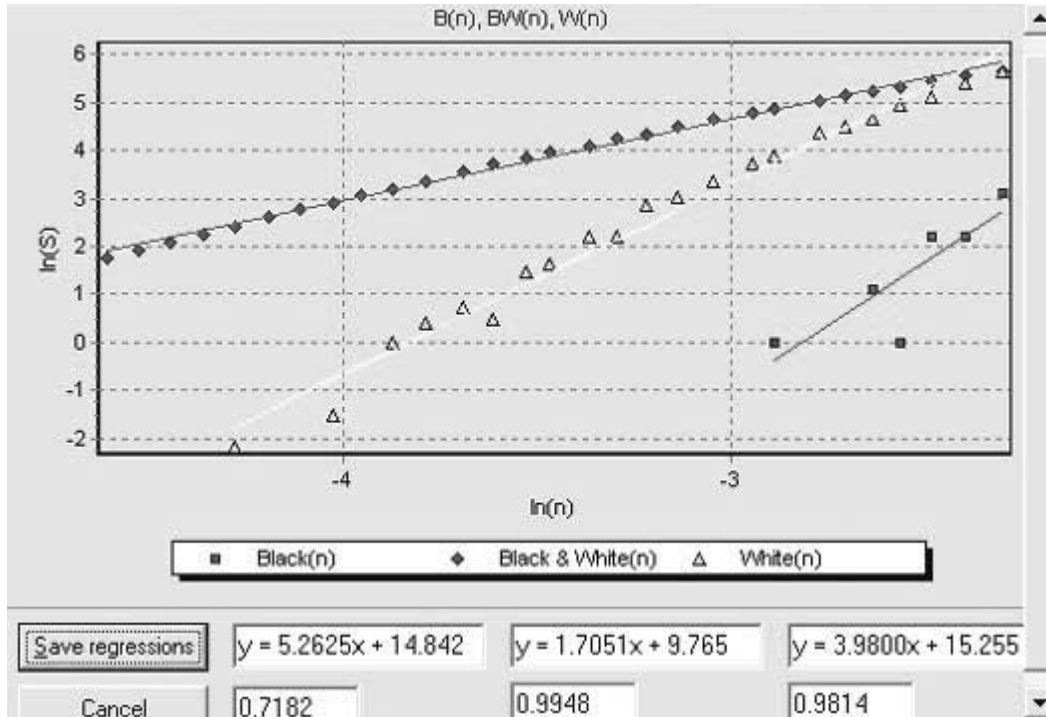


Figure 7. The fractal dimension for human blood sample (DBW=1.7051); 30 steps; minimum 10; maximum 100.

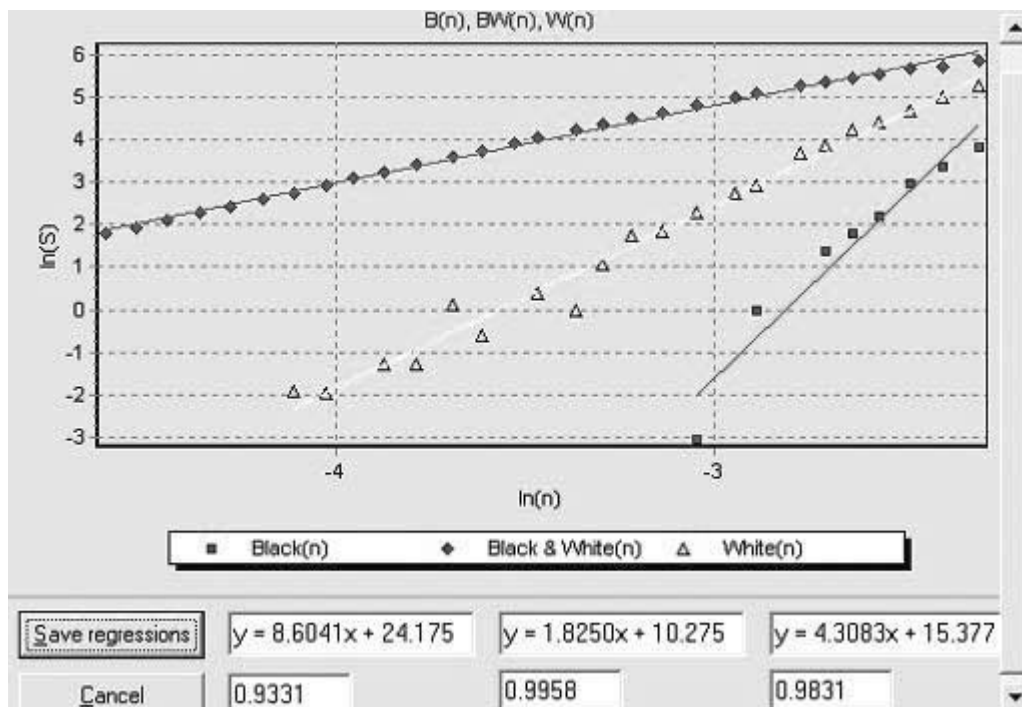


Figure 8. The fractal dimension for horse blood sample (DBW=1.825); 30 steps; minimum 10; maximum 100.

dextran solutions. Adsorption of polymer is not a prerequisite for RBC aggregation. Aggregate formation thus occurs when the Gibbs free energy difference, given by the osmotic pressure difference between the bulk phase and the polymer-depleted region between 2 RBCs, is larger than the electrostatic repulsive energy of the macromolecules present on the RBC surface. Our results are concordant with those presented by Windberger et al. (24).

To the best of our knowledge, no data are available to compare with our results on the aggregation of RBCs for different animals using fractal analysis. Despite the fact that the aggregation of the RBCs is one of the major factors that defines the rheological properties of the blood in the capillaries, there is not yet an adequate method to determine erythrocyte aggregability *in vivo*. We consider that the aggregation of RBCs is a reversible dynamic process determined by a non-linear phenomenon not yet understood. If in this process the laws of thermodynamics are valid, then the Newtonian aspects of blood are related to linear phenomena and the non-Newtonian behaviour is related to non-linear ones (self-organisation). That means the anatomic conditions of the RBC aggregation may be considered related to

deterministic chaos. That is the reason we consider that fractal analysis, which involves both experimental and theoretic aspects, is a convenient and efficient method to analyse the aggregability of RBCs. HarFA soft functioning on the basis of the box counting method yielded significant results for the 2 samples of aggregated RBCs analysed in this paper. The fractal dimension for the human blood sample was 1.7051 and for the horse blood sample it was 1.8250. The fractal dimension appears to be a measure of aggregation geometrical complexity since its values increase when the aggregation irregularities increase. The results obtained suggest that a higher fractal dimension corresponds to higher aggregability, meaning higher complexity of cells' properties of interaction with each other. In the present study we also suggested that fractal analysis might be a promising tool for studying RBC aggregation. We may assume that if we develop an easy method to measure the fractal dimension of images, it may be a good measurement of cell aggregability.

Our results showed that for cow, sheep, rabbit and rooster samples aggregation is practically absent but human and horse RBCs have high aggregability.

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