Morphology of Human Blood Cells

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INTRODUCTION

Blood contains three major cell types. Scanning electron microscopy (SEM) is an ideal method for visualizing cellular detail not otherwise obtainable by light microscopy and requires no special training to interpret micrographs. However, careful sampling and dehydration techniques are required in order to obtain undistorted cells representative of the living forms. Depending on the cell type, different methods for collecting blood cells were used. All samples were fixed in buffered 2% glutaraldehyde, dehydrated through ethanol, critical point dried, and sputter coated with gold-palladium prior to examination in the microscope. Besides gaining an appreciation of the variety of cellular types contained in circulating human blood, knowing the morphology of blood cells can provide valuable information for future studies evaluating extra-corporeal circulation induced changes in blood with SEM.

RED BLOOD CELLS (RBC)

Each mm^3 of blood contains approximately five million RBCs. Cells were obtained by drawing one cc of blood from a patient's arterial line into a heparinized sterile syringe prior to surgery. An equal amount of fixative was immediately drawn into the syringe. After fixation, the cells were washed by centrifugation and deposited onto glass slides that had been treated to retain particles. They are observed in Fig. 1 as smooth, uniform biconcave discs measuring approximately 5 μm in diameter and 1.2 μm in thickness. In Fig. 2 they are also observed in rouleaux formation stacked upon one another. RBCs are repeatedly deformed in flowing blood due to contact with other RBCs and vessel walls but resume the typical shape shown here when unstressed. If whole blood clots one can observe a classic RBC-fibrin meshwork as shown in Fig. 3. For this sample, one drop of blood was allowed to clot on a glass slide for five minutes prior to fixation. SEM reveals one manifestation of the cellular/plasmatic reaction when blood coagulation occurs. RBCs are entrapped by strands of polymerized fibrin. Such a reaction was initiated by blood contact with the glass surface and air. When this phenomenon occurs *in vivo* blood flow is slowed or stopped in the injured vessel preventing blood loss. A similar phenomenon can occur during extracorporeal circulation if adequate levels of heparinization are not maintained.

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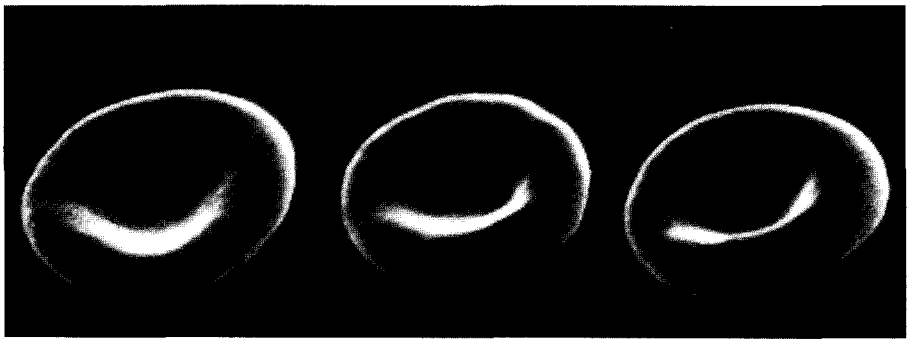

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

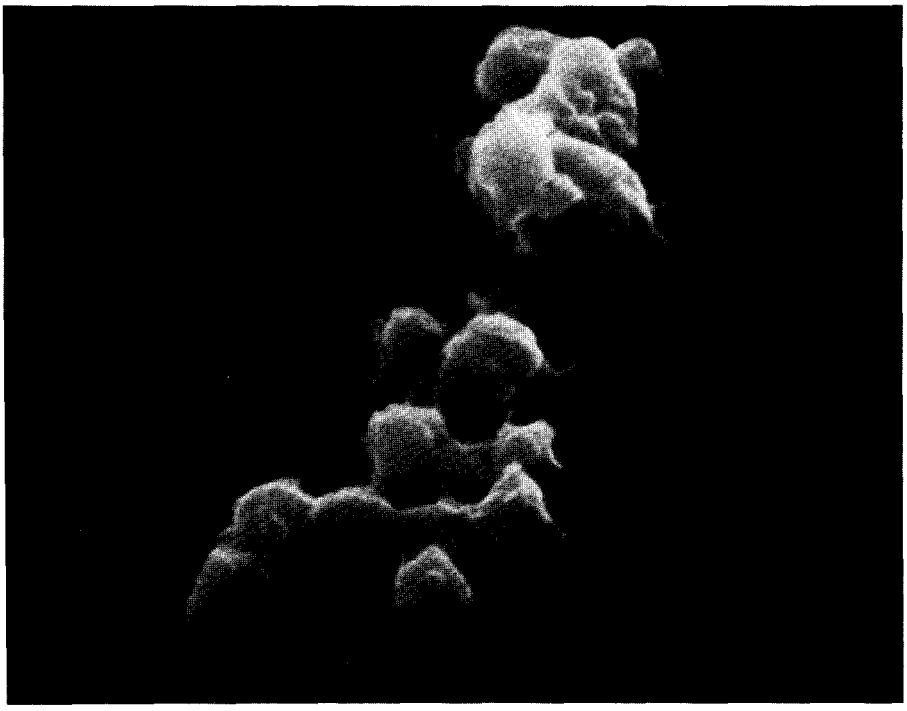

Figure 6

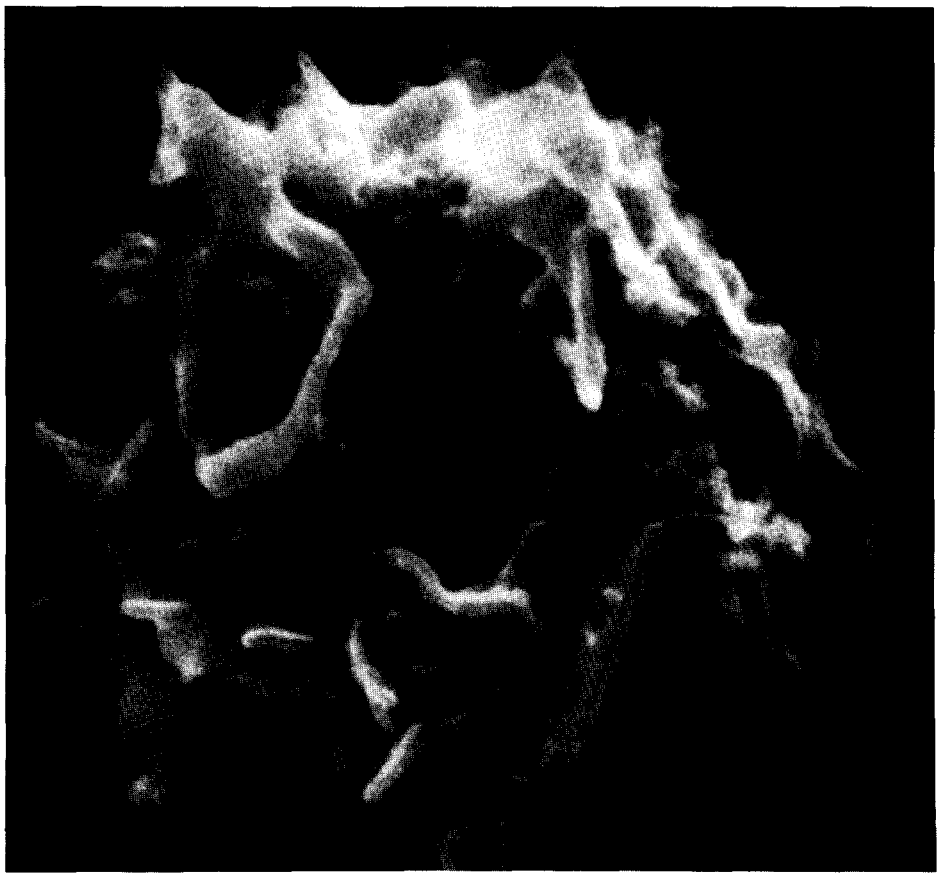

Figure 7

PLATELETS (PLT)

One mm^3 of blood contains 250–500 thousand PLTs, or one-twentieth the number of RBCs. One cc of PLT-rich plasma, anticoagulated in CPD solution, was obtained from the blood bank and fixed. After washing and centrifugation, PLTs were deposited onto glass slides. Fig. 4 shows three discoid PLTs measuring approximately 1.5 μm in diameter and .35 μm in thickness. This is the most common circulating form. They are passive because there is no pseudopod formation. PLTs participate in homeostasis by plugging vascular leaks at sites of injury and can initiate plasma coagulation through release of substances contained within and on the cell body. Such a reaction is accompanied by radical morphologic transformation termed active. The transformation from passive to active form may be triggered by tissue factors, foreign surfaces, collagen, ADP, or other stimuli. Fig. 5 shows two normal PLTs in the upper right corner and one active one in the lower left. When activated, PLTs tend to become attracted to other PLTs and can form aggregates. Pseudopod formation and aggregation may be reversible. Fig. 6 shows one form of PLT aggregation on glass. Note extensive pseudopod formation, sphering

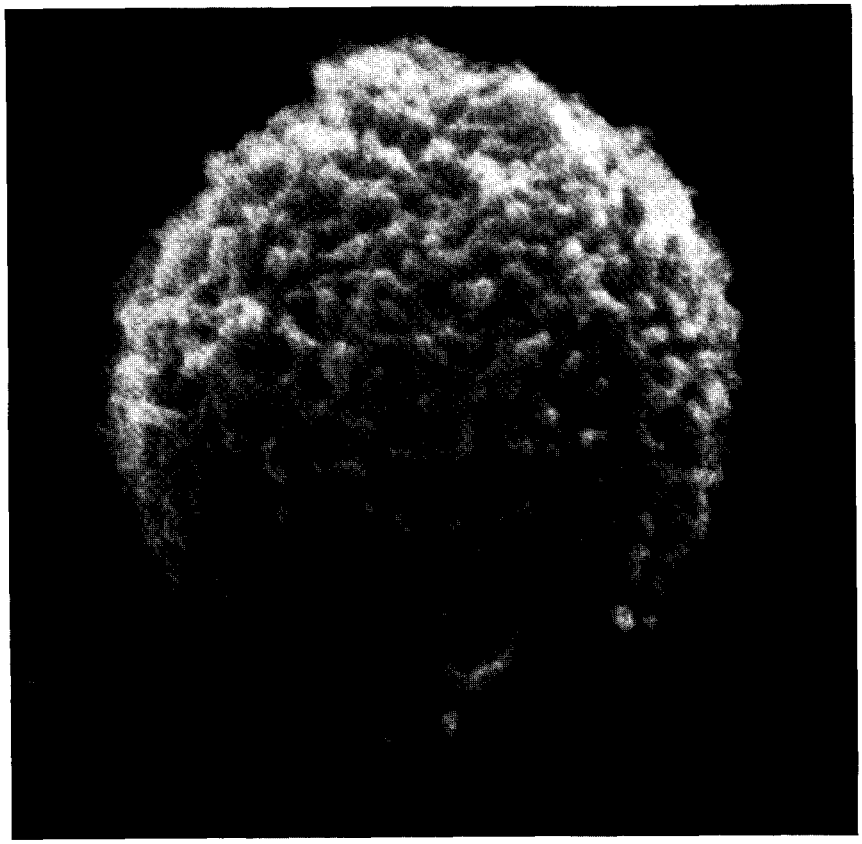

Figure 8

of the cell bodies, and adhesion to the surface and between cells. The drop in circulating PLTs that has been documented during extra-corporeal circulation is thought to be due primarily to reversible aggregation and sequestration of PLTs in the liver.

WHITE BLOOD CELLS (WBC)

WBCs were the most difficult cell type to isolate due to their chemotactic nature and the small number in circulation. Each mm^3 of blood contains only 5–8 thousand WBCs, or two for every 1000 RBCs. There are two major varieties: granular, of which neutrophils are the major type; and non-granular, primarily represented by lymphocytes. Precise differentiation of WBCs by SEM alone is not reliable, although distinctions between granular and nongranular varieties can be made. Two methods were used: first, in order to collect a high percentage of WBCs, nine ccs of whole blood was collected into a warm test tube containing one cc of sodium citrate solution. Centrifugation at 37° C. isolated a buffy coat which was then gently aspirated into buffer solution and centrifuged again to wash the cells. The cells were then fixed and deposited on glass slides. Fig. 7 shows a close-up view of a spherical neutrophil with ruffled surface features. It is approximately

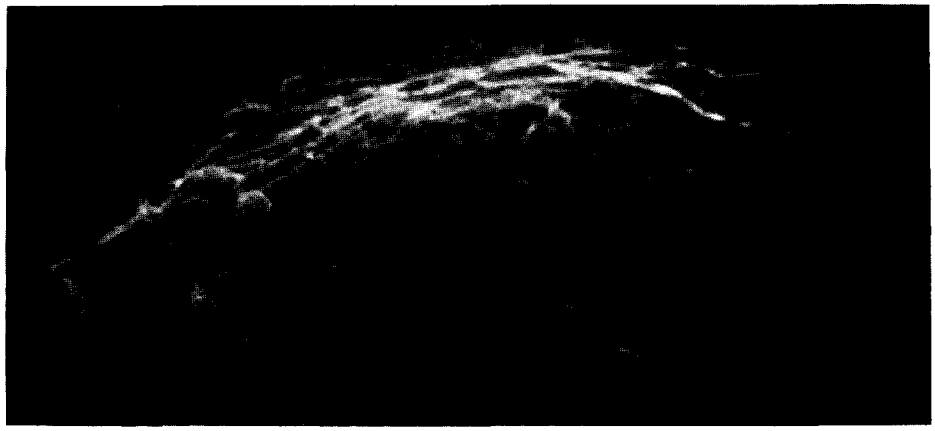

Figure 9

5.5 μm in diameter. Neutrophils are responsible for fighting infection by phagocytosis of foreign bodies and participate in the inflammatory process. A circulating lymphocyte is shown in Fig. 8. It is slightly smaller than the neutrophil measuring approximately 4.0 μm in diameter. The surface is microvillous with multiple small projections. Lymphocytes are involved in immune responses and scar tissue formation. The second method for collecting WBCs was chosen to show their ability to interact with a surface. *In vivo,* WBCs are capable of ameboid movement and can pass extravascularly into tissue by a process

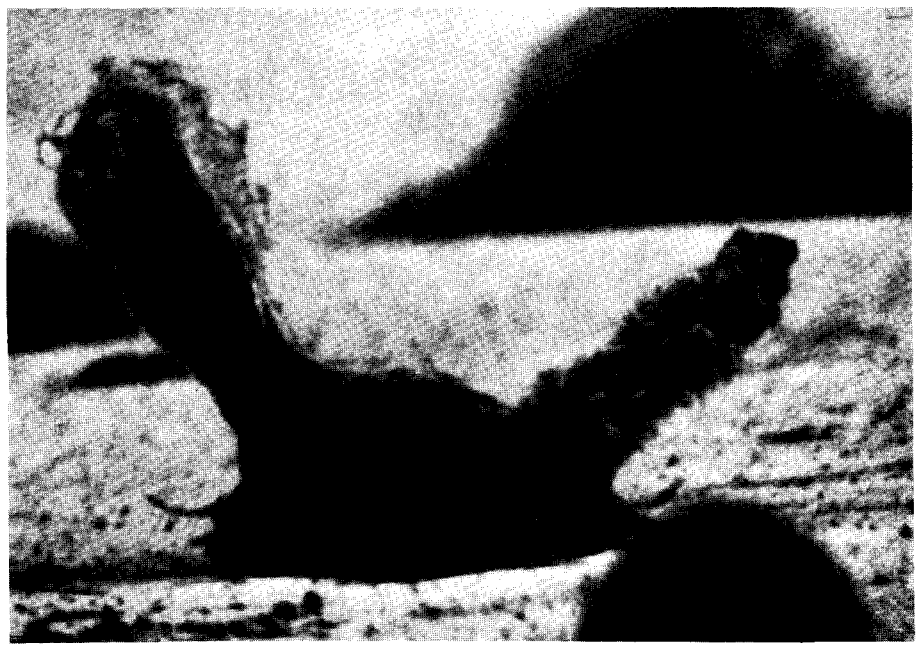

Figure 10

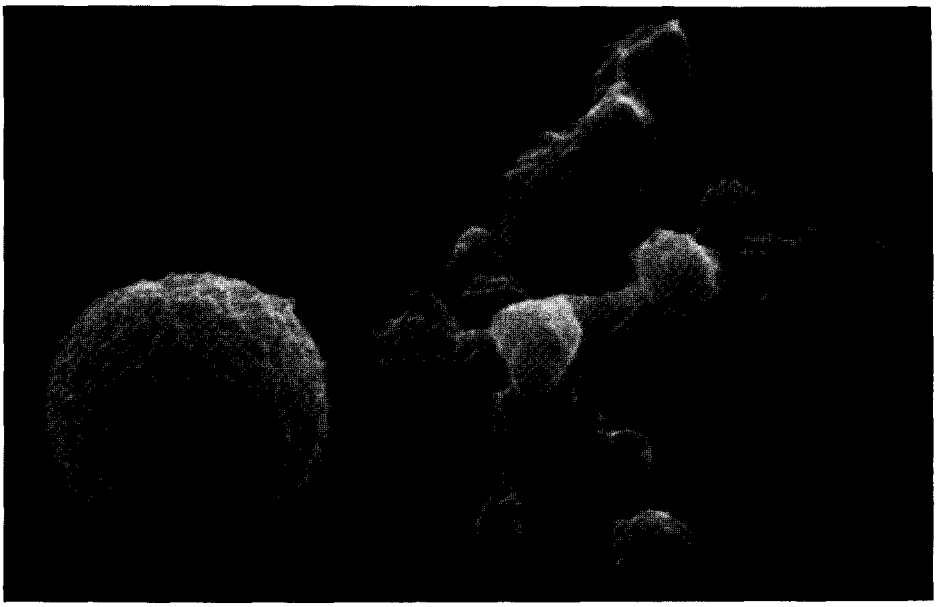

Figure 11

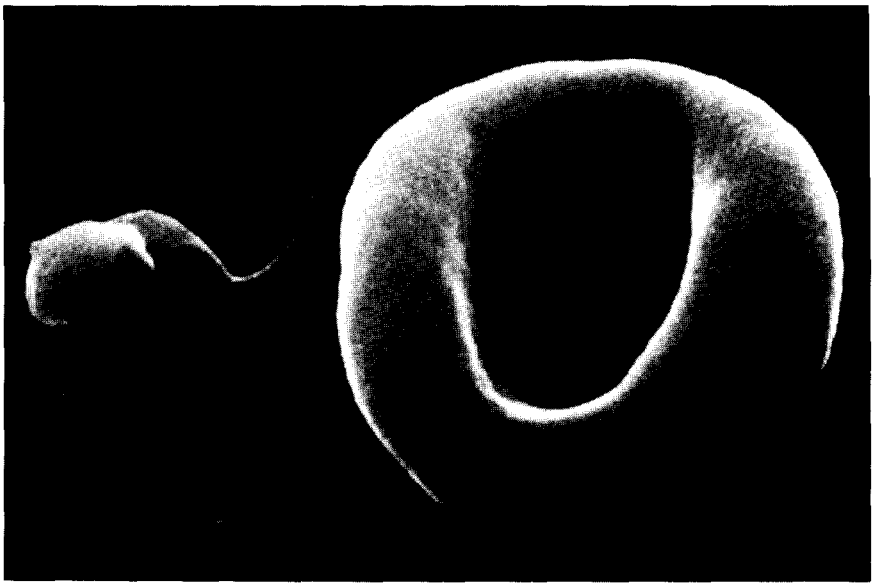

Figure 12

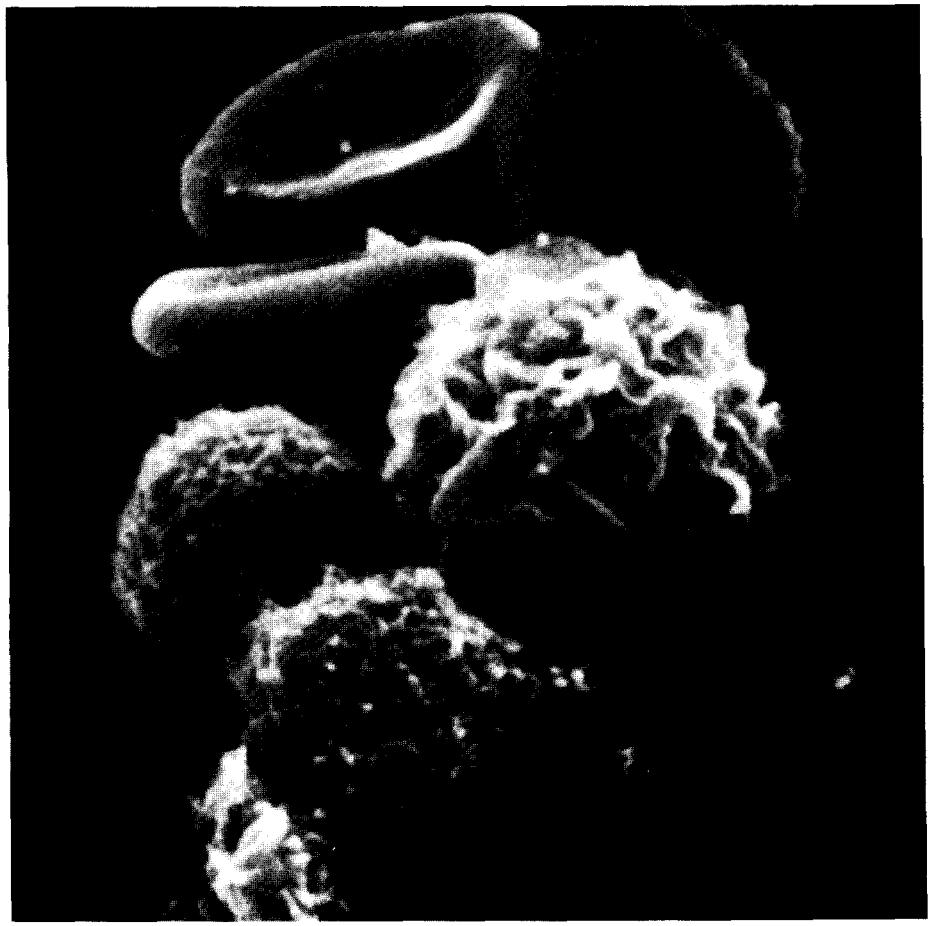

Figure 13

called diapedesis. Several drops of blood obtained by lancing the finger of a volunteer were allowed to drop onto glass coverslips and clot in a humidified 37° C. chamber. At ten minute intervals up to fifty minutes, the clot was carefully removed, and the coverslips were washed and fixed. Adherent WBCs, predominantly neutrophils, were observed interacting with the glass. Fig. 9 shows a spread WBC firmly adherent to the foreign surface and measuring approximately 16.0 μm in width. Fig. 10, taken at an angle of 88°, shows another WBC fixed in random movement on the surface. Tail-like spikes project from the main cell body.

RBC, PLT AND WBC RELATIVE SIZES

As can be seen in the preceeding micrographs, circulating blood cells are of different shapes and sizes. Figs. 11–13 were chosen to show cells of different types in close proximity

to one another and to illustrate their morphologic differences. Before continuing, test yourself by trying to identify the different cell types and forms.

Fig. 11 shows a single WBC with a PLT aggregate. Fig. 12 shows a single RBC with two active PLTs on top of one another. Note the relatively small size of the PLTs in both cases. Fig. 13 shows one neutrophil, three lymphocytes, and two RBCs.

SUMMARY

Besides gaining an appreciation of the variety of cellular types and forms contained in circulating human blood, the detailed morphology of normal blood cells provides valuable baseline information for future studies using SEM to evaluate extracorporeal circulation induced cellular changes. Preoperative detection of abnormal platelet morphology and function in the presence of normal platelet numbers could conceivably be attained. A better understanding of the morphologic cellular features of some hematologic disorders may also accrue.

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