*Cellular Engineering*

# Rheological modelling of leukocytes

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**Abstract**—*A three-layer Newtonian model is investigated using a combined Eulerian–Lagrangian computational method to describe the dynamic behaviour of leukocytes. The model, composed of a cell membrane (outer layer), cytoplasm (middle layer) and nucleus (inner layer), can better describe the recovery characteristics because large viscosity and capillarity differences between layers are considered, and both Newtonian and seemingly non-Newtonian behaviours reported in the literature can be reproduced. It is found that, to describe adequately the various rheological characteristics of leukocytes, the presence of the highly viscous nucleus and its deformation/recovery, as well as the surface energy stored in the fluid interfaces, are critical. Photographs from pipette experiments using a fluorescent technique confirm the theoretical finding of the important role played by the nucleus in cell deformation.*

**Keywords**—*Leukocytes, Rheological characteristics, Three-layer Newtonian model, Compound drop model*

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## 1 Introduction

RHEOLOGICAL STUDIES of leukocytes (white blood cells) are essential, not only for the comprehension of microculatory flow dynamics, but also for the understanding of their functions and behaviour in health and disease. The rheological properties of the leukocyte, together with its structural characteristics, determine the deformability of the cell, especially during large deformations, such as those involved in their release from the bone marrow or extravasation into the interstitium. This deformability is also critical in the leukocyte's response to disease/infection in humans, because it determines the ability of the cell to flow and deform in capillaries and/or migrate in tissue. Therefore obtaining the correct mechanical description for the leukocytes has been a focus of research in recent years.

There are several types of leukocytes: they are of different sizes, with a nucleus or nuclei of different size and shape. However, experimental observations show that leukocytes behave similarly under comparable, relative deformation, but have different surface tension and viscosity properties. Because neutrophils are the most common leukocytes, most modellings have been done on these cells. The intracellular components in the current leukocyte model used are assumed only to contribute to an increase in the cell apparent viscosity.

Much work has been done over the years on obtaining the correct rheological model for leukocytes. In earlier studies by SCHMID-SCHONBEIN *et al.* (1981), the leukocyte was modelled as a simple visco-elastic solid, with a Maxwell element in parallel with an elastic element, as shown in Fig. 1*a*. The elastic element was thought to impart shape memory that enabled the cell to recoil to the resting shape. However, EVANS and KUKAN (1984) showed that the deformation of a leukocyte into a pipette is a continuous flow process, with no approach to a static deformation limit. It was therefore suggested that the leukocyte be modelled as a Newtonian liquid drop with a prestressed cortical tension (YEUNG and EVANS, 1989), as shown in Fig. 1*b*, rather than a visco-elastic solid. The shape memory then would be induced in much the same way as in drops, by ascribing a cortical tension (corresponding to surface tension in drops) to the membrane enclosing the cytoplasm. Based on this representation, YEUNG and EVANS (1989) simulated the flow of a neutrophil into a pipette by imposing a constant 'ring' force at the pipette tip and a constant orifice pressure inside the pipette to satisfy the free slip condition.

Although the liquid-like behaviour of neutrophils is now widely accepted, the precise nature of this liquid is not established. In their experiments, NEEDHAM and HOCHMUTH (1990) observed that the cell exhibits a non-Newtonian shear-thinning behaviour when flowing into a pipette at high rates of

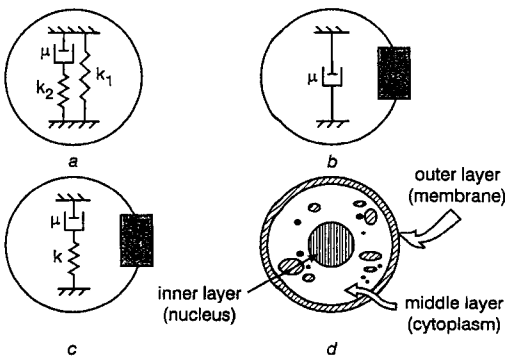


**Fig. 1** *Illustration of various rheological models for the leukocyte: (a) Standard visco-elastic solid model. (b) Newtonian liquid model with constant cortical tension. (c) Maxwell model with constant cortical tension. (d) Three-layer model*

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deformation produced by large aspiration pressures. The non-Newtonian nature of the cell is also suggested by its contents, for example actin filaments, microtubles and other suspended particles, even though their role may increase the cell viscosity only. Thus, the behaviour of neutrophils cannot be described in the same fashion as simple Newtonian liquid drops. These types of behaviour have been thought to indicate a 'fading elastic memory' reminiscent of Maxwell liquids. DONG *et al.* (1988) used a Maxwell liquid model with a constant cortical tension, as illustrated in Fig. 1*c*, to analyse the behaviour of neutrophils flowing continuously into a pipette. However, experimental data could be correlated with their model only if the material properties of the Maxwell liquid were continuously varied as the deformation proceeded. Hence, their model cannot be considered to yield a fundamental description of the leukocyte in terms of a Maxwell fluid.

Another attempt to obtain a non-Newtonian description of the leukocyte was made by TSAI *et al.* (1993) through a power-law representation of the cell apparent viscosity. The power-law model essentially tries to link the shear-rate dependency with the cytoplasmic viscosity to explain the shear-thinning behaviour observed in the micropipette experiments. Although the power-law fluid model could be matched, for suitable initial conditions, to a fairly wide region of the deformation path of leukocytes, the rapid recoil region could not be predicted.

It appears that the rate of deformation, the extent of deformation, the excess aspiration pressure and the holding time all affect the cell recovery characteristics, resulting in different apparent viscosities. Table 1 summarises the apparent viscosity values for neutrophils reported by various groups. These results were obtained from aspiration (flow into a pipette) and recovery experiments. Therefore these curious properties of the cell have yet to be fully explained by existing theoretical models based on the Newtonian or non-Newtonian framework.

It is clear that the existing viscoelastic solid, Newtonian and non-Newtonian liquid drop models fail to explain the complex rheological behaviour of leukocytes. This is because such models do not take into account the internal structure of the cells. As HOCHMUTH *et al.* (1993) point out, it seems that the correct model of the leukocyte can only be obtained when it is true to the morphology of the cell and its contents.

In the search for much a model, we have developed (KAN *et al.*, 1998) a three-layer or compound drop model to capture the cell's major mechanical features (Fig. 1*d*). The three-layer model gives a better representation of the morphology of the leukocyte and its interior than the existing ones. The model includes the most important physical components, namely the membrane cortex, the cytoplasm and the nucleus, and neglects the contributions of the smaller intracellular particles, including the microtubules and filaments. This is a good first-order approximation as the cytoplasm is mainly composed of water. The cytoplasmic elements are assumed only to produce a higher cytoplasmic viscosity.

The objectives of this paper are to show that the three-layer model is physical rheological model for the leukocyte and to demonstrate the importance of the nucleus in the cell deformation and overall rheological behaviour.

## 2 Materials and methods

### 2.1 *Leukocyte preparation*

The cell preparation procedure was designed to give a pure sample of leukocytes in as physiological an environment as possible, while minimising the cells' active response to the glass surfaces of the microchamber and the pipette. Human neutrophils and lymphocytes were isolated according to the procedure of ENGLISH and ANDERSEN (1974). Whole venous blood, anticoagulated with EDTA, was diluted to 50% (v.v.) with $Ca^{2+}$ and $Mg^{2+}$-free modified Hanks' balanced salt solution (HBSS, Sigma, pH 7.4, 300 mOsm) containing EDTA and carefully layered over Ficoll-Hypaque gradients (Sigma Histopaque-1077 and -1119) having densities of 1.077 and 1.119, respectively, at 25°C. After 20 min centrifugation at 800 g and 25°C, the neutrophils at the 1077/1119 interface and the lymphocytes at the 1119/plasma interface were collected and washed twice with 10x volume HBSS. The final suspension of leukocytes was in 50% autologous plasma/HBSS solution to prevent adhesion to glass surfaces (EVANS and KUKAN, 1984). The purity and viability of sample cells were determined by Wright stain and trypan blue exclusion tests.

### 2.2 *Nucleus/cell visualisation*

To observe, at the same time, the cell outer membrane and nucleus, a UV-excited, blue fluorescent bisbenzimidazole dye designated as Hoechst is used. This dye is reported to be cell-permeant and acts as a DNA-specific fluorochrome when

*Table 1 List of published values of neutrophils' cytoplasmic viscosity under different flow conditions*

| Authors | Experiments | Operating conditions | Cell apparent viscosity μ, (Poise) |
|---|---|---|---|
| DONG *et al.* (1988) | aspiration<br>recovery | small deformation | 100–300 |
| EVANS and YEUNG (1989) | aspiration | large deformation | 1000–2000 |
| NEEDHAM and HOCHMUCH (1990) | aspiration | high deformation rate<br>low deformation rate | 1000<br>2000 |
| DONG *et al.* (1991) | aspiration<br>recovery | high deformation rate<br>large deformation<br>large deformation | 200–2000 |
| TRAN-SON-TAY *et al.* (1991) | recovery | (short holding time)<br>large deformation<br>(long holding time) | 800–1500<br><br>1000–2000 |
| DONG and SKALAK (1992) | aspiration | large deformation | 100–700 |
| HOCHMUTH *et al.* (1993) | recovery | small deformation | 600 |
| TSAI *et al.* (1993) | aspiration | low aspiration pressure<br>high aspiration pressure | 5000<br>500 |
| WAUGH and TSAI (1994) | aspiration | high deformation rate | 1000–1500 |

bound to sequences of three A-T base pair in DNA. Hoechst 33342 (Sigma chemical) has been specifically chosen because it has slightly higher membrane permeability than Hoechst 33258 (ARNDT-JOVIN and JOVIN, 1989). Both dyes are water soluble and relatively non-toxic. Stoichometric vital staining of DNA by Hoechst 33342 generally requires exposure of the cells to the dye solution (5–10 $\mu$mol $l^{-1}$) for at least 30 min (SHAPIRO, 1995). Lower concentrations (1–2 $\mu$M) or shorter incubation periods can be used to demonstrate differences in rates of dye uptake between cell types (LALANDE *et al.*, 1980). This technique has been used to differentiate resting T lymphocytes (low uptake) from resting B cells and activated T cells (high uptake) (LOKEN, 1979).

A stock solution of Hoechst 33342 is made by dissolving 0.1 mg of the dye into 1 ml of Hanks' balanced salts solution. A working solution ($cc = 10\,\mu g\,ml^{-1}$, $pH = 7.3$) is freshly made by diluting 10 x the stock solution. The cells are incubated for 30 min at room temperature in a solution with a final concentration of 5 $\mu g\,ml^{-1}$ or 8.5 $\mu$M (50% working solution–50% cell solution).

After incubation, the cells are placed in the chamber under an inverted microscope equipped with fluorescent capability (Zeiss Axiovert 100, 100x oil immersion objective). To excite the Hoechst 33342 dye, a variable-intensity mercury arc lamp (Zeiss, HBO 100 W AttoArc) is used. The cells are first excited with a low light intensity (10%), and then the intensity is progressively increased during the experiment to allow visualisation of the nucleus. Cells are only illuminated for short periods to prevent quenching and cell damage due to long exposure to UV excitation.

Images are obtained with a high-sensitivity (0.0003 lx) television camera (CCD Video camera system, Model ZVS-47 DEC) and displayed on a colour TV monitor (Sony). The experiments are also recorded with a VCR (Panasonic, AG 1730), and pictures are grabbed through a Power Macintosh 7100/80 AV and analysed with the NIH image 1.61 software.

### 2.3 *Micromanipulation and recovery*

Micropipettes were made from 1 mm capillary glasss tubing, pulled to a fine point and broken by quick fracture to the desired tip diameter of 4.0 $\mu$m. Pipettes were filled with isosmotic buffer and were connected to a manometer via water-filled tubing. Pipette pressures ranging from 10 dyn $cm^{-2}$ to 100 000 dyn $cm^{-2}$ can be applied using micrometer-driven displacement of a water-filled reservoir or displacement of a simple suction syringe. Cells were manipulated at room temperature in a microchamber on the microscope stage by a micropipette that had been flushed previously with 50% autologous plasma solution to prevent adhesion of the cell to the glass pipette surface. Experiments were viewed with an interference contrast video microscope and recorded on video tape for subsequent geometric analysis using video calipers.

### 2.4 *Theoretical analysis*

The recovery characteristics of leukocytes were analysed using a mixed Eulerian–Lagrangian computational method. Detailed information on the algorithm (UDAYKUMAR *et al.*, 1998) and the theoretical analysis of the recovery (KAN *et al.*, 1997) can be found elsewhere. A short description of the method used is given here for the benefit of the reader. The main features of this method, the Eulerian-Lagrangian Algorithm for Interface Tracking (ELAFINT), are: a flow solver used to stimulate the incompressible fluid flows over a wide range of Reynolds numbers, and a Cartesian grid employed to solve the fluid equations; interactions of fixed arbitrary geometries and moving boundaries evolving with the flow field. The first is based on the well-developed pressure-based solution procedure (PATANKAR, 1980; SHYY, 1994). Both fluid–fluid and fluid–solid interfaces are described by means of markers indexed sequentially and separated into objects. A computational task when dealing with moving boundaries is handling the interactions between the interface and flow field. The flow solver can incorporate changes in the force term contributions in the governing equations, allowing the effects of moving boundaries to be communicated to the flow field. In turn, based on the flow field developed, the motion of the interface can be determined at every time instant. In this work, the immersed boundary technique (PESKIN, 1977) is used to treat moving fluid–fluid boundaries. The presence of the interface and its effects on the flow field are established via modified source terms in the governing equations. In this manner, the information of the interface component (the Lagrangian part) is redistributed to the flow field (the Eulerian part). This task can be achieved by use of a $\delta$-function (PESKIN, 1977).

## 3 Results and discussion

Figs. 2 and 3 indicate that the three-layer model can describe reported rheological behaviour with viscosity values that are more physiological.

Fig. 2 shows the three-layer model data with the recovery experiment of TRAN-SON-TAY *et al.* (1991) on a cell that has been held for more than 5s in the pipette before expulsion. Viscosity and surface tension values are selected so that the recovery time scales of the nucleus and cytoplasm are similar, as that is one condition for a Newtonian response. (The leukocyte shows Newtonian response under specific experimental conditions.)

In the Figure, $\mu_n$ and $\mu_c$ are the viscosity of the nucleus and cytoplasm, respectively, $\gamma_n$ and $\gamma_c$ are the surface tensions of the nucleus and cell membrane, and $L_n$ and $L_c$ are the lengths of the nucleus and overall cell as recovery proceeds. Viscosity and surface tension are scaled with respect to the suspending fluid viscosity and cytoplasm/cortex complex surface tension, respectively. The two cases shown indicate that the compound drop model is not too sensitive to the viscosity and surface tension values of the cytoplasm and nucleus, *per se*, as long as their time scales are equal.

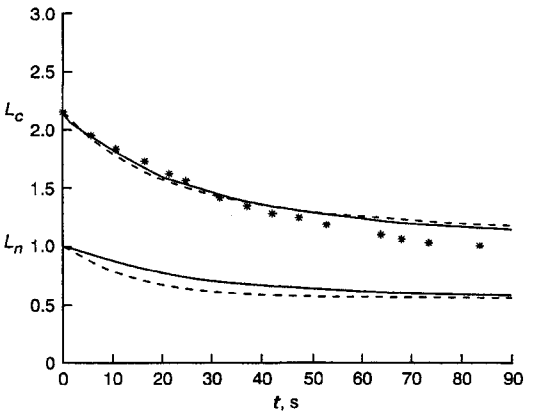


**Fig. 2** *Cell recovery: typical Newtonian behaviour reported for cell held inside pipette for over 5s. * Experimental data from Fig. 8 in TRAN-SON-TAY et al. (1991). (—) Case 2: $\mu_c = 40$; $\mu_n = 400$; $\gamma_c = 1$; $\gamma_n = 10$; $L_c = 2.103$; $L_n = 1.0$ (large deformed initial shape for nucleus). (- - -) Case 4: $\mu_c = 200$; $\mu_n = 2000$; $\gamma_c = 1$; $\gamma_n = 10$; $L_c = 2.152$; $L_n = 1.0$ (sausage-like initial shapes for cell and nucleus, large deformation for nucleus)*

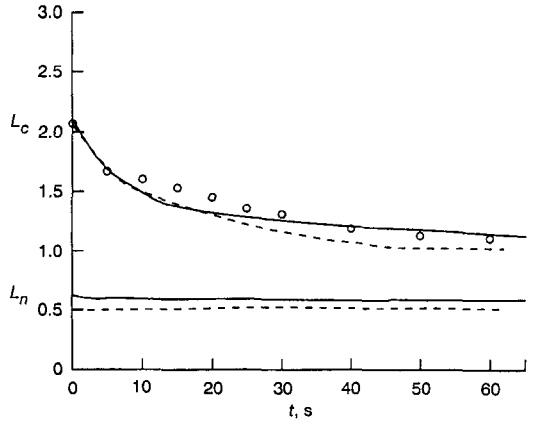


**Fig. 3** *Cell recovery: typical non-Newtonian behaviour reported for cell held inside pipette for less than 5s. ○ Experimental data from Fig. 9 in TRAN-SON-TAY et al. (1991) (—) Case 1: $\mu_c = 40$; $\mu_n = 400$; $\gamma_c = 1$; $\gamma_n = 10$; $L_c = 2.111$; $L_n = 0.618$ (slightly deformed initial shape for nucleus). (- - -) Case 3: $\mu_c = 10$; $\mu_n = 1000$; $\gamma_c = 1$; $\gamma_n = 100$; $L_c = 2.064$; $L_n = 0.5$ (sausage-like initial shape for cell, undeformed initial shape for nucleus)*

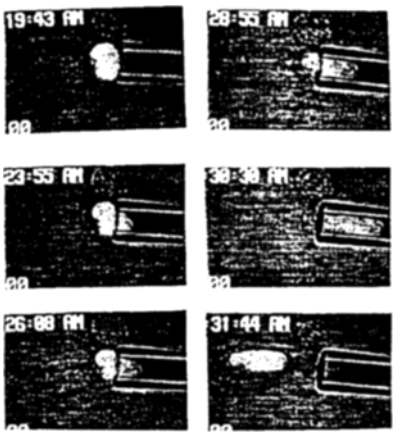


**Fig. 4** *Fluorescent technique experiment: sequence of pictures showing deformation and expulsion of lymphocyte in and out of 3.7 μm pipette. The outlines of the nuclear and cellular membranes can clearly be seen*

The Newtonian behaviour reported by TRAN-SON-TAY *et al.* (1991) in their Fig. 8 can be obtained by modifying the amount of extension of the nucleus and the value of the recovery time scale. The fit between the theory and data does not appear to be very good because the scale used magnifies the difference in the values. It was shown by TRAN-SON-TAY *et al.* (1991) that a Newtonian liquid drop model fits the experimental data very well. There are several conditions where a compound drop will behave as a homogeneous liquid drop: for example, when the inclusion is so small that it does not affect the dynamics of the compound drop, or when the inclusion is sufficiently deformed.

It is important to note that in a previous study (KAN *et al.*, 1998), we have found that the recovery rate of the cells depends on the history of deformation for both two-layer and three-layer models; the time-history of deformation can be stored in terms of the cell and nucleus shapes. (This accounts for the faster recovery of the cell after a high rate of stretching.) The theoretical work indicates that, unless the nucleus and its deformation are included in the analyses, neither Newtonian nor non-Newtonian drop models for leukocytes can yield a reliable picture of the hydrodynamics of such cells. It is found that a condition for a three-layer model to exhibit Newtonian behaviour is compatibility in the time scales between the nucleus and the cytoplasm/cortex complex. The time scale $\tau_i$ is defined as the ratio $\gamma_i/\mu_i$, where $\gamma_i$ and $\mu_i$ are the surface tension and viscosity of the layer $i$, respectively. The subscript $i$ will either be $n$ or $c$ to denote the nucleus or cytoplasm/cortex complex. It was also shown that there are an infinite number of combinations to describe a particular rheological behaviour. The rheological factors of importance are the time scales and the extents of deformation of the nucleus and the outer cell membrane. The rate and mode (i.e. Newtonian/non-Newtonian) of the three-layer leukocyte depend critically on these factors.

In Fig. 3, upon release of the cell, there is a fast recoil that produces an apparent anomalous behaviour of the cell. Fig. 3 shows that the non-Newtonian behaviour of the recovery reported by TRAN-SON-TAY *et al.* (1991) in their Fig. 9 could also be obtained. In particular, the initial rapid recoil phase of the recovery process is captured by the model. The physical explanation of the ability of the model to yield this behaviour can be found in KAN *et al.* (1998). A better fit could have been found by modifying the value of the time scale and the extent of deformation of the nucleus. However, because of the large viscosity differences that exist between the suspending fluid (order of 10 cP), cytoplasm and nucleus (order of 2000P), the computations are extremely slow, and a complete matching of the data was not pursued. In addition, the shape of the nucleus is not spherical and would also alter the results and affect any quantitative values. Nevertheless, the main findings are that the compound drop model can describe both the Newtonian and non-Newtonian responses of the leucocytes without having to change the mechanical values of the elements, and that the nucleus plays an important role in the cell's rheological behaviour.

Our results show that the cell cytoplasm does not have to be highly viscous, as reported previously (EVANS and YEUNG, 1989; NEEDHAM and HOCHMUTH, 1990; DONG *et al.*, 1991; TRAN-SON-TAY *et al.*, 1991; 1994; HOCHMUTH *et al.*, 1993; WAUGH and TSAI, 1994). For example, a cell composed of a nucleus with viscosity and surface tension values ten times those for the cytoplasm (cases 1 and 2 in Figs. 2 and 3) can describe the observed behaviour and explain the different apparent viscosities reported in the literature, based on the different experimental conditions. It should be noted that, to speed up the calculations, the suspending fluid is taken to be equal to 1 P. This means that a cell with a cytoplasm viscosity of 40 P and a nucleus of 400 P, corresponding to cases 1 and 2, will show the same Newtonian response as a homogeneous cell with an apparent viscosity of about 1500–2000 P. The exact value will depend on the value of the cell membrane surface tension. The values given by the compound drop model can be lowered by selecting a different combination that includes another ratio for the recovery time scale, a different extent of deformation for the nucleus, and a shape for the nucleus that is not a cigar shape or symmetric. However, these results demonstrate that the cytoplasm, which is mainly composed of water, does not have to be extremely viscous, as previously reported.

Fig. 4 clearly proves that the nucleus plays a major role in the cell deformation in the microcirculation. To observe both the cell outer membrane and nucleus, we use a fluorescent technique described in Section 2. Visualisation of the nucleus during leukocyte aspiration and recovery provides valuable information on the nucleus visco-elastic properties, and this information is essential to fully understand the cell's overall rheological behaviour. Fig. 4 depicts a sequence of pictures showing the aspiration of a 7.5 μm lymphocyte into a 3.7 μm pipette. The deformation of the nucleus and contour of the cell can clearly be seen.

It is evident that complete understanding and representation of the rheological behaviour of leukocytes in microcirculation require knowledge about the rheological behaviour of the nucleus. Our current efforts are now directed towards performing experiments to provide information about the nucleus and a quantitative rheological description of the properties of leukocytes.

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