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The Microfiltrometer (MicroFM): a new filtration device for the assessment of less deformable erythrocyte subpopulations

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The hardware of a new filtration device, the Microfiltrometer (MicroFM), is described. The different components of the device; impedance meter, power supply, measuring cell and its 5-micron Oligopore filter are described and it is shown how they are interrelated and interfaced to a computer for data acquisition. The properties of the filter and the general functioning principle of the device are also elucidated. For each run, the MicroFM generates elementary signals from individual passages of many hundreds of red blood cells (RBCs) through micropores of a given 5-micron Oligopore filter. Analysis of each elementary signal provides two complementary parameters, the transit time τ of the explored RBC and the change in electrical impedance ΔZ caused by the temporary flow of the considered RBC through a particular micropore of the filter. These two parameters can be utilized for reliable assessment of erythrocyte deformability on a cellular level.

Key words: Bioimpedance; capacitance; erythrocyte deformability; Microfiltrometer; Oligopore filter; resistive length

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1. INTRODUCTION

Many devices have been developed to investigate erythrocyte deformability [1 – 11]. However, according to Sutton *et al.* [12], "none of these techniques can as yet reliably detect the existence of subpopulations of more rigid red blood cells...". Indeed, most of these techniques yield a collective result for the explored red blood cells (RBCs). Unlike these techniques is the improved version of the Cell Transit Analyser (CTA) developed by Fisher *et al.* [13]. The main

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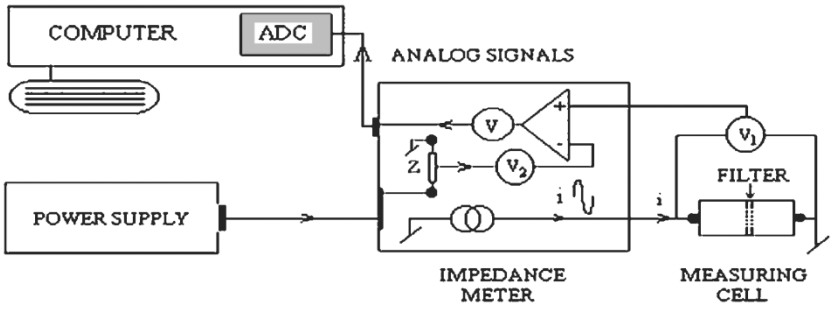

FIG. 1. The Microfiltrometer block diagram.

advantage of this device is to compute several parameters on many hundreds of individually explored erythrocytes for each run. A standardized parameter was recently proposed in order to assess the erythrocyte deformability on a cellular level with the CTA [14]. The parameter is independent of the specific 5-micron Oligopore filter used to carry out the measurements. Unfortunately, in routine use, this parameter is not convenient, because the protocol refers to a hypothetical control blood sample [15], which is a serious shortcoming of the calibration performed with the CTA. In this paper we present a new filtration device, the so-called "Microfiltrometer" (MicroFM), where this problem has been resolved.

2. MATERIALS AND METHODS

2.1. The components parts of the Microfiltrometer

The MicroFM consists of three components: a power supply, an impedance meter and a measuring cell. The Microfiltrometer block diagram shown in Figure 1 highlights how its components are interrelated and interfaced to a computer via an acquisition board.

The schematic diagram of the measuring cell is shown in Figure 2. It is divided into two compartments by a 5-micron Oligopore filter F (see section 2.2). The two compartments are made of plexiglass and graduated from 0 to 10 cm. The compartments A and B are designed to hold RBC suspension and the physiological serum, respectively. Each of the two compartments is connected to an electrode of the impedance meter. Under the effect of a driving pressure ΔP of a few centimetres of water, the RBCs run through the micropores of the filter.

The electrical circuit of the impedance meter and that of its power supply have already been described in a previous work [16]. The impedance meter generates an alternating current of adjustable frequency f (10 to 120 kHz) and adjustable constant amplitude i (0.1 to 1.2 mA peak-to-peak) delivered to the measuring cell. The voltage V_1 at the terminals of the

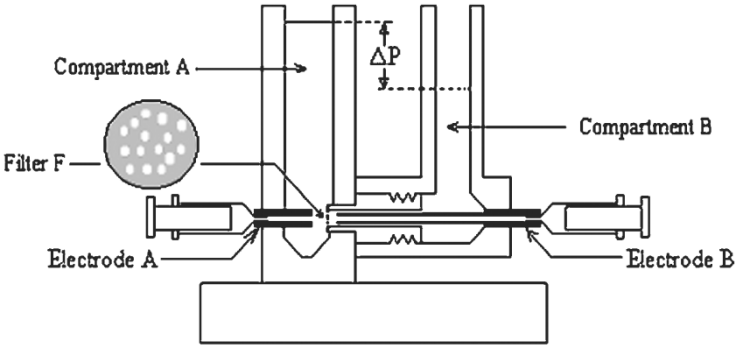

FIG. 2. Schematic diagram of the measuring cell.

measuring cell is measured, rectified and filtered, then compared with the baseline voltage V_2, a manually adjustable voltage equal to that at the terminals of the measuring cell in the absence of driving pressure through the Oligopore filter. The amplification of this differential voltage $\Delta V = V_1 - V_2$ yields voltage V which is digitized by an analog to a digital converter (ADC), and addressed to the computer for acquisition and processing. The ADC is a commercial data acquisition board "Data Translation 01 EZ", having a 12-bit resolution and a sampling frequency of 10 kHz.

2.2. Properties of the 5-micron Oligopore filters

The Oligopore filter is the main element of the measuring cell. Produced by an irradiation method described by Fleischer *et al.* [17, 18] through a polycarbonate membrane, the 5-micron Oligopore filters have initially been specially manufactured to be used with the Cell Transit Analyser (CTA®). These filters are marketed by ABX, Montpellier, France. Theoretically, each filter contains uniform cylindrical micropores, 5 μm in diameter and 11 μm long. For each measurement, the Oligopore filter was allowed to stabilize in a measuring cell filled with saline (NaCl, 300 mOsmol/L) with conductivity σ equal to 1.98 S/m at 25°C. Their properties have been studied, and presented in an earlier paper [19]. According to this work:

- The equivalent circuit of the Oligopore filter may be chosen as a resistor and a capacitor in parallel where the resistance R is mainly due to the saline-filled micropores and the capacitance C represents the influence of the polycarbonate membrane. The series resistance of the bulk solution is negligible in this case [20].
- If f is the frequency of the alternating current, i_R and i_C the current in the resistor and capacitor, respectively, and V the voltage across the electrodes of the measuring cell, then:

$$i_C = 2\pi f C V \tag{1}$$

$$i_R = i\cos\theta \tag{2}$$

and

$$R = \frac{V}{i_R} = \frac{V}{i_{cos\theta}} \tag{3}$$

where θ is the phase angle between currents i_R and i.

- The results from complex impedance measurements with a Solartron 1260 impedance analyser in the frequency range 25 – 126 kHz showed parallel resistance values in the range 16.8 to 27.8 kΩ for seven tested 5-micron Oligopore filters and parallel capacitance values of 38 ± 2.5 pF, nearly constant over the frequency range of interest. The corresponding phase angle θ was typically 12°, 22° and 27° at 40 kHz, 80 kHz and 100 kHz, respectively.
- The average number of micropores inside each of the 5-micron filters, as counted after using a JSM 6400 Scanning Electron Microscope, is equal to 30. Hence, since the resistance R of the whole filter behaves as a circuit of 30 saline-filled micropores in parallel, the resistance R_1 of a single cylindrical saline-filled micropore is given by $R_1 = 30\ R = \frac{L}{\sigma \pi r^2}$ where L is assumed to be the resistive length of the particular micropore with a real length $Lg = 11\ \mu m$ and radius $r = 2.5\ \mu m$. Since the diameter $d = 5\ \mu m$ is comparable to the real length $Lg = 11\ \mu m$, the resistive length L of these pores should be used instead of the geometrical micropore length when calculating the resistance, in order to account for end effects [21]. With the conductivity of the saline being $\sigma = 1.98\ S/m$ at 25°C, we may postulate that the resistive length of such a cylindrical conductor is given by a linear function of the electrical resistance R of the whole filter, that is $L = 30\ \pi\ \sigma\ r^2\ R$.

2.3. Functioning principle of the Microfiltrometer

The MicroFM exploits the insulating properties of the RBC and is intended to explore the distribution of erythrocyte deformability by measuring both erythrocyte transit time τ and transitory variation of the filter electrical impedance ΔZ when a RBC (approximately 8 μm in diameter) flows through a micropore (5 μm in diameter) under the influence of a driving pressure ΔP. When compared to the electrical resistance of such a micropore (approximately 700 kΩ), the RBC behaves like an insulator, since the electrical impedance of

the latter is around 20 GΩ [22]. When the measuring cell is fed with a current i of frequency f, the current through the capacitor (filter material) and the resistance (micropores) of the filter are i_C and i_R, respectively. So, the change of impedance ΔZ occurring from the temporary passage of one RBC through one micropore is given as $\Delta Z = \Delta V / i_R$, where ΔV is the voltage drop already defined in section 2.1.

2.4. Experimental procedure

Measurements are performed on very diluted suspensions of blood (1:2500) to avoid coincidences of the flow of two RBCs at the same time through two micropores of the filter; 2 μL of whole blood taken by finger capillary puncture was directly suspended in a volume of 5 mL isotonic saline (NaCl, 300 mOsmol/L, 25°C, conductivity $\sigma = 1.98$ S/m, viscosity $\acute{\eta} \approx 1$ mPoiseuille), which gives a suspension hematocrit of around 0.02%. Before each exploration, the filter was carefully cleaned by ultrasound for 30 s. The frequency f and amplitude i of the feeding current were adjusted to 40 kHz and 300 μA (p–p), respectively. The driving pressure ΔP between the two sides of the Oligopore filter was set to 5 cm H_2O favouring the compartment containing the blood suspension. Each run entails acquiring 1000 elementary signals generated by 1000 individually explored erythrocytes.

3. RESULTS

A typical acquired elementary signal is presented in Figure 3.

Let us define the marks B, M and E of each elementary signal (ξ) to be:

B = beginning of ξ;
M = maximum of ξ;
and
E = end of ξ.

These marks can be interpreted as follows:

B = marks the RBC's entry into the lines of field of micropore resistive length;
M = marks the RBC's entry into the geometrical section of the micropore;
E = marks the RBC's exit from the lines of field of micropore resistive length.

Let us also define parameters τ and ΔR, where:

- τ = duration BE = transit time of the RBC through the micropore resistive length;
- ΔR = magnitude of $\xi = \Delta V / i_R$ = increase in electrical impedance occurring during the temporary passage of the considered RBC through one micropore where i_R is the calculated current with Eq. (2).

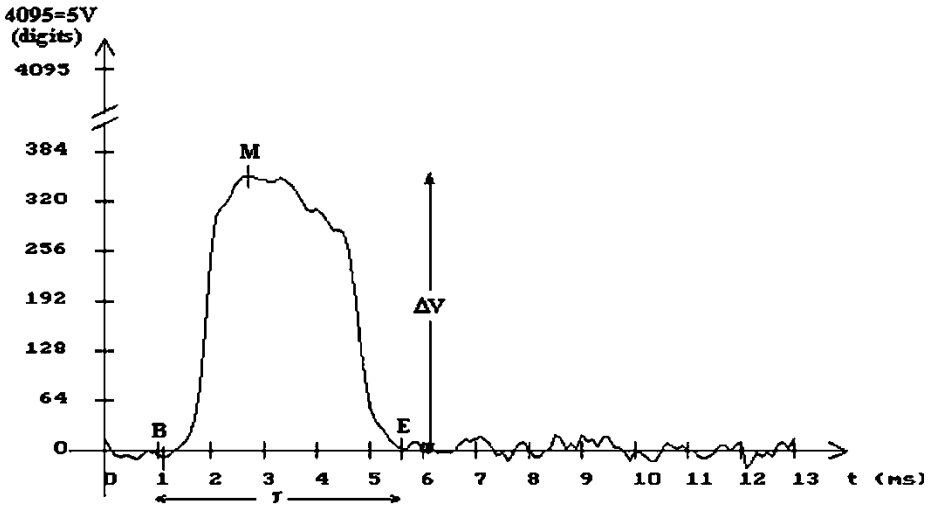

FIG. 3. A typical elementary signal generated by the Microfiltrometer.

4. CONCLUSION

The MicroFiltrometer is a well-qualified filtration device based on kinetic impedancemetry during temporary flow of one erythrocyte through a micropore. Unlike the passive components of this device (measuring cell and 5-micron Oligopore filter), which are the same as those used by the CTA, we have a specially designed impedance meter, a generator of alternating current of known frequency and known amplitude and a voltage detector across the measuring cell. The properties of the 5-micron Oligopore filter used by this device are well controlled. Thus, each explored RBC generates a specific elementary signal where two parameters are defined, the transit time τ and the impedance drop ΔZ occurring during the temporary passage of the considered RBC through a particular micropore. The ease with which the MicroFM assesses these parameters for each explored RBC is a significant improvement compared with the performance of existing devices. The procedure of erythrocyte deformability qualification is detailed in another paper.

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