NUMERICAL MODELLING OF DEFORMATION BEHAVIOUR OF RED BLOOD CELLS IN MICROVESSELS USING THE COUPLED SPH-DEM METHOD

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Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

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Numerical Modelling of Deformation behaviour of Red Blood Cells in Microvessels using the coupled SPH-DEM Method
Dedicated to

my dear parents and my dear wife with love
Numerical Modelling of Deformation behaviour of Red Blood Cells in Microvessels using the coupled SPH-DEM Method
Keywords

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Abstract

Blood can be considered as a suspension of different blood cells. Among all the blood cells, about 99% of blood cells are red blood cells (RBCs) and it is equivalent to nearly 45% of total blood volume. The microcirculation of blood plays a vital role in the human body. When blood flows through the cardiovascular network, RBCs in blood provide oxygen and nutrients to cells and remove carbon dioxide and waste from them. Due to the viscoelastic membrane of the RBCs, the healthy RBCs are highly deformable and can thus easily squeeze through the smaller blood vessels like capillaries. On the other hand, RBCs infected by some diseases, for example malaria, are stiffer and so less deformable. Thus, it is harder for them to flow through the capillaries. Less deformable RBCs slow down the blood flow rate. As a result of it, the provision of oxygen to human cells decreases and this causes illnesses. In order to understand and predict the mechanical behaviour of both healthy and infected RBCs critically and realistically, a number of experimental studies have been carried out. However, the experiments on motion and deformation of RBCs are somewhat difficult, due to the micro-dimensions of the RBCs and the blood vessels. In this context, numerical modelling techniques have a good potential to explain and predict the motion and deformation behaviour of the RBCs in the smaller blood vessels, like capillaries.

Among all the numerical methods, meshfree particle methods have several advantages over the conventional grid-based methods in modelling the behaviour of RBCs. In particular, smoothed particle hydrodynamics (SPH), as one of the well-established meshfree particle methods, has been proposed by researchers to solve the micro-scale hydrodynamics problems. Furthermore, it has been found that the
discrete element method (DEM) models, based on spring networks combined with a minimum energy approach, provides a more realistic initial geometrical shape for the RBC membrane, which is closer to the matured healthy RBCs’ shape. Therefore, in this research, a coupled SPH-DEM approach was developed to model and investigate the behaviour of RBCs in capillaries.

As the first step of this study, a modified SPH algorithm was developed to simulate the micro scale hydrodynamics problems. The validity of this SPH algorithm was examined against the classical benchmarking problems. Then, a two-dimensional RBC model was developed based on the coupled SPH-DEM. In order to validate the SPH-DEM model, the behaviour of a single RBC in a shear flow was examined and the results compared with the published data. Simulation results of two-dimensional RBC in uniform capillaries revealed that there are some critical parameters that directly affect the behaviour of the RBCs.

After that, the SPH-DEM model was employed to investigate the motion and deformation behaviour of a single RBC in a stenosed capillary. Simulation results provided some asymmetrical deformed shapes when the RBC passed through the stenosed capillary. Furthermore it was revealed that the deformation index is not reliable enough to measure the deformability of the RBCs when they exhibit highly asymmetrical deformed shapes. The asymmetrical shapes of the deformed RBCs highlighted the necessity of the three-dimensional RBC model to capture their behaviour more accurately.

The developed SPH-DEM model was improved to capture the hydrodynamic interaction between multiple RBCs. Using the developed multiple RBC model, the influence of the hydrodynamic interaction between RBCs on their behaviour was
comprehensively explained. It was found that the hydrodynamic interaction between two RBCs is significant when they get closer to each other and as a result of that, two RBCs tend to depart from each other. Furthermore, results revealed that the properties of the leading RBC of two RBCs directly affect the motion and deformation behaviour of the trailing RBC.

Finally a three-dimensional SPH-DEM model was developed to capture the three-dimensional (3D) deformation behaviour of RBCs. In order to validate the developed three-dimensional RBC model, a similar approach as used in the validation of two-dimensional RBC was used. The model was used to predict the 3D deformation nature of the RBCs and simulation results showed highly asymmetrical and complicated deformed shapes, when the RBCs pass through narrower sections of the capillaries. Finally, numerical simulations were carried out to predict the critical diameter of a capillary, which stops the blood flow in that capillary.

In summary, the developed coupled SPH-DEM approach has been proven to be a powerful tool to model motion and deformation of the RBCs in capillaries. The coupled SPH-DEM models were able to replicate the hydrodynamic interaction between RBCs and three-dimensional deformation behaviour of the RBCs. Furthermore, the developed models were successfully employed to determine the critical diameter of the stenosed capillaries.
List of Publications

Journal Articles


5. **Polwaththe-Gallage, H. N., Saha, S. C., Sauret, E., Flower, R., Senadeera, W., & Gu, Y.** Application of SPH DEM approach to numerically simulate the deformation of three dimensional RBCs in non-uniform capillaries (Under preparation).
Conference Articles


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<td>BEM</td>
<td>Boundary Element Method</td>
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<td>DEM</td>
<td>Discrete Element Method</td>
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<td>DI</td>
<td>Deformation Index</td>
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<td>DPD</td>
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<td>Moving Particle Semi-implicit</td>
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<td>RBCs</td>
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Chapter 1: Introduction

This chapter outlines the background of this study (Section 1.1), the context/problem of this study (Section 1.2) and its objectives (Section 1.3). Section 1.4 describes the significance and contribution of this research. Section 1.5 explains the outline of the remaining chapters of the thesis. At the end of this chapter, the conceptual framework of this research is illustrated (Section 1.6)

1.1 Research background

The microcirculation of blood plays a vital role in the human body by providing oxygen and nutrients to its cells while at the same time removing wastes and carbon dioxide (Fisher, 2014; Klinken, 2002; Yousefi et al., 2014). This process is greatly affected by the physiological properties of the blood, especially by the rheological properties of the red blood cells (RBCs), such as deformability of the RBCs (Almac et al., 2007; Tripette et al., 2009; Tsai et al., 2010). Changes in the rheological properties of the RBCs are caused by certain human diseases, such as malaria and sickle cell disease (Dao et al., 2003). According to the World Health Organisation’s latest Malaria report (World-Health-Organisation., 2014), annually about 216 million Malaria cases are predicted and it is estimated that 655,000 deaths (Keating, 2012) will occur from malaria. Therefore, it is important to understand the motion and deformation behaviour of RBCs in order to predict and diagnose the type and severity of these diseases.

Most of the previous studies on microcirculation have proven that blood is a non-Newtonian and inhomogeneous fluid (Pontrelli, 1998; Wada et al., 2008). Blood
can be considered as a suspension of different blood cells: Red Blood Cells (erythrocytes), White Blood Cells (leukocytes) and Platelets (thrombocytes), in a fluid called plasma, which is composed mainly of water (Milcic, 2010; Tavares-Dias, 2006). Among all the blood cells, more than 99% of blood cells are RBCs (Asharani et al., 2010) and they occupy about 45% of total blood volume (Jung et al., 2012; Shvartsman et al., 2003; Skalak et al., 1989; Tsubota et al., 2006b).

Figure 1.1: The typical biconcave shape of the healthy matured RBCs

There are about $10^{10}$ capillary blood vessels, whose diameters are in the range of 5 $\mu$m to 10 $\mu$m, (Dzwinel et al., 2003). In the capillary vessels, RBCs reveal a number of interesting shapes and dynamics in response to the flow conditions, which are crucial for optimal mass transfer. The abnormal motion and deformation of RBCs are mainly related to several diseases, such as sickle cell anemia (Ye et al., 2010). In order to diagnose and treat these diseases, the mechanical behaviour of the RBCs and their responses to the changes in rheological properties of blood and RBCs should accurately and quantitatively be understood. There are certain diseases such as malaria, cancer and sickle cell anemia, which lead to altering the deformability of the RBCs (Jiang et al., 2013). Once the RBCs are infected by
malaria or sickle cell anaemia, the RBCs do not deform enough to pass through the narrower capillaries (Fisseha et al., 2012). Further, if the blood vessel is stenosed, there is a high risk of microvascular blockage (Cooke et al., 2001), which may lead to stopping the blood flow in that capillary.

The importance of understanding the mechanical behaviour of the RBCs and the mass transfer, such as oxygen and carbon dioxide exchange between the RBCs and tissues through capillaries, motivates a number of experimental (in vivo and in vitro), theoretical and numerical studies. However, the studies on motion and deformation of RBCs are somewhat difficult, due to the micro-dimensions of the RBCs and the blood vessels. In this context, numerical modelling techniques have a very high potential to explain and predict the behaviour of the RBCs in the capillaries, especially when the RBCs are infected by diseases such as malaria and cancers. Therefore, currently computational models are widely used to model, interpret and predict the RBCs’ mechanical behaviour.

1.2 Research problem

It can be seen that the RBCs play a major role in the human body and it is generally assumed that RBCs are predominant in blood rheology. The healthy RBCs are highly deformable and can thus easily squeeze through the small capillaries having internal diameters less than their characteristic size. On the other hand, RBCs infected by malaria or other diseases are stiffer and so, less deformable, thus it is harder for them to flow through the smaller capillaries. Therefore, it is very important to critically and realistically investigate the motion and deformation behaviour of both healthy and infected RBCs, which form a current gap in knowledge.
This research aims to investigate the motion and deformation behaviour of RBCs in blood vessels, especially in capillaries, to understand the more realistic behaviour of RBCs and their responses as the rheological properties of RBCs and the geometry of the capillary are changed. Therefore, the key research problem in this research is:

“Can an effective numerical model be developed to model and investigate how the RBCs’ motion and deformation behaviour changes with the different rheological properties of the RBCs and with different flow conditions?”

1.3 Research aims and objectives

This research aims to develop an effective numerical modelling technique to investigate RBCs’ motion and deformation behaviour and their responses for different blood flow conditions. This model will also be used to investigate the motion and deformation behaviour of RBCs, when their deformability is changed due to infection or aging. The key objective of this research is to use the coupled smoothed particle hydrodynamics (SPH) and discrete element method (DEM) to develop a novel RBC model for analysis of the motion and deformation behaviour of the RBCs in capillaries. This objective will be achieved via the following steps:

- Develop a general improved SPH algorithm to simulate micro-scale hydrodynamics problems and validate the improved SPH algorithm, by comparing the SPH results with the analytical solutions for classical hydrodynamics problems.
- Develop a two-dimensional mathematical model to obtain a more realistic shape of an RBC, when it is at rest, with the aid of existing discrete element method (DEM) models based on spring networks,
and couple the developed two-dimensional mathematical model of RBC with the SPH algorithm developed in FORTRAN platform.

- Simulate and visualise the motion and deformation of a single two-dimensional RBC when it flows through a uniform micro capillary under the Poiseuille flow, and to qualitatively validate the developed model using the experimental results (Jeong et al., 2006) and the published data (Kaoui et al., 2011).

- Quantify (mean velocity, energies of the RBC and deformability) and predict the motion and deformation of the RBCs, when they flow through different geometrical microvessels, having uneven cross sections or stenoses. Further studies will be done to predict the motion and deformation behaviour of RBC infected by diseases like malaria.

- Investigate and quantify the effect of hydrodynamic interaction between RBCs on motion and deformation behaviour of RBCs, when multiple RBCs interact with each other.

- Develop a thee-dimensional SPH-DEM model for an RBC and validate the developed three-dimensional RBC model qualitatively and quantitatively. The developed three-dimensional model will be used to predict the three-dimensional deformation properties of the RBCs and, finally, the critical diameter of a capillary, which stops the blood flow, will be predicted.

1.4 Innovation, contribution significance and

Up until now, there has been limited research exploring the motion and deformation of RBCs. According to the best of the author’s knowledge, no realistic
RBC model has been successfully developed incorporating SPH concepts and DEM based on a spring network with minimum energy concepts, in order to examine and predict the motion and deformation of RBCs in capillaries. In this research, a number of unique contributions can be achieved, which are listed as below:

This research will develop novel two-dimensional and three-dimensional RBC models using coupled SPH-DEM for better understanding of the motion and the deformation behaviour of elastic RBCs. These models will be used to replicate the hydrodynamics interaction between multiple RBCs. Furthermore the three-dimensional deformation behaviour of infected RBCs will be modelled. This three-dimensional coupled SPH-DEM model will be the very first three-dimensional RBC model to investigate the behaviour of RBCs in capillaries with stenosed sections.

- The RBCs’ behaviour will be systematically and comprehensively investigated for different flow velocities, rheological properties of RBC membrane and the different geometrical configurations of the capillaries by alternating the values of the developed computer code, especially the behaviour of the infected RBCs in stenosed capillaries.

- The three-dimensional coupled SPH-DEM model will be employed to examine the critical diameter of a stenosed capillary, at which the blood flow through that stenosed capillary will be stopped.

- The RBC models could be used to diagnose the infected RBCs and predict the stiffness of the infected or abnormal RBCs, by comparing the morphology of the real RBCs and model predictions. In addition to that,
the simulation results obtained in this research could be used to design a blood filter to filter out the infected RBCs.

Though this research will not focus on the mass transfer through the RBC membrane, the model developed in this research will be advantageous for future researchers on mass transfer through the RBC membrane (how RBCs transfer oxygen through RBC membrane), since the SPH method is a meshfree particle method. This model would be improved to explain and predict the changes in the morphology of the RBCs when the RBCs are ageing. This research uses the fundamental concepts of hydrodynamics, the SPH method and DEM. Therefore, this RBC model can be modified or adapted for different simulation conditions. Once the developed model of a single RBC is validated, numerical simulations for multiple RBCs can be carried out with some alternations and modifications of the developed SPH-based FORTRAN code. Moreover, the simulations can be extended for different flow conditions such as RBC motion in capillaries with larger diameters, and RBC motion in bifurcating blood vessels.

This research will provide a comprehensive and deep understanding of the motion and the deformation behaviour of viscoelastic RBCs, which will greatly extend and enhance the existing knowledge and understanding of RBCs. It will lead to predicting pathological conditions such as abnormal motions and deformations of RBCs, as well as providing detailed knowledge to diagnose vascular diseases related to RBCs. The modelling techniques and concepts used in this research should also be applicable to future studies in similar research areas. The development of a new RBC meshfree model will contribute to further improvements of meshfree modelling at the micro scale level, using DEM and SPH methods.
1.5 Thesis outline

In Chapter 1: (this chapter), the research background and context/problem are discussed. The objectives and aims of this research are also elaborated with details of the significance and contribution of this research.

In Chapter 2: a contemporary literature review is presented. This chapter begins with the previous experiments carried out on microcirculation and blood. The numerical techniques applied to RBCs and blood in order to model the motion and deformation of RBCs are also discussed in this chapter. Finally, the literature review will be summarised and the implications from the literature will be highlighted at the end of the chapter.

In Chapter 3: the emphasis will be on the introduction of the modelling implementation techniques. The basics of the SPH method will be briefly introduced, together with introduction of two-dimensional and three-dimensional RBC models, DEM based on spring networks and a minimum energy approach. Additionally, the basic simulating parameters used in the following chapters will be presented. Finally, the approach employed to validate the developed models will be presented.

In Chapter 4: the motion and deformation of a single two-dimensional RBC in a uniform capillary will be studied. At the end of this chapter, the key parameters of the RBC, plasma flow and capillary geometry greatly affecting the motion and deformation of the RBC will be identified.

In Chapter 5: the motion and deformation of a single two-dimensional RBC in a stenosed capillary will be studied. In this chapter, how the membrane energy of the RBC varies when it passes through the stenosed section will be presented. Finally,
the importance of the three-dimensional simulations will be emphasised at the end of the chapter.

In Chapter 6: the simulation results obtained on motion and deformation behaviour of multiple two-dimensional RBCs will be explained. The hydrodynamic interactions between multiple RBCs and how they affect the motion and deformation behaviour of multiple RBCs will be comprehensively discussed.

In Chapter 7: the deformation behaviour of three-dimensional RBCs will be presented. In this chapter, the motion of multiple three-dimensional RBCs in narrow capillaries which are narrower than the diameter of the RBCs at rest will be presented. Finally, the approach used to determine the critical diameter of a stenosed capillary, which stops the blood flow, will be described.

In Chapter 8: the major conclusions, limitations, and recommendations of this research will be presented.
1.6 Framework of this research

1. Background
2. Research problem
3. Objectives & Aims
4. Significance & contribution
5. Thesis Outline
6. Research Framework

1. Introduction
2. Experimental studies
3. Numerical studies
4. Summary & Implication

1. Literature Review
2. Introduction
3. Experimental studies
4. Numerical studies

1. Modelling Implementation & Validation
2. SPH approach & Validation
3. 2-d RBC model & Validation
4. 3-d RBC model & Validation
5. Time integration technique
6. Convergence Study

1. Research Schedule
2. Behaviour of a single 2-d RBC in a uniform capillary
3. Behaviour of a single 2-d RBC in a stenosed capillary
4. Behaviour of multiple 2-d RBCs in capillaries
5. Behaviour of 3-d RBCs in capillaries

1. Conclusions & Suggestions
2. Research Summary
3. Limitations
4. Suggestions
Chapter 2: Literature Review

This chapter provides a summary of the knowledge on microcirculation. The major part of this literature review was done to review the current knowledge on the motion and deformation behaviour of the RBCs and the methodologies used by the previous studies. This chapter begins with an introduction to RBCs and their properties (Section 2.1). Then, previous experimental studies on RBCs and the major findings will be explained in Section 2.2. Section 2.3 details the numerical studies on microcirculation and in this section, meshfree particle methods and their advantages will be explained. At the end of this chapter, the implications from the literature are illustrated (Section 2.4)

2.1 Introduction

Bone marrow produces all the blood cells of the blood including RBCs (Perrod, 2013). RBCs eject their nuclei in the early stages of maturity (Freund et al., 2010; Jenkins, 1977; Pozrikidis, 2003; Undi et al., 2013). Healthy human RBCs have a biconcave disk shape (see Figure 1.1) with a mean diameter of about $8\,\mu m$ and a mean thickness of about $2\,\mu m$ at rest (Dupire et al., 2012). The average surface area and the average volume of a healthy RBC are about $135\,\mu m^2$ and $90\,\mu m^3$ respectively (Shi et al., 2012). The biconcave shape of the RBCs will remain only over the course of its life span of 120 days (Suresh et al., 2005). This biconcave shape of the RBCs provides a high surface-to-volume ratio and it aids in increasing the efficiency of oxygen diffusion through the RBC membrane. The viscoelastic membrane of the RBC consists of a lipid bilayer, and it is supported by a mesh-like cytoskeleton. The mesh-like cytoskeletons are formed by a network of spectrin...
proteins linked by short filaments of actin (Fedosov et al., 2010; Pozrikidis, 2003). This biological membrane contains an incompressible Newtonian fluid called cytoplasm, which contains large amount of haemoglobin, which is highly efficient at binding oxygen (Korin et al., 2007).

![Diagram of Blood Circulation](image)

**Figure 2.1: Blood circulation within the cardiovascular network**

Blood flows continuously within the human cardiovascular network as shown in Figure 2.1, as blood flows from the heart to arteries, capillaries, veins and then flows back to the heart (Fouras et al., 2012). The heart pumps oxygen-rich blood to tissues and the organs of the body through arteries (Garza et al., 1984). In the tissues, blood moves through the capillaries, which are the smallest blood vessels in the body, in where the haemoglobin of RBCs releases oxygen to the cells and absorbs carbon dioxide from cells. Carbon dioxide rich blood then travels through the veins to the heart, which pumps the blood to the lungs. In the lungs, haemoglobin absorbs oxygen from the air (Jones, 1925) and releases carbon dioxide, due to the high
concentration of oxygen in lungs. Finally, blood moves back to the heart. However, diseases like malaria change the properties of the RBCs, which lead to change in the rheological properties of the blood. As a result of that, blood flow rate and the ability of providing oxygen to the tissues of the human body are changed. The importance of understanding mechanical behaviour of the RBCs and the mass transfer, such as oxygen and carbon dioxide exchange between the RBCs and tissues through capillaries, motivates a number of experimental (in vivo and in vitro), theoretical and numerical studies.

2.2 Experimental studies on microcirculation

The invention of the microscope in the seventeenth century, led to the discovery of the network of capillaries in the human body. In 1830, Poiseuille conducted a number of experiments using liquids and cylindrical tubes, which contributed to understanding the blood flow in the large vessels and in the microcirculation (Popel et al., 2005). Fahraeus observed that the average RBC concentration in the blood flow decreases when the capillary diameter decreases below 300 µm (Pozrikidis, 2005a). As a result, discharge hematocrit (overall volume fraction of RBCs) is greater than the tube hematocrit (volume fraction of RBCs inside the capillary). This behaviour is known as the Fahraeus effect. Furthermore, Fahraeus reported that the apparent viscosity of the blood decreases in the blood vessels having a diameter of between 8 µm to 500 µm. Further decrease in capillary diameter shows a rapid increase in apparent viscosity, which is known as the Fahraeus-Lindqvist effect (Bagchi, 2007). In order to understand the RBCs’ deformation and different rheological properties, a number of experiments have been conducted with the aid of optical tweezers (Dao, et al., 2003), Micropipette
aspiration (Artmann et al., 1997), and optical magnetic twisting cytometry (Fedosov, et al., 2010).

Many researchers reported three different types of RBC motions (Figure 2.2) in a linear shear flow, namely, tank treading, tumbling, and vacillating breathing (Danker et al., 2008; Shi, et al., 2012; Veerapaneni et al., 2011). For higher shear rates and higher viscosities of plasma, RBCs show a tumbling motion, in which the RBCs undergo a flipping motion while hardly changing their shapes. On the other hand, for the lower plasma viscosities or lower shear rates, RBCs deform into ellipsoidal shapes with constant inclined angles, while the membrane circulates around the cytoplasm, which is known as a tank treading motion (Keller et al., 1982). Depending on the degree of confinement and the maximum flow velocity, the vacillating breathing motion of RBCs can be seen, in which the major axis of the RBC oscillates around the shear direction, accompanied by a breathing-like motion (Shi, et al., 2012; Vitkova et al., 2008). Furthermore, in a Poiseuille flow, three different shapes (Figure 2.3) of RBCs including Parachute shape, bullet-like shape and slipper shape can be found in literature, depending on the rheological properties of the RBCs and flow conditions (Hosseini et al., 2009; Kaoui et al., 2009).

![Figure 2.2: The three types of RBC's motion in capillaries; (a) Tank treading, (b) Tumbling and (c) Vacillating breathing](image)

Since it is difficult to study the microcirculation and rheological properties of RBCs quantitatively, due to the micro sized dimensions, most recent research has
been conducted based on numerical modelling. Further, to understand these types of motions and deformations of RBCs, a number of numerical models were presented.

![Image of RBC shapes](image)

**Figure 2.3:** The three types of deformed RBC’s shapes in capillaries; (a) Parachute shape, (b) Bullet-like shape and (c) Slipper shape

### 2.3 Numerical studies on microcirculation

#### 2.3.1 Finite element methods to study RBC behaviour in microvessels

In the last few decades, a number of numerical models have been introduced to study the RBCs’ behaviour in blood vessels. Pozrikidis (2003) developed a numerical model based on the Boundary Element Method (BEM) to simulate the RBCs’ motion in a Poiseuille flow. Pozrikidis (2003) revealed that diskoidal RBCs, initially placed at the major axis of the capillary tube, achieve an axisymmetric parachute shape as RBCs advance the capillary tubes, while the RBCs placed at intermediate orientation angles attain a slipper-like shapes (Pozrikidis, 2005b). But in the BEM, flow is restricted on a Stokes flow region. Therefore, it is not possible to extend the simulation for large vessels, where the temporal unsteady effect should be considered (Ii et al., 2012). Furthermore, Pozrikidis (2005a) revealed that the initial cell spacing, or the tube hematocrit for a fixed capillary diameter, influences the mean flow velocity and the deformation of the RBCs. However, their study did not focus closely on the effect of the mean velocity of the flow and deformation behaviour of the RBCs when the properties of a specific RBC are changed due to the infection by a disease. Sun et al. (2005) employed the Lattice Boltzmann approach to
simulate the two-dimensional blood flow in a blood vessel, considering blood cells as a suspension of blood cells in plasma. They reproduced the motion of blood cells in plasma to explain the experimental results, such as the Fahraeus-Lindqvist effect and Fahraeus effect (Sun, et al., 2005). However, in this model the deformation characteristics of the RBCs were ignored, since all the blood cells were modelled as rigid bodies (Tsubota et al., 2006c).

Eggleton et al. (1998) used the Immersed Boundary Method (IBM) to simulate the deformation of a capsule in a simple shear flow and revealed that the RBC membrane shape begins to asymptotically approach a steady-state value as the ratio between the dilation modulus to extensional modulus is increased. However, the capsule response was followed for a short time (Sui et al., 2008). Shi et al. (2012) employed IBM to study the deformation of a single RBC in the Poiseuille flow and found that the RBCs tend to move towards the axis of the microvessel. Further, he stated that the steady state shape of the RBCs in the Poiseuille flow depends on the swelling ratio, initial inclined angle of the RBC, maximum velocity of the Poiseuille flow, the height of the capillary, and the bending stiffness of the RBC membrane. In addition, the same method has been used by Takagi et al. (2009) to analyse the deformation of blood cells.

The above methods divide the continuum materials into discrete elements and the individual elements are interconnected by a topological map, known as a mesh or grid. Then a suitable interpolation function is built upon the mesh (Ii, et al., 2012; Tsubota et al., 2006a) and the solutions are obtained by solving partial differential equations. However, it is very time consuming to use these Finite Element Methods (FEM) for the problems with complex geometries with moving boundaries (Gu,
2005). Moreover, Stokes et al. (Stokes et al., 2010) reported pressure instabilities of FEM results for simulating fluid transport behaviours of biological soft tissues. In addition, these studies have been conducted without considering the influence of the rheological properties of the intercellular fluid on the deformation mechanism of the RBCs and they have not addressed the inhomogeneous nature of the RBCs. On top of that it is very complicated and time consuming to employ these FEMs to model the mass exchange through the RBCs’ wall. For example, diffusion of oxygen particles and carbon dioxide particles through the RBCs’ wall is a relatively challenging task to be modelled, using FEMs. In order to understand the RBCs behaviour in microvessels more accurately, it is essential to develop models that are able to capture and describe more subcellular details, such as RBC wall, interactions between RBCs and the differences in the RBCs’ shapes and sizes (Van Liedekerke et al., 2010). In this context, recently developed Meshfree Particle Methods (MPMs) have a very high potential to explain and predict the behaviour of the RBCs in the capillaries, especially when the RBCs are infected by diseases such as malaria and cancers.

### 2.3.2 Meshfree particle methods and their advantages

Meshfree particle methods (MPM) are a particular class of numerical simulation algorithms and they employ a set of finite number of discrete particles to represent the state of a system. The main feature of MPMs is that they do not use a predefined mesh for the domain discretisation (Gu, 2005). There are some unique advantages of MPMs over the conventional grid-based methods (Li et al., 2002);

- They can effortlessly handle very large deformations.
• Meshfree discretisation can provide accurate representation of a geometric object.

• MPMs can be easily used to model deformable moving boundaries.

• Without much effort, inhomogeneous objects and free surfaces can be modelled by MPMs.

There are quite a lot MPMs that can be found in the literature, such as Smoothed Particle Hydrodynamics (SPH) method, Moving Particle Semi-implicit (MPS) method, Dissipative Particle Dynamics (DPD) method, Discrete Element Method (DEM) and Particle In Cell (PIC) method (Kondo et al., 2011; Monaghan, 1992; Scholtès et al., 2012; Warren, 1998). Among them, SPH is the oldest MPM and it is approaching its mature stages. Due to the continual improvement and modification of the concepts of the SPH method, the accuracy and the stability of the SPH method have reached an accepted level (Liu et al., 2003).

2.3.3 Particle methods to study RBC behaviour in microvessels

MPMs directly address the inhomogeneous nature of the blood flow and they can be used to simulate single and multi-phase fluid dynamics. Using particle methods, it is convenient to model the internal structure of the blood cells. A two-dimensional model was presented by Tanaka et al. (2005) using SPH to demonstrate the tank treading behaviour of a single RBC under shear flow and the axial migration of RBCs under the Poiseuille flow. In this model, both Plasma and cytoplasm were discretised into fluid particles in the SPH sense while the RBC membrane was discretised into solid particles, which were interconnected by elastic springs. The forces acting on the RBC membrane were calculated by the pressure difference between the plasma and the cytoplasm, while the forces acting on the fluid particles,
by the membrane particles, were calculated by the interaction forces based on the SPH method. Then the system was mathematically modelled by the Navier-Stokes equations with the external forces (More details are discussed in Chapter 3)

Tanaka et al. (2005) recognised that cytoplasm viscosity makes a significant contribution for the deformation of the RBC membrane. But the simulated results showed a significant disagreement with the experimental results, due to the use of fewer particles to represent the RBCs’ membrane (Tanaka, et al., 2005) and initial coarse shape of the RBC (see Figure 2.4). Further, they emphasised that there might be mismatches between the experimental results and the analytical results, if the two-dimensional simulations were carried out.

![Cell internal fluid particles][1]
![Elastic membrane particles][2]
![Plasma fluid particles][3]

**Figure 2.4: Coarse nature of the RBC model (Tanaka, et al., 2005)**

Tsubota et al. (Tsubota, et al., 2006a; Tsubota, et al., 2006b; Tsubota, et al., 2006c) employed the Moving Particle Semi-implicit (MPS) method to analyse the motion and the deformation of an RBC. The RBC membrane and the plasma domain were discretised into particles and the membrane particles were interconnected to the neighbouring particle by the elastic springs, in a similar way to the model proposed by Tanaka et al. (2005). This RBC model considers the elastic energy stored in the springs due to the stretch/compression and the elastic bending energy stored in the springs due to the bending. Furthermore, they introduced a penalty function to make up the area optimisation. This term is considered as the energy associated with the
incompressibility of the RBC membrane. The total energy of the RBC membrane is the sum of the above energies: elastic spring energy, elastic bending energy and energy related to the area optimisation.

The forces acting on each membrane particle are calculated based on the principle of virtual work. The initial shape of the RBC at rest is obtained, as the total energy is minimised. Finally, the motion of the RBC membrane particles is modelled by,

\[ m_i \ddot{r}_i + \gamma \dot{r}_i = F_i \]  

(2.1)

where the \( m_i \) and \( \gamma \) is the mass and membrane viscosity of each RBC membrane particle. However, two-dimensional simulation of the blood flow represents rather qualitative results on the motion and the deformation of the RBCs. In addition, the formation of a blood clot, due to the aggregation of platelets, was modelled. In order to simulate the aggregation of platelets, each platelet was modelled by a single particle.

Li-Guo et al. (2010) employed coarse-grained molecular dynamics (MD) simulations to obtain the equilibrium shape of the RBC. They revealed that the RBCs become flatter with shorter persistence lengths and this leads to decrease the deformability of the RBCs when the RBCs are infected by malaria. However, their study was a very brief one (Li-Guo, et al., 2010). Imani et al. (2009) developed a three-dimensional numerical model to simulate the Malaria Infected Red Blood Cell (IRBC). In their model, all the components of the blood are modelled by discrete particles, while the Malaria parasites inside the RBCs are represented by a cluster of rigid particles. For this simulation, the biconcave-shaped healthy RBC and the spherical shape of IRBC are used, as the IRBCs become stiffer and less deformable,
to qualitatively examine the flow in a narrow 6 $\mu$m square channel. Results revealed that IRBC cannot flow through the narrow channels (Imai, et al., 2009). Hosseini et al. (2009) proposed a two-dimensional particle-based model, in which plasma and cytoplasm were discretised into the particles in the SPH procedure. Initial biconcave shape of the RBC membrane at rest is obtained by the widely used geometrical function,

$$Z = \pm 0.5R_0\left[1 - R^2\right]^{0.5}\left[C_0 + C_1R^2 + C_2R^4\right]$$  \hspace{1cm} (2.2)

where $R_0 = 3.91 \mu m$, $C_0 = 0.02072$, $C_1 = 2.00256$, $C_2 = -1.1228$ and

$R^2 = (x^2 + y^2) / R_0^2 \leq 1$; $x$ and $y$ are the coordinates of $x$ and $y$ axes. When $y = 0$, the two-dimensional biconcave shape of the RBC can be derived. Under the assumption that both the plasma and cytoplasm have the same viscosity, the tank treading motion of the RBCs in the simple shear flow and RBCs’ parachute shape in Poiseuille flow were observed. But the initial biconcave shape of the RBC membrane at rest used in this approach shows a significant mismatch with the initial shape obtained based on minimum energy and the principle of virtual work (Figure 2.5). Tosenberger et al. (2011) compared the applicability of Molecular Dynamics (MD) and Dissipative Particle Dynamics (DPD) to model RBC motion in microvessels and noted that the DPD model converges faster to a stable flow. Furthermore, they reported the bending coefficient of the RBC membrane greatly affects the RBC deformability.
A hybrid grid based particle level set method was proposed by Ye et al. (2010) to study the motion and deformation of the RBCs in capillaries. They widely used a geometrical function (Eq. (2.3)) to investigate the effect of crucial parameters including radius of the RBC, elastic modulus and bending stiffness of the RBC membrane.

\[
\begin{align*}
  x &= x_0 + r \cos \theta (0.1035 + 1.0013 \sin^2 \theta - 0.5614 \sin^4 \theta) \\
  y &= y_0 + r \sin \theta
\end{align*}
\]  

(2.3)

where \( r \) was taken as the half of the RBC diameter (7.42 \( \mu \)m). Furthermore, they studied the effect of the density ratios and the viscosity ratios of inside fluid and outside fluid (Cytoplasm and Plasma). However they limited their studies only to two-dimensional RBC models.

Fedsov et al. (2010) confirmed that the shape deformation of RBCs decreases for larger values of the RBCs’ membrane bending rigidity and the simulation results revealed that the RBCs’ membrane viscosity is 0.02-0.06 Pa.s, with respect to the cytoplasm viscosity of \( \eta_i = 5 \times 10^{-3} \) Pa.s and plasma viscosity of \( \eta_o = 1 \times 10^{-3} \) Pa.s. A modified MPS method was introduced by Ahmadian et al. (2012) to investigate the motion of the RBC though microvessels. The proposed method reduced the
computational time by more than a factor of twenty without affecting the accuracy of the results. Recently a three-dimensional model was proposed by Nagayama et al. (2012) to simulate the RBC behaviour in the capillary blood flow using the MPS method. But instead of solving Navier-Stokes equations, which were used in the above particle methods, a momentum equation for the RBC was developed, considering the inter-particle force, viscous diffusion and external force. Results show that the RBCs can have three types of shapes - Rocket shape, Zipper shape and Parachute shape - which comply with the previous, depending on the internal diameter of the microvessel and tube hematocrit. Further, they studied the motion of RBCs in bent channels. However, the complete RBC behaviour in microvessels was not explained by this model.

Tsubota and Wada (2010) proposed a three-dimensional spring network model to estimate the elastic membrane force of an RBC membrane during its tank treading motion. In their model, the RBC membrane is discretised into triangular elements. Assuming a simple shear flow, a small external force was introduced on each node to reproduce the tank treading motion. This model was further improved by Nakamura et al. (2013) to simulate the mesoscopic blood flow. However, they assumed that RBCs do not disturb the surrounding flow and a one-way coupling was implemented for the flow-RBC by predefining the macroscopic flow field. Recently, the dissipative particle dynamic (DPD) method was employed by Ye et al. (2014) to develop a three-dimensional RBC model to predict the tube flow containing interacting RBCs.

2.4 Summary and implications

Based on the above literature review, several points are summarised:
• Even though a number of models have been developed to describe the RBC behaviour in microvessels, the models developed based on the grid generation methods, such as BEM and IBM, have several limitations (Tsubota, et al., 2006a; Tsubota, et al., 2006c) compared with the recently developed meshfree particle methods. Constructing a regular grid for complex geometry such as for RBC is very time consuming task and these grid-based methods cannot handle large deformations of RBCs. Furthermore, the subcellular details, such as RBC wall and cytoplasm interactions, cannot be completely included in the simulation. In order to overcome these limitations, several meshfree particle-based models have been proposed to investigate the behaviour of RBCs in microvessels.

• In the literature, several particle methods can be found, such as SPH, DPD, and MPS methods, which have been used to analyse RBCs’ motion and deformation. However, the previous studies used simplified geometrical functions (Hosseini, et al., 2009; Ye, et al., 2010) to initiate the biconcave shape of the healthy RBC. These functions provide unrealistic biconcave nature for the RBC at rest. The deformability of the RBCs is generally calculated with the aid of the geometrical measurements of the deformed RBC and the initial shape of the RBCs is one of the important geometrical parameters, which directly affects the deformability of the RBC. Therefore, the initial geometry of the RBCs should be accurately established in order to compare the deformability of the RBCs under different conditions. On the other hand, the recently developed minimum energy approach
provides a more realistic initial geometrical shape for the RBC membrane that is closer to the matured healthy RBCs’ shape. However, limited research has been conducted using this minimum energy approach.

- Most of the research was carried out to investigate the behaviour of RBCs in uniform capillaries. However, in the cardiovascular network, capillaries with the stenosed sections can often be found. There is a high risk of microvascular blockage, which even leads to stopping the blood flow in capillaries depends on the severity of the stenosis (Cooke, et al., 2001). However, to the best of the author’s knowledge, there has been very little work done to investigate the motion and deformation of RBCs through stenosed capillaries.

- In addition, very few three-dimensional particle-based RBC models, which were developed to simulate RBCs’ behaviour in microchannels using the MPS method and DPD method, can be found in the literature. However, these models do not explain the complete behaviour of the RBCs in the blood vessels, especially in the capillaries with stenosed sections.

- The numerical modelling techniques have a very high potential to explain and predict the behaviour of the RBCs in the capillaries. Among all the numerical modelling techniques, recently developed MPM are currently used effectively to analyse the problems related with large deformations such as the deformations of RBCs (Liu et al., 2006). In particular, SPH, as one of the popular and well-established meshfree particle approaches, has received widespread attention and
been proposed by researchers to solve and analyse the micro-scale hydrodynamics problems. In addition, spring network models based on the minimum energy concepts are extensively used to model the elastic membrane of the RBCs.

Therefore, in this research, coupled SPH and DEM models, based on a spring network and minimum energy approach, have been employed to explore the motion and deformation of RBCs in capillaries.
The SPH is one of the popular and well-established meshfree particle approaches, which can be employed to explore the motion and deformation of RBCs in capillaries. In addition, it was revealed from the literature review that the recently developed minimum energy approach provides a more realistic initial geometrical shape for the RBC membrane. In this chapter, the modelling implementation techniques employed to model the behaviour of RBCs in capillaries will be documented concisely. The basic concepts of the SPH method will be presented in Section 3.1. The approach employed to validate the SPH method for micro-scale hydrodynamics problems will be explained later in this section. Section 3.2 will describe the methodology employed to model and validate the two-dimensional RBC. The approach used to model and validate the three-dimensional RBC will be explained in Section 3.3. The time integration technique and the study on convergence will be described in Section 3.4 and Section 3.5 respectively. At the end of this chapter, a concise summary will be presented (Section 3.6).

3.1 SPH approach for hydrodynamics problems

SPH is a meshfree (grid-less), Lagrangian particle method, basically developed for hydrodynamics problems. In SPH, any fluid domain can be discretised into a set of a finite number of particles. Each particle represents a finite mass, associated with
density and pressure. The system is evolved due to the interaction between discretised particles and due to the externally exerted forces (M. Liu & Liu, 2005).

3.1.1 Basic concepts of the SPH method

3.1.1.1 Particle Approximation

In the SPH method, any field function value \( f \) of a specific particle \( i \) is approximated from the same field function value of surrounding neighbouring particles (inside of the influence domain, in Figure 3.1), using a smoothing or kernel function \( W \) by,

\[
f_i = \sum_{j=1}^{N} \frac{m_j}{\rho_j} f_j W_{ij}
\]

(3.1)

where, \( m_j, \rho_j \) and \( f_j \) are the mass, density and field function value of the neighbouring particle \( j \) respectively, while \( N \) is the number of neighbouring particles in the influence domain (see Figure 3.1) and \( W_{ij} \) is the kernel function value. The value of \( W_{ij} \) depends on \( R \); where \( R = |r_i - r_j| / h \) (here, \( |r_i - r_j| \) is the distance between the \( i^{th} \) particle and the \( j^{th} \) particle, while \( h \) is the smoothing length, which defines the influence domain of the \( i^{th} \) particle, as can be seen in Figure 3.1).

![Figure 3.1: Particles inside of the influence domain contribute to determine the field function value of \( i^{th} \) particle](image)

Similarly, the first derivative of that field function can be approximated by,

\[
\nabla_i \cdot f_i = \sum_{j=1}^{N} \frac{m_j}{\rho_j} f_j \cdot \nabla_i W_{ij}
\]

(3.2)
In this study, the popular cubic spline smoothing kernel function (see Eq. (3.3)) is used as it has a narrower compact support over other popular smoothing functions (Liu, et al., 2003).

\[
W(R, h) = \alpha_d \times \begin{cases} 
\frac{2}{3} - R^2 + \frac{1}{2} R^3 & \text{if } 0 \leq R \leq 1 \\
\frac{1}{6} (2 - R)^3 & \text{if } 1 \leq R \leq 2 \\
0 & \text{if } R \geq 2 
\end{cases}
\]

where, \(\alpha_d\) is \(1/h, 15/7\pi h^2\) and \(3/2\pi h^3\) in one-dimensional, two-dimensional and three-dimensional space.

![Figure 3.2: Behaviour of the cubic spline smoothing kernel (W) and its first derivative (\(\nabla W\))](image)

Generally, Navier-Stokes equations in Lagrangian form are used to model fluid dynamics problems. In this study, the fundamental physical laws of conservation of mass,

\[
\frac{D\rho}{Dt} = -\rho \nabla \cdot \mathbf{v}
\]  

(3.4)

and conservation of momentum,

\[
\frac{D\mathbf{v}}{Dt} = -\frac{1}{\rho} \nabla p + \frac{\mu}{\rho} \nabla^2 \mathbf{v} + \mathbf{f}
\]  

(3.5)
based on the Navier-Stokes equations, are used under the assumption that the system is isothermal. Where, \( \rho \) and \( \mu \) are the density and the dynamic viscosity of the fluid in that order, \( \mathbf{v} \) is the velocity vector and the \( p \) is the pressure while \( \mathbf{f} \) is the vectorial external force acting on the fluid particles respectively.

The equations for conservation of mass [Eq. (3.4)] and conservation of momentum [Eq. (3.5)] are rewritten by,

\[
\frac{D\rho}{Dt} = \sum_{j=1}^{N} m_j (\mathbf{v}_i - \mathbf{v}_j) \cdot \nabla_i W_{ij} \tag{3.6}
\]

and

\[
\frac{D\mathbf{v}_i}{Dt} = -\sum_{j=1}^{N} m_j \left( \frac{p_j}{\rho_j^2} + \frac{p_i}{\rho_i^2} \right) \cdot \nabla_i W_{ij} \tag{3.7}
\]

\[
+ \sum_{j=1}^{N} m_j \frac{\mu_j + \mu_i}{\rho_j \rho_i} \frac{||\mathbf{r}_i - \mathbf{r}_j||}{\rho_j \rho_i ||\mathbf{r}_i - \mathbf{r}_j||^2} \nabla_i W_{ij} (\mathbf{v}_i - \mathbf{v}_j) + \mathbf{f}
\]

respectively using SPH concepts and a cubic spline smoothing kernel, where \( i \) represents the particle on focus and \( j \) represents the considered neighbouring particle in the influence domain (see Figure 3.1).

### 3.1.1.2 Artificial Compressibility

In the SPH method, theoretically incompressible fluids are actually considered as compressible fluids. Artificial compression is introduced to the system to produce a pressure disturbance via a quasi-incompressible equation of state.

\[
p = B \left( \left( \frac{\rho}{\rho_0} \right)^\gamma - 1 \right) \tag{3.8}
\]

Where \( B \) is a problem dependent parameter, that usually is the initial pressure (Liu, et al., 2003), \( \rho_0 \) is the initial density and \( \gamma = 7 \). This ensures large pressure variation
corresponding to the variations in density, which maintains the density variation at less than 1% within the system.

### 3.1.1.3 Boundary Treatment

In SPH, when the field function values (e.g., density; $\rho$, pressure; $p$ or velocity; $v$) of the particles near or on the boundary are calculated, only the particles inside the boundary contribute to the approximation of that field function value, since there are no particles beyond the boundary (see Figure 3.3). This kind of one-sided contribution from neighbouring particles gives wrong approximations.

\[
F_j = \begin{cases} 
D \left( \frac{r_0}{r_i - r_j} \right)^{12} - \left( \frac{r_0}{r_i - r_j} \right)^{4} & \text{if } \left( \frac{r_0}{r_i - r_j} \right)^{12} \geq 1 \\
0 & \text{if } \left( \frac{r_0}{r_i - r_j} \right)^{12} \leq 1 
\end{cases}
\]  

(3.9)

![Figure 3.3: One sided contribution from the neighbouring particles](image)

In order to avoid this numerical error, virtual particles are employed symmetrically outside of the boundary, as shown in Figure 3.4. These virtual particles have the same density and pressure as the corresponding real particles. However, they produce a sufficient Lenard-Jones (LJ) type repulsive boundary force to avoid the penetration of real particles through the boundary, when real particles approach the boundary. The LJ type repulsive force is calculated by,
where $D$ is a problem dependent parameter, that usually is equal to the square of the maximum velocity and $r_0$ is usually selected approximately close to the initial particle spacing.

![Application of virtual boundary particles](image)

**Figure 3.4**: Application of virtual boundary particles

### 3.1.1.4 Average Velocity

In the application of artificial compression to the incompressible fluids, it is important to employ the average velocity concept to the problem, which includes the contribution from neighbouring particles. The average velocity of a given particle is calculated by,

\[
\frac{dx_i}{dt} = v_i - \varepsilon \sum_{j=1}^{N} \frac{m_j}{\rho_j} v_{ij} W_{ij}
\]  

(3.10)

where $x_i$, $v_i$, $m_j$ and $\rho_j$ are the position vector velocity of the $i^{th}$ particle, velocity vector of the $i^{th}$ particle, the mass of the neighbouring $j^{th}$ particles and velocity of the neighbouring $j^{th}$ particles respectively. While $v_{ij}$ is the velocity difference between the particles referred $(i$ and $j)$ and $W_{ij}$ is the smoothing function value. In Eq. (3.10) $\varepsilon$ is a problem dependent parameter and it depends on the initial particle spacing, time interval and the $B$ value in Eq. (3.8). This makes the particles move in a velocity closer to the average velocity of the neighbouring particles.
3.1.2 Validation of SPH approach

A general basic SPH code is modified and improved with the help of previous work done by Liu et al. (Liu, et al., 2003) using FORTRAN language. The hydrodynamics problems that involved low Reynolds numbers and laminar flows are mainly considered, when modifying and improving the basic SPH code. The accuracy of the developed code is checked against the results of some classical benchmark problems and the results are presented in the following sections.

3.1.2.1 Poiseuille Flow

The flow between two parallel stationary plates is simulated. The dimensions of the fluid domain is set as \( L = 4 \, \mu m \) (x-directional distance; length) and \( D = 10 \, \mu m \) (y-directional distance; diameter). The fluid domain between two parallel plates is modelled by 1000 particles, such that the minimum distance between two neighbouring particles is equal to \( 0.2 \, \mu m \). In addition, 40 wall particles are employed to model the top and bottom boundaries (walls) of the flow domain as shown in Figure 3.5.

![Initial particle configuration for Poiseuille flow](image_url)
A constant pressure difference is assigned between inlet and outlet of the fluid domain, as the inlet and outlet normal stresses are set to 6.4 N/m² and 0 N/m² respectively. The assigned pressure difference is then converted into the body force by,

\[
\frac{F_x}{m} = -\frac{1}{\rho} \frac{dp}{dx}
\]

where \( F_x \) is the body force acting on a unit mass, \( \rho \) is the density of the flow media and \( dp/dx \) is the pressure gradient. The calculated body force, \( 1.6 \times 10^3 \) N/kg is then applied to all the plasma particles in the fluid domain. The behaviour of the plasma flow is analysed and the particle velocity at a range of time intervals is compared with the analytical solution \([\text{Eq. (3.12)}]\) provided by Morris et al. (Morris et al., 1997):

\[
v_x(y,t) = F_x y(D - y)
+ \sum_{n=1}^{\infty} \frac{4F_x D^2}{\nu \pi^2 (2n+1)^3} \sin \left( \frac{\pi y}{D} (2n+1) \right) \exp \left( -\frac{(2n+1)^2 \pi^2 \nu}{D^2} t \right)
\]

where \( D \) is the height of the flow domain, \( y \) is the \( y \)-directional coordinate while \( \nu \) is the kinematic viscosity of the fluid, which is equal to the ratio between the dynamic viscosity (\( \mu \)) and density (\( \rho \)) of the fluid. The density and the dynamic viscosity of the fluid are set to \( 1 \times 10^3 \) kg/m³ and \( 1 \times 10^{-3} \) Pa.s respectively. The time step is set to \( 1 \times 10^{-8} \) s and the periodic boundary conditions are applied to the inlet and the outlet.

The initially stationary fluid is driven by the applied body forces and gradually, the fluid particle starts to flow between the two plates. After about 6000 time steps \( (t = 6 \times 10^{-5} \) s), the fluid particles arrive to a steady state and the particles located at the centre of the microchannel obtain the maximum velocity of \( 2 \times 10^{-2} \) m/s. This corresponds to a Reynolds number of \( 2 \times 10^{-2} \). A velocity profile of the particles
located in-between \( x = 2.0 \mu m \) and \( x = 2.2 \mu m \) is obtained from the SPH results and analytical solution at \( t = 1\times10^{-6} \) s, \( 1\times10^{-5} \) s and \( 1\times10^{-3} \) s. The results are then compared and plotted in Figure 3.6. It is found that the SPH results show a very good agreement with less than 1% deviation with the analytical solution.

![Figure 3.6: Comparison of velocity profiles between analytical solution and SPH solution for Poiseuille flow](image)

### 3.1.2.2 Couette Flow

The Couette flow between two parallel plates is simulated. Initial particles are distributed, the same as in the case of the Poiseuille flow (Figure 3.5). The Couette flow is generated by the sudden motion of the upper plate at a constant velocity of \( 2\times10^{-2} \) m/s. The time step is set to \( 1\times10^{-8} \) s and the periodic boundary conditions are applied. After about 6000 time steps (when \( t = 6\times10^{-5} \) s), the simulation results reach the steady state. Morris et al. (1997) suggested a series solution for the time dependent behaviour of the Couette flow by,

\[
v_{x}(y,t) = \frac{v_0}{D} y + \sum_{n=1}^{\infty} \frac{2v_0}{n\pi} (-1)^{y} \sin \left( \frac{n\pi}{D} y \right) \exp \left( -\nu \frac{n^2 \pi^2}{D^2} t \right)
\]

(3.13)
where $v_0$ is the maximum velocity of the upper plate while $v$ is the kinematic viscosity of the fluid. In Eq. (3.13), $D$, $y$ and $t$ are the height of the fluid domain, the $y$-directional coordinate, and the time respectively.

![Figure 3.7: Comparison of velocity profiles between analytical solution and SPH solution for Couette flow](image)

The velocity profile of the Couette flow is obtained from the SPH results for the particles located in-between $x = 2.0 \mu m$ and $x = 2.2 \mu m$ at $t = 1 \times 10^{-6}$ s, $1 \times 10^{-5}$ s and $1 \times 10^{-3}$ s. The analytical results for the Couette flow are calculated by Eq. (3.13) for the same time intervals to compare the results and are plotted in the Figure 3.7. The determined SPH results are deviated from the analytical solution by no more than 2%.

### 3.2 Two-dimensional SPH-DEM Model for Red Blood Cell

A two dimensional DEM model based on a spring network is used to represent the RBC membrane, as used in previous studies (Pan et al., 2009; Tsubota, et al., 2006c). In this RBC model, it is hypothesised that the RBCs attain their biconcave shape due to the ejection of their nuclei. In a two-dimensional RBC model, in order
to obtain the RBC membrane shape, the initial shape of the RBC is assumed to be a circle, which is corresponding to a state of the RBC, before ejecting the nucleus. Then the circular RBC membrane is discretised into a finite number of point masses as shown in Figure 3.8. The radius of the initial circle should be chosen such that the diameter of the final shape of the RBC gives the average diameter of RBCs, about 7.6 μm. Therefore, the radius of the initial circle is assumed to be 2.8 μm. In addition, the number of particles in the RBC membrane should be chosen ensuring that the minimum distance between two neighbouring particles is equal to the particle minimum spacing in the fluid (plasma) domain, in order to simulate the problem effectively and efficiently by employing the SPH method. Therefore, 88 particles are used and they are interconnected by 88 elastic stretching/compression springs; this number is chosen so that the distance between two neighbouring particles is approximately 0.2 μm. In the following sections, the geometrical shapes of the RBC membrane for the different number of particles are revealed. In addition to the elastic springs, bending springs are used to avoid the folding of the membrane (see Figure 3.9).

In order to obtain a stable RBC membrane shape the total energy of the RBC membrane is considered. The total energy of the RBC consists of two components;

- Elastic stretching/compression energy
- Elastic bending energy
Chapter 3: Development, Implementation and Validation of SPH-DEM Models for Red Blood Cells

In the following sections the approach used to calculate the above energies is described.

3.2.1 Computation of forces in two-dimensional RBC model

3.2.1.1 Forces due to elastic stretching/compression energy

The total elastic energy stored in the springs (Tsubota, et al., 2006b) due to stretching/compression is

$$ E_i = \frac{1}{2} K_i \sum_{i=1}^{N} \left( \frac{l_i - l_0}{l_0} \right)^2 $$

(3.14)

where $l_i$, $l_0$, $N$ and $K_i$ are the length of the $i$th spring, the reference length (the length with no deformation) of the $i$th spring, the number of springs and the spring constant for stretching/compression respectively. The reference length is set to the initial length, when the springs are at rest. Assume that the 1st membrane particle ($P_1$) is connected to the 2nd membrane particle ($P_2$) and 88th membrane particle ($P_{88}$) by the 1st spring ($S_1$) and 88th spring ($S_{88}$) respectively (see Figure 3.9).
The force acting on the 1\textsuperscript{st} membrane particle due to the change in the length of $S_1$ and $S_{88}$ is then calculated based on the principle of virtual work by

$$\mathbf{F}_{l,1} = -K_l \frac{\partial}{\partial \mathbf{r}_1} \left[ \left( \frac{l_1 - l_0}{l_0} \right)^2 + \left( \frac{l_{88} - l_0}{l_0} \right)^2 \right]$$

(3.15)

where $l_1$, $l_{88}$ and $\mathbf{r}_1$ are the length of the 1\textsuperscript{st} spring, the length of the 88\textsuperscript{th} spring and the position vector of the $P_1$. The initial lengths ($l_0$) of all the springs are identical.

The new stretched/compressed lengths of the $S_1$ and $S_{88}$ are found from the coordinates of the connected particles ($P_1$, $P_2$ and $P_{88}$) in $xy$-plane. The coordinates of $P_1 (x_1, y_1)$, $P_2 (x_2, y_2)$ and $P_{88} (x_{88}, y_{88})$ are employed to calculate the $\mathbf{F}_{l,1}$. Therefore, the force acting on the 1\textsuperscript{st} membrane particle due to the change in the length of $S_1$ and $S_{88}$ is

$$\mathbf{F}_{l,1} = -K_l \frac{\partial}{\partial \mathbf{r}_1} \left[ \left( \frac{\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} - l_0}{l_0} \right)^2 + \left( \frac{\sqrt{(x_{88} - x_1)^2 + (y_{88} - y_1)^2} - l_0}{l_0} \right)^2 \right]$$

(3.16)

Finally, the $x$-directional component of the $\mathbf{F}_{l,1}$ is found as,
\[
F_{i,l,s} = -K_i \frac{\partial}{\partial x_i} \left[ \left( \frac{\sqrt{(x_i - x_2)^2 + (y_i - y_2)^2} - l_0}{l_0} \right)^2 \right] + \left( \frac{\sqrt{(x_{ss} - x_i)^2 + (y_{ss} - y_i)^2} - l_0}{l_0} \right)^2 \right] (3.17)
\]

and it can be simplified into,

\[
F_{i,l,s} = -K_i \left[ \left( \frac{l_i - l_{0i}}{l_i l_{0i}^{1/2}} \right) (x_i - x_2) + \left( \frac{l_{ss} - l_0}{l_{ss0} l_0^{1/2}} \right) (x_{ss} - x_i) \right] (3.18)
\]

In the same way, the \( y \)-directional component of the \( F_{i,1} \) is found as,

\[
F_{i,l,s} = -K_i \left[ \left( \frac{l_i - l_{0i}}{l_i l_{0i}^{1/2}} \right) (y_i - y_2) + \left( \frac{l_{ss} - l_0}{l_{ss0} l_0^{1/2}} \right) (y_{ss} - y_i) \right] (3.19)
\]

Similarly, forces acting on all the other particles due to the stretching/compression of elastic springs are calculated

### 3.2.1.2 Forces due to elastic bending energy

The elastic bending energy stored in the RBC membrane (Tsubota, et al., 2006b) due to the bending of the membrane is then calculated by

\[
E_b = \frac{1}{2} K_b \sum_{i=1}^{N} \tan^2 \left( \frac{\theta_i}{2} \right) (3.20)
\]

where \( \theta_i \) and \( K_b \) are the external angle between a pair of consecutive springs and the spring constant for bending. Assume that the external angle between \( S_1 \) and \( S_{ss} \) is \( \theta_1 \).

This angle \( (\theta_1) \) is found from the coordinates of \( P_1 \) \((x_1, y_1)\), \( P_2 \) \((x_2, y_2)\) and \( P_{ss} \) \((x_{ss}, y_{ss})\) in \( xy \)-plane with the aid of the law of cosine:

\[
\theta_i = \pi - \cos^{-1} \left[ \frac{\left( x_i - x_2 \right)^2 + \left( y_i - y_2 \right)^2 + \left( x_{ss} - x_i \right)^2 + \left( y_{ss} - y_i \right)^2 - \left( x_{ss} - x_2 \right)^2 - \left( y_{ss} - y_2 \right)^2}{2 \times \sqrt{\left( x_i - x_2 \right)^2 + \left( y_i - y_2 \right)^2} \times \sqrt{\left( x_{ss} - x_i \right)^2 + \left( y_{ss} - y_i \right)^2}} \right] (3.21)
\]
The $x$-directional and $y$-directional force components acting on each membrane particle due to the membrane bending are separately calculated based on the principle of virtual work, similar to the above calculations.

$$\mathbf{F}_i = -\frac{\partial E}{\partial \mathbf{r}_i}$$  \hspace{1cm} (3.22)

In addition to the stretching/compression energy and bending energy, in order to maintain a constant membrane area, an energy penalty function (Tsubota, et al., 2006b) is introduced:

$$E_i = \frac{1}{2} K_s \left( \frac{s - s_e}{s_e} \right)^2$$  \hspace{1cm} (3.23)

where $s$ and $s_e$ are the cross sectional area of the RBC and the equivalent cross sectional area of the RBC respectively, and $K_s$ is the penalty coefficient. The value for $s_e$ is chosen such that it is approximately equal to the actual cross sectional area of a healthy matured RBC. The cross sectional area of the RBC is approximated by

$$s = \sum_{i=1}^{N} (y_i - y_{i+1}) \left( \frac{x_i + x_{i+1}}{2} \right)$$  \hspace{1cm} (3.24)

The accuracy of this approximation increases with the number of particles ($N$) employed to represent the RBC membrane. However, it is computationally more expensive to employ a large number of particles to represent the RBC membrane. The $x$-directional and $y$-directional force components acting on each particle to maintain a constant membrane area are separately calculated using the principle of virtual work, as described earlier (see Eq. (3.22)).

The total force acting on the $i^{th}$ particle is the sum of all above mentioned forces:
\[ \mathbf{F}_i = \mathbf{F}_{l,i} + \mathbf{F}_{b,i} + \mathbf{F}_{s,i} \]  \hspace{1cm} (3.25)

where \( \mathbf{F}_{l,i}, \mathbf{F}_{b,i} \) and \( \mathbf{F}_{s,i} \) are the force vectors acting on \( i \)th particle due to the membrane stretching/compression, membrane bending and change in cross sectional area of the RBC respectively.

### 3.2.2 Validation of two-dimensional RBC model

In order to validate the two-dimensional RBC model, initially, the biconcave shape of the RBC membrane is generated. In Eq. (3.14) \( K_l \) is set to \( 5 \times 10^{-8} \) N.m and \( K_b \) in Eq. (3.20) is set to \( 5 \times 10^{-10} \) N.m (Tsubota, et al., 2006c). The energy penalty coefficient \( (K_s) \) is set to \( 1 \times 10^{-5} \) N.m (Tsubota, et al., 2006c) while \( s_e \) is set to 50% of the initial area of the circle, which gives the physiological shape of a healthy RBC very well. A FORTRAN code is developed to calculate all the forces acting on the membrane particles. The velocity and the position of the each particle are updated by using the acceleration \( (\mathbf{r}_i) \), which is related to the total force by

\[ \mathbf{F}_i = m_i \mathbf{r}_i + c \mathbf{r}_i \]  \hspace{1cm} (3.26)

where \( \mathbf{r}_i \) and \( m_i \) are the velocity and the mass of the \( i \)th particle, while \( c \) is the damping coefficient and is set to \( 1 \times 10^{-2} \) N.s/m (Tsubota, et al., 2006c). The mass of each particle is set to \( 2 \times 10^{-10} \) g (Tsubota, et al., 2006c) and the time step is set to \( 5 \times 10^{-9} \) s.

Initially, the total energy of the RBC membrane is very high (see Figure 3.10) due to the difference in \( s \) and \( s_e \) and the forces with high magnitudes are acting on the membrane particles (see Figure 3.11). The membrane particles start to move due to those forces; as a result of this, the cross sectional area reduces. After about 0.1 s the typical biconcave shape of the RBC is obtained (see Figure 3.12). As can be seen
in Figure 3.10 and Figure 3.11 \( t = 0.1 \) s the total energy of the RBC and the total resultant force acting on the RBC membrane have reached stable minimum values at \( t = 0.1 \) s.

![Figure 3.10](image1.png)

Figure 3.10: Change in total energy of the two-dimensional RBC with time.

![Figure 3.11](image2.png)

Figure 3.11: Change in total resultant force acting on the two-dimensional RBC with time.

Since the total energy of the RBC and the total resultant force acting on the RBC membrane do not change with time after 0.1 s, the RBC membrane does not show any shape change. Therefore, after 0.1 s, the RBC membrane has reached a stable shape. This biconcave shape of the RBC membrane matches very well with the shape of a healthy matured RBC.
3.2.2.1 Qualitative validation

The obtained geometry of the two-dimensional RBC membrane is then rotated, such that the major axis of the RBC membrane is vertical. Finally, the RBC membrane is put into a plasma domain in a capillary with the diameter \( D \) of 9.6 \( \mu \text{m} \) and the length \( L \) of 50 \( \mu \text{m} \). In order to represent the plasma component, the outside of the RBC membrane in the problem domain is discretised into a set of particles such that the particle spacing is equal to 0.2 \( \mu \text{m} \) (see Figure 3.13). The internal component of the RBC (hemoglobin) is represented by the same manner as the plasma component in the problem domain. A set of wall particles are used to represent the wall of the capillary, through which plasma flows. The density and the dynamic viscosity of the wall particles are set as similar to the plasma particles.
In order to validate the two-dimensional RBC model, pressure driven Poiseuille flow is applied to the fluid domain and the behaviour of the RBC morphology is analysed. The inlet pressure is set to 512.5 Pa, while the outlet pressure is set to zero. The pressure is then converted into the body forces and applied on the plasma particles. Periodic boundary conditions are applied to the inlet and the outlet. The time step is $1\times10^{-9}$ s. The dynamic viscosity of plasma, cytoplasm and RBC membrane particles is assumed to be $1\times10^{-3}$ Pa.s and other parameters are given in Table 3.1.

Lennard-Jones type repulsive forces, ($\mathbf{RF}$) are applied pair-wisely as in Eq. (3.9) to the fluid and membrane particles (see Figure 3.14) in to avoid the penetration of fluid particles (plasma and hemoglobin) through RBC membrane (Polwaththe-Gallage et al., 2014). Here, $\mathbf{RF}_p$ is force acting pair-wisely on RBC membrane and Plasma particles to avoid the penetration of plasma particle through the RBC membrane. Similarly, $\mathbf{RF}_c$ force avoids hemoglobin particles to escape the
RBC membrane boundary. In Figure 3.14, $F_p$ is the force acting on each particle due to the pressure and viscosity. This force can be calculated using SPH methodology.

In Figure 3.14, $F_b$, $F_s$, $F_{la}$, and $F_{lb}$ are the forces acting on the RBC membrane particles; due to the bending spring between $la$ and $lb$ springs, due to the areal conservation and due to the stretching/compression of $la$ and $lb$ springs respectively. While $i$, $j$ and $k$ are three consecutive RBC particles in that order and $c$ and $p$ are hemoglobin and plasma particles.

The RBC shape gradually deforms from its initial biconcave shape to a parachute shape, as the RBC advances in the Poiseuille flow (see Figure 3.15). At the steady state, the RBC attains a parachute shape, which even allows it to move through capillaries that have smaller diameters than the diameter of the RBC at rest. The parachute of the deformed RBC achieved in this study is comparable with the
shapes reported by Jeong et al. (2006) and Tsubota et al. (2006b). This deformation phenomenon is very crucial for the effective mass transfer in microcirculation (Vadapalli et al., 2002).

Table 3.1: Simulation parameters for two-dimensional RBC model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_l$</td>
<td>Spring constant for stretching/compression</td>
<td>$5 \times 10^{-8}$ N.m</td>
<td>(Shi, et al., 2012)</td>
</tr>
<tr>
<td>$K_b$</td>
<td>Spring constant for bending</td>
<td>$5 \times 10^{-10}$ N.m</td>
<td>(Shi, et al., 2012)</td>
</tr>
<tr>
<td>$K_s$</td>
<td>Energy penalty coefficient</td>
<td>$1 \times 10^{-5}$ N.m</td>
<td>(Shi, et al., 2012)</td>
</tr>
<tr>
<td>$\rho_{RBC}$</td>
<td>Density of RBC membrane particles</td>
<td>1098 kg/m$^3$</td>
<td>(Sun, et al., 2005)</td>
</tr>
<tr>
<td>$\rho_{plasma}$</td>
<td>Density of RBC plasma particles</td>
<td>1025 kg/m$^3$</td>
<td>(Frcitas, 1998)</td>
</tr>
<tr>
<td>$\rho_{cytoplasm}$</td>
<td>Density of RBC cytoplasm particles</td>
<td>1050 kg/m$^3$</td>
<td>(Le et al., 2009)</td>
</tr>
</tbody>
</table>

3.2.2.2 Quantitative validation

Fischer et al. (Fischer et al., 1978) experimentally observed that the RBCs gradually deform and elongate from their biconcave shape to an ellipsoidal shape when they are subjected to a shear flow. At the steady state, the deformed shape of the RBCs does not change and the RBCs make a constant inclination angle with the flow direction. Meanwhile, the RBC membrane circulates around the cytoplasm, known as a tank treading motion. In order to verify the developed two-dimensional RBC model, the RBC membrane is rotated, such that the major axis of the RBC membrane is horizontal and is put into a plasma domain. The initial particle distribution of plasma and cytoplasm is set similar to that in the previous section. The height and the length of the channel are set to 7 $\mu$m and 12 $\mu$m, respectively.
The shear flow is generated by the sudden motion of the upper and lower boundaries of the flow channel in opposite directions at a constant velocity of $2 \times 10^{-2}$ m/s. In this simulation, the time step is set to $1 \times 10^{-9}$ s.

![Tank treading motion of the RBC membrane](image)

Simulation results reveal that, when the RBC is subjected to a shear flow, the membrane rotates around the cytoplasm, while the RBC makes a constant angle with the horizontal direction (see Figure 3.16). In Figure 3.16, the black dot represents the first particle of the RBC membrane. With time that particle rotates in a clockwise direction along the membrane. Furthermore, the velocity distribution of the whole
flow field confirms the tank treading behaviour of the RBC membrane. The inside fluid of the RBC (cytoplasm) also circulates inside the membrane, due to the tank treading motion of the RBC membrane (see Figure 3.17).

![Figure 3.17: Velocity distribution of flow field at t = 0.003 s](image)

The model considered here is validated against the simulation results published by Kaoui et al. (2011). They have established the steady state inclination angle for different area ratio ($s^*$) values in RBC, when the RBC is subjected to a shear flow. The shape of the RBC membrane can be directly changed, by changing the equivalent area ($s_e$) of the RBC membrane. The area ratio ($s^*$) is equal to $s_e/s$, where $s$ is the area of the initial circle. When the area ratio is increased, RBC membrane gives a more circular shape. Four shapes for different area ratios ($s^* =$ 0.6, 0.7, 0.8 and 0.9) are considered and the relevant RBC membrane shapes are obtained (see Figure 3.18).
Then, the RBC behaviour is simulated under a simple shear flow. The time step is set to $1 \times 10^{-9}$ s. The dynamic viscosity of plasma, hemoglobin and RBC particles is assumed to be $1 \times 10^{-9}$ Pas and other parameters are given in Table 3.1. The equilibrium inclination angles for four shapes were compared with the previously published results (Kaoui, et al., 2011). Our simulation results show a good agreement with previous results with less than 5% difference as shown in Figure 3.19.

Figure 3.18: Equilibrium RBC membrane shape for different area ratios

Figure 3.19: Equilibrium inclination angle of RBC for different area ratios
3.3 Three-dimensional SPH-DEM Model for Red Blood Cell

In this research, a number of asymmetrical behaviours of the two-dimensional RBC model are observed. Therefore, it is essential to carry out three-dimensional study to investigate more precise behaviours of the RBCs. For that purpose, a three-dimensional RBC model is developed based on the knowledge of two-dimensional model development. In the three-dimensional RBC model, a similar approach is used as in the two-dimensional model, to generate the biconcave discoidal shape of the RBC.

The three-dimensional RBC membrane is modelled by a DEM based on a spring network (Tsubota, et al., 2010). Similar to the two-dimensional RBC model, it is hypothesised that the RBCs attain their discoidal biconcave shapes due to the ejection of their nuclei. Initially, it is assumed that the shape of the RBC membrane is to be a sphere with a radius of 3.1 µm, which is corresponding to a state of the RBC, before ejecting the nucleus. The spherical geometry is built by COMSOL Multiphysics 4.2a software, using a “surface” option for the object type. Then, a user controlled mesh is generated for the spherical surface with the same minimum and maximum element sizes (0.4 µm), to ensure the size and the shape of the triangles remain as similar as possible (see Figure 3.20).
Finally, the mesh is exported as a “.mphtxt” file to obtain the node coordinates and the node numbers. The exported mesh file shows that the spherical surface is divided into 954 mesh points, or nodes, and 1904 elements. The coordinates of the 954 nodes and the node numbers, which generate 1904 triangular elements, are extracted for further processing with a FORTRAN code. In order to form the three-dimensional geometry of the RBC membrane, three types of energies are considered (Tsubota, et al., 2010):

- Elastic stretching/compression energy
- Elastic bending energy
- Energy related to area incompressibility

### 3.3.1 Computation of forces in three-dimensional RBC model

#### 3.3.1.1 Forces due to elastic stretching/compression energy

The RBC membrane shows in-plane deformation (stretching/compression) when the membrane is subjected to an external force field (Tsubota, et al., 2010). To represent the in-plane deformation, elastic springs ($S$) are used to interconnect the
particle on each node, which generates the triangular elements of the mesh. The length changes in these springs, changes the stored energy

\[ E_l = \frac{1}{2} K_l \sum_{i=1}^{NS} (l_i - l_{i,0})^2 \]  

(3.27)

where \( K_l \) is the spring constant for stretching/compression and \( NS \) is the number of springs, while \( l_i \) and \( l_{i,0} \) are the deformed length and the reference length of the \( i^{th} \) spring respectively. The reference length is set to the initial length, when the springs are at rest. Assume that the \( P_1 \) particle is connected to six neighbouring particles by six elastic stretching/compression springs as shown in Figure 3.21.

![Figure 3.21: Three-dimensional spring network and particle locations.](image)

The forces acting on the \( P_1 \) particle due to any change in the length of the springs are calculated on the basis of the principle of virtual work.

\[ \mathbf{F}_{i,1} = -K_l \frac{\partial}{\partial \mathbf{r}_1} \sum_{i=1}^{6} (l_i - l_{i,0})^2 \]  

(3.28)

where \( \mathbf{r}_1 \) is the position vector of the \( P_1 \) particle. The force component in the \( x \)-direction, acting on \( P_1 \) particle due to the change in the length of the \( S_1 \) spring, is calculated as a function of the coordinated of the \( P_1 (x_1, y_1) \) and \( P_2 (x_2, y_2) \) particles,

\[ \mathbf{F}_{i,1,x} = -K_l (l_{s1} - l_{s1,0}) \frac{x_1 - x_2}{l_{s1}} \]  

(3.29)
where \( l_{S1} \) and \( l_{S1,0} \) are the deformed and original length of the \( S_1 \) spring respectively.

### 3.3.1.2 Forces due to elastic bending energy

The RBC membrane seeks to minimise the bending energy to obtain a stable shape, so that the membrane is locally flat. The elastic bending energy (Tsubota, et al., 2010) stored in the three-dimensional RBC membrane due to the bending is

\[
E_b = \frac{1}{2} K_b \sum_{n=1}^{NB} L_n \tan^2 \left( \frac{\theta_n - \theta_{n,0}}{2} \right)
\]  
\[ (3.30) \]

where \( \theta_n \) is the angle between two normal vectors formed of neighbouring triangles formed by elastic springs for stretching/compression and \( \theta_{n,0} \) is the reference angle above two triangles without deformation (see Figure 3.22).

![Figure 3.22: Angle between two neighbouring triangles.](image)

Also, \( L_n \) is the length of the common side of two triangles and \( K_b \) is the spring constant for bending. The number of neighbouring triangles is \( NB \) and is equal to the number of elastic springs used to represent the stretching/compression forces. Here, \( \theta_{n,0} \) is set to zero and Eq. (3.30) is rewritten as

\[
E_b = \frac{1}{2} K_b \sum_{n=1}^{NB} L_n \frac{1 - \hat{n}_{ijk} \cdot \hat{n}_{ij}}{1 + \hat{n}_{ijk} \cdot \hat{n}_{ij}}
\]  
\[ (3.31) \]
where $\mathbf{n}_{ijk}$ and $\mathbf{n}_{ilj}$ are the unit normal vectors for two neighbouring triangles $\triangle IJK$ and $\triangle JIL$ respectively, as shown in Figure 3.22. Then, the force acting on the $i^{th}$ particle due to the bending is

$$ F_{b,i} = -K_B \frac{\partial}{\partial r_i} \sum_{i=1}^{NB} L_i \frac{1 - \mathbf{n}_{ijk} \cdot \mathbf{n}_{ilj}}{1 + \mathbf{n}_{ijk} \cdot \mathbf{n}_{ilj}} $$

(3.32)

### 3.3.1.3 Forces related to area incompressibility

The number of lipids per area of the RBC membrane is constant and the RBC membrane shows a high resistance to change in the surface area. The elastic energy generated by the RBC membrane (Tsubota, et al., 2010) due to a change in total RBC membrane area from reference area $A_0$ to $A$ is

$$ E_A = \frac{1}{2} K_A A_0 \left( \frac{A - A_0}{A_0} \right)^2 $$

(3.33)

where $K_A$ is the area expansion modulus for the whole membrane. Further, local area incompressibility of the RBC membrane should be considered and the energy generation due to the changes in the local area of triangles ($A_n$), formed by the elastic springs for stretching/compression is

$$ E_a = \frac{1}{2} K_a \sum_{n=1}^{N_t} \left( \frac{A_n - A_{n,0}}{A_{n,0}} \right)^2 A_{n,0} $$

(3.34)

where $A_{n,0}$ is the reference value for the initial local area of the $n^{th}$ triangle and $K_a$ is the area expansion modulus for the triangular elements. The forces acting on the $i^{th}$ particle due to the local area incompressibility and total area incompressibility ($\mathbf{F}_{i,a}$ and $\mathbf{F}_{i,A}$ respectively) are calculated based on the principle of virtual work (see Eq. (3.22)).
3.3.1.4 Volume constraint

The total volume ($V$) enclosed by the RBC membrane is equal to the volume of a healthy matured RBC. In addition to the above mentioned energy types, in order to maintain a constant enclosed volume, an energy penalty function is introduced (Tsubota, et al., 2010). The energy generation due to the change in the total enclosed volume is

$$E_v = \frac{1}{2} K_v V_0 \left( \frac{V - V_0}{V_0} \right)^2$$  \tag{3.35}

where $V_0$ is the reference volume and $K_v$ is the penalty coefficient to maintain the $V$ as $V_0$. To calculate the total volume of the RBC, the RBC is divided into triangular oblique prisms as shown in Figure 3.23. The volumes of the prisms are individually calculated, and then the sum is taken. Assume that the particles $I (x_I, y_I, z_I)$, $J (x_J, y_J, z_J)$, and $K (x_K, y_K, z_K)$ generate the triangle $IJK$. The projected area vector $A_p$ of $IJK$ triangle on the $xy$ plane is equal to the area of $I'J'K'$ triangle (see Figure 3.23) and is proportional to the cross product of $I'J'$ and $J'K'$:

$$A_p = \left( \frac{I'J' \times J'K'}{2} \right)$$  \tag{3.36}

To calculate the projected area, counter-clockwise orientation of the three triangle points and the real value of the cross product are used. Thus, the error due to the orientation is omitted. Then the volume of the prism is

$$V_p = \left( \frac{I'J' \times J'K'}{2} \right) \left( z_I + z_J + z_K \right)$$  \tag{3.37}
The force acting on the $i^{th}$ particle due to the volume constraint ($F_{v,i}$) is calculated on the basis of the principle of virtual work $k$ (see Eq. (3.22)).

### 3.3.2 Validation of three-dimensional RBC model

In order to validate the three-dimensional RBC model, initially, the biconcave discoidal shape of the RBC membrane is generated. The mass of each particle is $1 \times 10^{-9}$ g and the time step is $1 \times 10^{-3}$ s. The velocity and the position of the each particle are updated by using the acceleration ($\ddot{\mathbf{r}}$), with the aid of Eq. (3.26) and damping coefficient ($c$) is set to $1 \times 10^{-7}$ N.s/m (Tsubota, et al., 2010). In Eq. (3.27) $K_i$ is set to $1 \times 10^{-6}$ N/m and $K_b$ in Eq. (3.30) is set to $1 \times 10^{-11}$ N (Tsubota, et al., 2010). The area expansion modulus for the whole RBC membrane ($K_A$) and the area expansion modulus for the triangular elements ($K_a$) are set to $5 \times 10^{-3}$ N/m and $5 \times 10^{-5}$ N/m respectively (Tsubota, et al., 2010). For the three-dimensional RBC model $V_0$ in Eq. (3.35) is set to 60% of initial volume of the sphere, which gives the physiological shape of a healthy RBC (Nakamura, et al., 2013) while $K_V$ is set to $2 \times 10^{-5}$ N/m$^2$ (Tsubota, et al., 2010). The FORTRAN 90 computer code is developed...
to calculate the forces acting on the RBC membrane particles and Intel Visual FORTRAN Composer XE compiled the code. Results are analysed using Tecplot 360 software.

![Figure 3.24: Change in total energy of the three-dimensional RBC with time.](image)

Initially, the RBC membrane experiences very high forces, and then gradually the total resultant force acting on the membrane decreases (see Figure 3.25). After about 20 s the forces acting on the membrane become stable and do not show any variation with time. The variation of the energy with time exhibits similar behaviour, as shown in Figure 3.24. The typical biconcave discoidal shape of a matured healthy RBC is obtained at $t = 40$ s (see Figure 3.26). Since the total energy and the total resultant force acting on the RBC membrane do not change with time after $t = 20$ s, the RBC does not show any shape change. Therefore, after 20 s, the RBC membrane has reached a stable shape. This biconcave discoidal shape matches with scanning electron microscope images of RBCs (Bessis, 1973). A cross section of the final three-dimensional shape of the RBC membrane gives the two-dimensional biconcave shape of the RBC and its aspect ratio is close to the aspect ratio of an average healthy RBC (Tsukada et al., 2001).
3.3.2.1 Qualitative validation

To validate the three–dimensional RBC model, the motion and deformation of the RBC in a uniform capillary is examined. The RBC membrane is put into a plasma domain in a capillary with the diameter \((D)\) of 9.6 \(\mu\text{m}\) and the length \((L)\) 31.6 \(\mu\text{m}\). In order to represent the plasma component, the fluid particles are arranged outside the RBC membrane, such that the particle spacing is equal to 0.4 \(\mu\text{m}\). The cytoplasm particles in the RBC interior are arranged in the same manner. A set of wall particles are used to represent the wall of the capillary, through which plasma flows. The density and the dynamic viscosity of the wall particles are set as similar
to the plasma particles. Lennard-Jones type repulsive forces (Liu, et al., 2003) are applied to plasma and cytoplasm particles to avoid the penetration of fluid particles through the capillary walls and the RBC membrane (Polwaththe-Gallage, et al., 2014). A pressure driven Hagen-Poiseuille flow is applied to the fluid domain and the behaviour of the RBC morphology is analysed. The inlet pressure is set to 200 Pa, while the outlet pressure is set to zero. The pressure is then converted into the body forces and applied to the plasma particles. Periodic boundary conditions are applied to the inlet and the outlet. The time step is $1 \times 10^{-8}$ s and other parameters are set as in Table 3.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_l$</td>
<td>Spring constant for stretching/compression</td>
<td>$1 \times 10^{-6}$ N/m</td>
<td>(Tsubota, et al., 2010)</td>
</tr>
<tr>
<td>$K_b$</td>
<td>Spring constant for bending</td>
<td>$1 \times 10^{-11}$ N</td>
<td>(Tsubota, et al., 2010)</td>
</tr>
<tr>
<td>$K_A$</td>
<td>Area expansion modulus for the whole membrane</td>
<td>$5 \times 10^{-3}$ N/m</td>
<td>(Tsubota, et al., 2010)</td>
</tr>
<tr>
<td>$K_{A}$</td>
<td>Area expansion modulus for the triangular elements</td>
<td>$5 \times 10^{-5}$ N/m</td>
<td>(Tsubota, et al., 2010)</td>
</tr>
<tr>
<td>$K_V$</td>
<td>Energy penalty coefficient</td>
<td>$2 \times 10^{-5}$ N/m$^2$</td>
<td>(Tsubota, et al., 2010)</td>
</tr>
<tr>
<td>$\rho_{RBC}$</td>
<td>Density of RBC membrane particles</td>
<td>1098 kg/m$^3$</td>
<td>(Sun, et al., 2005)</td>
</tr>
<tr>
<td>$\rho_{plasma}$</td>
<td>Density of RBC plasma particles</td>
<td>1025 kg/m$^3$</td>
<td>(Frcitas, 1998)</td>
</tr>
<tr>
<td>$\rho_{cytoplasm}$</td>
<td>Density of RBC cytoplasm particles</td>
<td>1050 kg/m$^3$</td>
<td>(Le, et al., 2009)</td>
</tr>
<tr>
<td>$\mu_{RBC}$</td>
<td>Dynamic viscosity of RBC membrane particles</td>
<td>$20 \times 10^{-3}$ Pa.s</td>
<td>(Fedosov, et al., 2010)</td>
</tr>
<tr>
<td>$\mu_{plasma}$</td>
<td>Dynamic viscosity of plasma</td>
<td>$1 \times 10^{-3}$ Pa.s</td>
<td>(Fedosov, et al., 2010)</td>
</tr>
</tbody>
</table>
$\mu_{\text{cytoplasm}}$ & Dynamic viscosity of cytoplasm particles & $5 \times 10^{-3}$ Pa.s & (Fedosov, et al., 2010)

Figure 3.27: Deformed shape of the three-dimensional RBC in Hagen-Poiseuille flow at $t = 0, 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, 0.21, 0.24, 0.27, 0.30, 0.33, 0.36, 0.39, 0.42, 0.45, \text{ and } 0.48 \text{ ms}$

Similar to the two-dimensional RBC, the three-dimensional RBC gradually deforms from its initial biconcave shape to a parachute shape, as the RBC advances in the Hagen-Poiseuille flow (see Figure 3.27). At the steady state, the RBC attains a parachute shape and it is comparable with the shapes reported by Tsubota et al. (2006b).

3.3.2.2 Quantitative validation

Similar to the two-dimensional RBC model validation, to validate the three-dimensional RBC model, the deformation behaviour of the RBC is examined when the RBC is subjected to a linear shear flow. In order to generate the linear shear flow, the RBC is put into the plasma domain within a rectangular channel, then, the top and bottom plates of the rectangular channel are moved at a same velocity $V$, but in opposite directions. Periodic boundary conditions are applied to the inlet and outlet of the channel. Due to the motion of the top and bottom plates of the flow channel, plasma particles start to move and generate a pressure on the RBC.
As a result, the RBC elongates and shows a deformed shape (see Figure 3.29); the deformation index ($DI$) of the RBC is calculated for different shear stress values.

In this section, the $DI$ of the RBC is the ratio between the lengths of the RBC in $z$-direction to $y$-direction. Simulation results reveal that the $DI$ increases with the shear stress as shown in Figure 3.30. It agrees very well with the previous
experimental results with about 10% difference. In this study, Eq. (3.38) is used to calculate the shear stress ($\tau$)

$$\tau = \frac{v}{h} \mu$$

(3.38)

where $\mu$ and $h$ are the dynamic viscosity of the plasma and height of the channel in $y$-direction respectively. Simulation results show that the $DI$ of the RBC increases with the shear rate and the results obtained from this technique agree with the previous experimental results (Schauf et al., 2003).

![Figure 3.30: Initial RBC position in the rectangular channel](image)

**3.4 Time integration technique**

The Leap-Frog (LF) algorithm is used in the time integration technique, due to its low memory storage requirement and high computational efficiency. In this technique, at the end of the first time step ($t_0$), the change in density and the velocity of the particles are used to advance the density and velocity at a half time step, while the positions of the particles are advanced in a full time step.
\[ t = t_0 + \Delta t / 2 \]
\[ \rho_i(t_0 + \Delta t/2) = \rho_i(t_0) + D\rho_i(t_0) \cdot \Delta t / 2 \] (3.39)
\[ \mathbf{v}_i(t_0 + \Delta t/2) = \mathbf{v}_i(t_0) + D\mathbf{v}_i(t_0) \cdot \Delta t / 2 \]
\[ \mathbf{x}_i(t_0 + \Delta t) = \mathbf{x}_i(t_0) + \mathbf{v}_i(t_0 + \Delta t/2) \cdot \Delta t \]

With the purpose of maintaining the calculation consistent at each following time step, at the start of each following time step, the density and velocity of each particle is required to be calculated at a half time step to correspond to the position.

\[ \rho_i(t) = \rho_i(t - \Delta t/2) + D\rho_i(t - \Delta t) \cdot \Delta t / 2 \] (3.40)
\[ \mathbf{v}_i(t) = \mathbf{v}_i(t - \Delta t/2) + D\mathbf{v}_i(t - \Delta t) \cdot \Delta t / 2 \]

Finally, at the end of each following time step, the density, velocity and the position of each particle are advanced in the standard Leap-Frog scheme.

\[ t = t + \Delta t \]
\[ \rho_i(t + \Delta t/2) = \rho_i(t - \Delta t/2) + D\rho_i(t) \cdot \Delta t \] (3.41)
\[ \mathbf{v}_i(t + \Delta t/2) = \mathbf{v}_i(t - \Delta t/2) + D\mathbf{v}_i(t) \cdot \Delta t \]
\[ \mathbf{x}_i(t + \Delta t) = \mathbf{x}_i(t_0) + \mathbf{v}_i(t + \Delta t/2) \cdot \Delta t \]

### 3.5 Convergence study

The accuracy of the SPH results highly depends on the number of particles per unit area or number of particles in unit volume. This is similar to the FEM, in which results obtained by finer meshes are more accurate compared to the results obtained by coarse meshes. In the SPH method, the number of particles in the problem domain can be directly varied by changing the minimum distance between two neighbouring particles in the problem domain. Although the minimisation of inter-particle distance gives more accurate results, it leads to an increase in the total computational time. In order to study the convergences of the results, the Poiseuille flow example is used with different values for minimum distance between
neighbouring particles; 0.1 µm, 0.2 µm, 0.25 µm, and 0.4 µm. The time steps are also changed according to the minimum particle distance. For example, when the minimum distance between neighbouring particles is 0.1 µm, the time step is set to $5 \times 10^{-9}$ s and for the other cases, the usual $1 \times 10^{-8}$ s time step is used (see Table 3.3).

Table 3.3: Time steps and elapsed times for different minimum particle spacing

<table>
<thead>
<tr>
<th>Minimum distance between neighbouring particles ($\mu$m)</th>
<th>Number of particles</th>
<th>Time step (s)</th>
<th>Elapsed time to simulate $1 \times 10^{-5}$ s (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluid</td>
<td>Wall</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>4000</td>
<td>168</td>
<td>$5 \times 10^{-9}$</td>
</tr>
<tr>
<td>0.2</td>
<td>1000</td>
<td>88</td>
<td>$1 \times 10^{-8}$</td>
</tr>
<tr>
<td>0.25</td>
<td>640</td>
<td>68</td>
<td>$1 \times 10^{-8}$</td>
</tr>
<tr>
<td>0.4</td>
<td>250</td>
<td>44</td>
<td>$1 \times 10^{-8}$</td>
</tr>
</tbody>
</table>

Figure 3.31: The variation of CPU elapsed time with the minimum distance between neighbouring particles ($dx$)

When the minimum distance between neighbouring particles is 0.2 µm the total number of particles including the wall particles is 4168 and it takes 20.13201 s to simulate the problem for $1 \times 10^{-5}$ s. When the minimum distance between
neighbouring particles is reduced by 2, the total number of particles in a unit area is increased by 4. However, this geometrical configuration takes more than 188 s for the simulation of $1 \times 10^{-5}$ s and it is an increment of more than 9 times of the value for 0.2 s (see Figure 3.31). The error percentage of the SPH results for different minimum particle distances is investigated. The error of the velocity obtained by SPH results is calculated for the particles located in-between $x = 2.0 \mu m$ and $x = 2.2 \mu m$ at steady state by,

$$\text{Error}\% = \frac{1}{n} \sum_{i=1}^{n} \left| \frac{v_{\text{sph}} - v_{\text{analytical}}}{v_{\text{analytical}}} \right|$$ (3.42)

where $v_{\text{sph}}$ is the velocity obtained from the SPH method, while $v_{\text{analytical}}$ is the velocity obtained from the analytical solution [Eq. (3.12)], $n$ is the number of fluid particles taken into consideration to calculate the percentage of error.

![Figure 3.32: The error percentage of velocity obtained by SPH compared to the analytical solution against the minimum distance between neighbouring particles](image)

When the minimum distance between neighbouring particles is $0.2 \mu m$ the error of the velocity by SPH results is less than 1% (Figure 3.32). Increase in the minimum distance between neighbouring particles in the initial particle configuration causes a drastic increase in the error percentage in velocity. However, when the minimum
particle distance is reduced from 0.2 \( \mu m \) to 0.1 \( \mu m \), the results do not show great change. On the other hand, CPU elapsed time is increased by more than 9 times. This is mainly due to the boost in the total number of fluid particles and is due to the reduction of the time step. Therefore, for the following two-dimensional simulations the minimum distance between neighbouring particles is set to 0.2 \( \mu m \). However, the number of particles in the problem domain show extreme increase in the three-dimensional simulations when the minimum distance between neighbouring particles is kept to 0.2 \( \mu m \). This leads to a drastic increase in the computational time (CPU elapsed time). Therefore, the minimum distance between neighbouring particles in the three-dimensional simulations is increased up to 0.4 \( \mu m \) to reduce the computational time. Thereby the cost computation is significant without greatly affecting the accuracy of the results. Later in this thesis, the results obtained from the three-dimensional simulations will be presented.

3.6 Summary

In summary, the SPH method was used to simulate the motion and deformation of a single RBC with the aid of existing spring network models based on DEM. The minimum energy principle was used to determine the RBC membrane shape. A modified FORTRAN code for the SPH approach was used to simulate the plasma flow in microchannels. The velocity profiles of plasma in Poiseuille and Couette flows were compared, with the analytical results presented by previous work to verify the SPH concepts.

Initially, the shape of the two-dimensional RBC membrane was assumed to be a circle and the membrane was discretised into particles based on DEM. Then, the energy functions related with the membrane are considered to obtain the biconcave
shape of the RBC membrane. The developed model provides more realistic and accurate shape for the RBC membrane, since the aspect ratio of this RBC model is closer to the aspect ratio of the matured healthy RBCs. For the first time, a minimum energy concept was used with the SPH concepts to simulate the motion and the deformation of the RBC. In order to validate the developed two-dimensional model, the deformation of a single RBC was examined, in Poiseuille and linear shear flows. Results revealed that the two-dimensional RBC attains a parachute shape, when it flows in a Poiseuille flow. Furthermore, the two-dimensional RBC showed a tank treading motion in linear shear flow while the cytoplasm particles show a rotational-like motion. Then, the model was numerically validated, by comparing the steady state inclination angle of the RBC for differently reduced area, when the RBC is subjected to a linear shear flow. The results show a good agreement again with previously reported results in the literature.

Finally, the two-dimensional RBC model was improved to a three-dimensional model. At this time, the initial shape of the RBC membrane was assumed to be a sphere. A similar approach used to validate the two-dimensional model was used to validate the three-dimensional RBC model. The use of a minimum energy principle with SPH concepts was successful, as the simulation results show a good agreement with previously reported results in the literature. It can be concluded that using the SPH method, RBC membrane-fluid interactions can be easily simulated.
Chapter 4: Deformation Behaviour of a Single Two-Dimensional Red Blood Cell in a Uniform Capillary

According to the literature review, it is found that the behaviour of the RBCs should be comprehensively studied in order to explain the motion and deformation of the RBCs. This chapter details the qualitative and quantitative analysis of the motion and deformation of a single two-dimensional RBC in a uniform capillary under different conditions. Although in blood there are a number of RBCs, at the beginning a single two-dimensional RBC in a uniform capillary is considered to analyse the behaviour of the RBC. The effect of the RBC’s properties, capillary geometry and the flow conditions on the motion and deformation of a single RBC in a uniform capillary is broadly discussed under this chapter.

In detail, Section 4.1 will present the velocity profile of the RBC at the steady state and how the velocity of the flow field changes due to the existence of the RBC. Section 4.2 and Section 4.3 will discuss the effect of the initial diameter and the area ratio of the RBC on the motion and deformation behaviour. After that, the influences of the inlet pressure and the diameter of the capillary on the behaviour of the RBC in the capillary will be discussed in Section 4.4 and Section 4.5 respectively. Finally, the impact of the membrane bending stiffness, spring constant for stretching/compression and penalty coefficient on the motion and deformation of the RBC will be explored systematically in Section 4.6 and Section 4.7. A concise summary is presented in Section 4.8 to close this chapter.
4.1 Velocity profile of the flow field

The velocity profile of the flow field is examined, when a single RBC moves in a uniform capillary. The length \( L \) and diameter \( D \) of the capillary is set to 50 \( \mu \text{m} \) and 9.6 \( \mu \text{m} \) respectively. The inlet pressure is set to 512.5 Pa, while the outlet pressure is set to zero. The dynamic viscosity \( \mu \) of plasma, cytoplasm and RBC membrane particles is assumed to be \( 1 \times 10^{-3} \) Pa.s and all the other parameters are set as described in Table 3.1. Due to the applied pressure at the inlet, the RBC with plasma advances in the capillary. However, in contrast to the Poiseuille flow, the velocity of neither the RBC nor the plasma flow can be accurately calculated in terms of analytical functions. The RBC acts as a barrier to plasma flow and distorts the Poiseuille velocity profile. If there are no objects within the plasma domain, then a pure parabolic velocity profile is seen. However, due to the existence of the RBC within the plasma domain, the pure parabolic shaped velocity profile is not seen (see Figure 4.1) and a blunt velocity profile is observed in the flow domain closer to the RBC. The velocity analysis shows that the plasma particles reach a higher velocity, when there is no RBC in the capillary (see Figure 4.1).

![Figure 4.1: Velocity of flow field with and without the RBC](image-url)

Figure 4.1: Velocity of flow field with and without the RBC
At the beginning, RBC membrane particles follow a parabolic velocity profile, similar to the velocity profile of a Poiseuille flow. Due to this parabolic velocity profile RBC particles move at different velocities; particles located near to the centreline of the capillary move faster, while the particles closer to the capillary walls move slower. The differences in the velocity of the membrane particles cause deformation of the RBC membrane. However, gradually all the RBC membrane particles reach a unique equilibrium velocity (see Figure 4.2 and Figure 4.3).

![Figure 4.2: Velocity profile of the RBC](image)

This indicates that still there is a relative motion of the RBC particles with respect to the plasma particles. The velocity profile of the plasma domain reveals that the plasma particles next to the capillary wall boundary have almost zero velocity, while the plasma particles at the centreline of the fluid flow reach maximum velocity. Shi et al. (Shi, et al., 2012; Ye, et al., 2010) reported similar behaviour.
4.2 Effect of the RBC’s undeformed diameter on the deformation behaviour

The effect of the initial diameter of the undeformed RBC on the deformation behaviour of a single RBC is studied. In this study, four RBCs with four different diameters are considered. In order to generate the geometry of four RBCs, four different values (2.8 µm, 2.61 µm, 2.26 µm and 1.94 µm) are chosen for the diameter of the initial circle (see Section 3.2). However, to maintain the distance between two consecutive membrane particles a constant, the number of particles employed to represent the RBC membrane is changed, as seen in Table 4.1. The diameter and the total length of the capillary are 9.6 µm and 50 µm respectively. The inlet pressure is set to 512.5 Pa. All the other parameters are kept constant.

Table 4.1: Number of particles employed for the RBCs with different diameter values.

<table>
<thead>
<tr>
<th>Diameter of the initial circle (µm)</th>
<th>Number of particles</th>
<th>Initial RBC diameter (µm)</th>
<th>Initial RBC width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC membrane</td>
<td>Hemoglobin</td>
<td>Plasma</td>
</tr>
<tr>
<td>2.8</td>
<td>88</td>
<td>271</td>
<td>11901</td>
</tr>
<tr>
<td>2.61</td>
<td>82</td>
<td>237</td>
<td>11947</td>
</tr>
</tbody>
</table>

Figure 4.3: Velocity of the particles at steady state
Generally, all the RBCs deform from their biconcave shapes to the parachute shapes. However, the deformation of the RBC with higher initial undeformed diameter is greater, compared with the RBCs with lower initial undeformed diameters. As can be seen in Figure 4.4a, the RBC with the highest initial undeformed diameter is subjected to the highest deformation and at the steady state the RBC shows fully symmetrical parachute shape. Furthermore, the thickness of the RBC is less at the two ends (top and bottom of the RBC) compared with the middle part of the RBC. Similar kind of behaviour is observed in the RBC with the initial undeformed diameter of 7.12 µm (see Figure 4.4b). The RBC with the lowest initial undeformed diameter (5.28 µm) shows an asymmetric deformed shape at the steady state (see Figure 4.4e). Furthermore, the thickness of the deformed shape is almost the same throughout its diameter and it does not exhibit observable thicker parts at the centre of the cell.

<table>
<thead>
<tr>
<th>2.26</th>
<th>71</th>
<th>180</th>
<th>12013</th>
<th>6.16</th>
<th>1.63</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.94</td>
<td>61</td>
<td>132</td>
<td>12087</td>
<td>5.28</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Figure 4.4: Deformed RBC shape at $t = 0, 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, 0.21, 0.24, 0.27, 0.30, 0.33, 0.36, 0.39, 0.42,$ and $0.45$ ms for the RBC with the initial diameter of (a) $7.64 \mu m$, (b) $7.12 \mu m$, (c) $6.16 \mu m$ and (d) $5.28 \mu m$.

Figure 4.5: Variation of the deformation index ($DI$) of the RBC with time for different RBC’s diameters.

Figure 4.5 shows the variation of the $DI$ of the RBC with time. In this study, the $DI$ of the RBC is calculated by $l/d$ as Eq (4.1),

$$E_b = \frac{1}{2} K_b \sum_{n=1}^{NB} L_n \tan^2 \left( \frac{\theta_n - \theta_{n,0}}{2} \right)$$

(4.1)

where $l$ and $d$ are defined in Figure 4.6.
Chapter 5: Deformation Behaviour of a Single Two-Dimensional Red Blood Cell in a Uniform Capillary

Figure 4.6: The deformation index (DI) of the RBC; \( DI = \frac{l}{d} \)

It can be seen that the RBC with the highest initial undeformed diameter deforms quickly, as the initial gradient (from \( t = 0 \) s to \( t = 0.0002 \) s) of the curve is greater than the gradients of the other curves (see Figure 4.5). On the other hand, the RBCs with lower initial undeformed diameter deforms slowly. As can be seen in Figure 4.5, at the steady state the RBC with the highest initial undeformed diameter shows the highest \( DI \). The \( DI \) of the other RBCs at the steady state reduces with the initial undeformed diameter.

Figure 4.7: Variation of the RBC’s mean velocity with time for different RBC’s diameters

The variation of mean velocities of the RBCs exhibit slight differences. The mean velocity of the smallest RBC is higher compared with the other RBCs (see Figure 4.7). Since the smallest RBC make a less disturbance on the plasma flow, the
flow field is not greatly affected by the existence of the RBC. On the other hand, when an RBC with a larger diameter exists in the capillary, the plasma flow is disturbed more and it reduces the mean velocity of the RBC (see Figure 4.7).

### 4.3 Effect of the area ratio of the RBC on the deformation behaviour

The effect of the area ratio of the RBC on the deformation behaviour of the RBC is studied. The area ratio \( s^* \) is equal to \( s_e/s \), where \( s \) is the area of the initial circle and \( s_e \) is the equivalent cross sectional area of the RBC. In this study, five RBCs with different area ratio values are considered. The diameter of the initial circle is set to 2.8 \( \mu \text{m} \). However, due to the differences in the cross sectional area of the RBCs, the number of particles employed to represent the cytoplasm and plasma are changed, as seen in Table 4.2. The diameter and the total length of the capillary are 9.6 \( \mu \text{m} \) and 50 \( \mu \text{m} \) respectively. The inlet pressure is set to 512.5 Pa. All the other parameters are kept constant.

<table>
<thead>
<tr>
<th>Area ratio ( s^* = s_e/s )</th>
<th>Number of particles</th>
<th>Initial RBC diameter (( \mu \text{m} ))</th>
<th>Initial RBC width (( \mu \text{m} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 0.45 )</td>
<td>88</td>
<td>254</td>
<td>11927</td>
</tr>
<tr>
<td>( 0.5 )</td>
<td>88</td>
<td>271</td>
<td>11901</td>
</tr>
<tr>
<td>( 0.55 )</td>
<td>88</td>
<td>316</td>
<td>11875</td>
</tr>
<tr>
<td>( 0.6 )</td>
<td>88</td>
<td>347</td>
<td>11839</td>
</tr>
<tr>
<td>( 0.65 )</td>
<td>61</td>
<td>367</td>
<td>11809</td>
</tr>
</tbody>
</table>

Table 4.2: Number of particles employed for the RBCs of different area ratio.
Simulation results reveal that the RBCs with different area ratios ($S^*$) generally deform from their biconcave shape to the parachute shape in a similar trend. However, the final deformed shapes are quite different from each other. The RBC with $S^* = 0.45$ shows very thin parachute shape at $t = 0.45$ ms (see Figure 4.8a), while the RBC with $S^* = 0.65$ shows thick parachute shape at the same time (see Figure 4.8e). The $DI$ of the RBC with $S^* = 0.65$ reaches the highest value, while the RBC with the least area ratio gains the lowest $DI$ at the steady state (see Figure 4.9). This difference in the $DI$ happens due to the difference in the initial $DI$ (considered as the aspect ratio at rest) at $t = 0$. The RBC with the highest area ratio ($S^* = 0.65$) has the highest aspect ratio at rest and the aspect ratio of the RBCs reduces with the area ratio of the RBC. Therefore, the RBCs have higher aspect ratio at $t = 0$ higher $DI$ while the RBCs with initial lower aspect ratios gain less $DI$ (see Figure 4.9).
The mean velocities of the RBCs with different area ratios do not show any significant variation and they gain a steady state mean velocity of about 0.085 m/s, when the RBCs flow in the capillary (see Figure 4.10). Therefore, it can be concluded that the mean velocity of the RBC is independent from the initial aspect ratio of the RBC.

Figure 4.9: Variation of the deformation index of the RBC with time for different swelling ratios
4.4 Effect of the inlet pressure on the deformation behaviour of the RBC

The effect of the inlet pressure of the capillary on the deformation behaviour of the RBC is studied. Inlet pressure of the capillary is changed to 307.5 Pa, 410 Pa, 615 Pa and 820 Pa. In this section, the membrane of the RBC is generated by a circle with the diameter of 2.8 μm and the reduced are ($s^*$) of 0.5 (see Section 3.2). The diameter and the total length of the capillary are 9.6 μm and 50 μm respectively. All the other parameters are unchanged.
The simulation results show that at the beginning all the RBCs deform at equal deformation rates. However, when the RBCs reach about 15 $\mu$m, the rates of deformation of the RBCs deviate from each other (see Figure 4.11). The RBC in capillary with largest inlet pressure (820 Pa) shows the highest rate of deformation and it gains the largest $DI$ at the steady state while the RBC subjected to the lowest pressure gradient gains the lowest $DI$ at the steady state (see Figure 4.11). However, the changes in the $DI$ values at the steady state for different inlet pressures are not that significant.

The mean velocity of the RBCs increases with the inlet pressure of the capillary. The mean velocity of the RBC in the capillary with the highest pressure gradient reaches the highest mean velocity about 0.15 m/s, while the RBC in the capillary with the inlet pressure of 307.5 Pa and zero outlet pressure reaches the lowest mean velocity of about 0.05 m/s. Therefore, it can be concluded that both the $DI$ and the mean velocity of the RBC directly depend on the inlet pressure of the capillary or the pressure gradient of the capillary.
4.5 Effect of the diameter of the capillary on the deformation behaviour of the RBC

The effect of the capillary diameter on the deformation behaviour of the RBC is studied. The capillary diameter is changed to 9.6 µm, 10.4 µm, 11.2 µm, 12.0 µm and 12.8 µm. The RBC described in the previous section (see the Section 4.4) is used for the simulations described in this section. The total length of the capillary and inlet pressure is set to 50 µm and 512.5 Pa. respectively, while keeping all the other parameters constant.

The RBC in the capillary with the smallest diameter \( (d = 9.6 \, \mu \text{m}) \) gains the highest \( DI \) at the steady state (see Figure 4.13). Moreover, from the beginning (form \( t = 0 \)) the rate of deformation of the RBC is higher in the capillary with the diameter of 9.6 µm. On the other hand, the RBC in the capillary with the highest diameter \( (d = 12.8 \, \mu \text{m}) \) shows the lowest deformation rate and at the steady state it gains the lowest \( DI \) (see Figure 4.13).

![Figure 4.13: Variation of the deformation index of the RBC with its position for different capillary diameter values](image)

Figure 4.14 shows the variation of the RBC’s mean diameter with its position for different capillary diameters. It can be seen from Figure 4.14 that the mean
velocity of the RBC increases proportionally with the capillary diameter. However, as can be seen in Figure 4.13, the $DI$ of the RBC decreases with the increase in the capillary diameter. The RBC in the capillary with the diameter of 12.8 $\mu$m gains the minimum $DI$, while it reaches the maximum mean velocity among all five cases. In the previous simulation, when the inlet pressure increases, both $DI$ and RBC’s mean velocity at the steady state increase. However, in this simulation, even though the mean velocity of the RBC increases in the capillaries with larger diameter values, the $DI$ of the RBC decreases. Therefore, it can be concluded that the capillary diameter plays a vital role in motion and deformation behaviour of the RBCs.

![Graph](image_url)

Figure 4.14: Variation of the deformation index of the RBC with its position for different capillary diameter values

### 4.6 Effect of the membrane bending stiffness of the RBC on the deformation behaviour

The effect of the membrane bending stiffness ($K_B$) on the deformation behaviour of the RBC is studied. The $K_B$ value is changed from $K_B = 5 \times 10^{-10}$ Nm ($K_{b1} = K_{b0}$) to $K_B = 5 \times 10^{-9}$ Nm ($K_{b1} = 10K_{b0}$), $25 \times 10^{-9}$ Nm ($K_{b1} = 50K_{b0}$), $50 \times 10^{-9}$ Nm ($K_{b1} = 100K_{b0}$), $5 \times 10^{-11}$ Nm ($K_{b1} = 0.1K_{b0}$), and $5 \times 10^{-12}$ Nm ($K_{b1} = 0.01K_{b0}$). The
diameter and the total length of the capillary are 9.6 µm and 50 µm respectively. The inlet pressure is set to 512.5 Pa. All the other parameters are set as described earlier.

Simulation results reveal that the DI of the RBC decreases with the membrane bending stiffness value (see Figure 4.15). When the membrane bending stiffness is less than its typical value (when $K_{b,l} = 0.1K_b$ and $K_{b,l} = 0.01K_b$) the RBCs do not show any considerable change in the deformed shape (see Figure 4.15e and Figure 4.15f). The RBCs with higher $K_b$ values (i.e. $K_{b,l} = 10K_b$, $K_{b,l} = 50K_b$ and $K_{b,l} = 100K_b$) exhibit rounder shape when they flow further downstream (see Figure 4.15 b, Figure 4.15c and Figure 4.15d). Furthermore, their thickness at the top and bottom is higher compared with the other cases. The RBCs with the highest $K_b$ values ($K_{b,l} = 50K_b$ and $K_{b,l} = 100K_b$) do not show typical deformed shape (i.e. parachute shape) and the RBC with $K_{b,l} = 100K_b$ shows a slight asymmetric behaviour, when it flows further downstream (see Figure 4.15 (d) when $t = 0.45$ s).
Figure 4.15: Deformed RBC shape at $t = 0, 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, 0.21, 0.24, 0.27, 0.30, 0.33, 0.36, 0.39, 0.42$ and $0.45$ ms for the RBC with (a) $K_b = K_{b_\text{r}}$, (b) $K_b = 10K_{b_\text{r}}$, (c) $K_b = 50K_{b_\text{r}}$, (d) $K_b = 100K_{b_\text{r}}$, (e) $K_b = 0.1K_{b_\text{r}}$ and (f) $K_b = 0.01K_{b_\text{r}}$.

Figure 4.16 shows variation of the $DI$ of the RBCs with their position. It confirms that the $DI$ of the RBC decreases when the membrane bending stiffness increases. Furthermore, the $DI$ of the RBCs with lower $K_b$ values ($K_{b1} = 0.1K_b$ and $K_{b1} = 0.01K_b$) do not show significant change in the $DI$ compared to the $DI$ of the RBC with the typical membrane bending stiffness value (see Figure 4.16).

Figure 4.16: Variation of the deformation index of the RBC with its position for different bending
The mean velocities of the RBCs with lesser $K_b$ values are nearly the same as the mean velocity of the RBC with typical membrane stiffness value (see Figure 4.17). However, the mean velocity of the RBC decreases when the $K_b$ value increases. It can be seen from Figure 4.17 that the mean velocity of the RBCs with higher $K_b$ values further decreases when they reach the downstream of the capillary.

The membrane bending stiffness significantly influences the RBC’s $DI$ and mean velocity when the RBCs are flowing through the smaller blood vessels, capillaries. This is mainly due to the biconcave shape of the RBC. In addition, in capillaries blood flow attains a blunt parabolic velocity profile and RBCs attempt to comply with the flow pattern. Therefore, in smaller blood vessels with uniform cross sectional areas, RBCs exhibit bending deformation and it is dominated (Puig-de-Morales-Marinkovic et al., 2007).
4.7 Effect of the spring constant for stretching/compression and penalty coefficient on the deformation behaviour

The effect of the spring constant for stretching/compression \(K_l\) on the deformation behaviour of the RBC is studied. The \(K_l\) value is changed from \(K_l = 5\times10^{-8}\) Nm \((K_{ll} = K_l)\) to \(K_l = 5\times10^{-7}\) Nm \((K_{ll} = 10K_l)\), \(25\times10^{-7}\) Nm \((K_{ll} = 50K_l)\), \(50\times10^{-7}\) Nm \((K_{ll} = 100K_l)\), \(5\times10^{-9}\) Nm \((K_{ll} = 0.1K_l)\), and \(5\times10^{-10}\) Nm \((K_{ll} = 0.01K_l)\). All the other parameters are set as described in the previous section (see Section 4.6). Simulation results show that the DI of the RBCs does not change considerably for different \(K_l\) values (see Figure 4.18a). The RBC with the least spring constant for stretching/compression \((K_l)\) shows a higher deformation rate starting from the beginning until about 0.25 ms. However, after about 0.25 ms, the rate of deformation of that RBC decreases and at the steady state DI of the RBC reaches a stable value, which is slightly less than the values of other RBCs. Furthermore, the variation of the RBCs’ mean velocity shows similar behaviour, except for the RBC with \(K_l = 5\times10^{-10}\) Nm \((K_{ll} = 0.01K_l)\). The RBC with the smallest spring constant for stretching/compression \((K_l)\) shows a slight decrease in the mean velocity at the steady state (see Figure 4.18b).

![Figure 4.18: (a) Variation of the deformation index of the RBC with time for different \(K_l\) values](image-url)
Finally, the effect of the penalty coefficient \(K_s\) on the deformation behaviour of the RBC is studied. The \(K_s\) value is changed from \(K_s = 1 \times 10^{-5} \text{Nm} \ (K_{sl} = K_s)\) to \(K_s = 1 \times 10^{-4} \text{Nm} \ (K_{sl} = 10K_s)\), \(5 \times 10^{-4} \text{Nm} \ (K_{sl} = 50K_s)\), \(10 \times 10^{-4} \text{Nm} \ (K_{sl} = 100K_s)\), \(2 \times 10^{-6} \text{Nm} \ (K_{sl} = 0.2K_s)\), and \(1 \times 10^{-7} \text{Nm} \ (K_{sl} = 0.01K_s)\), while keeping the other simulation parameters unchanged. Any considerable change in either \(DI\) or the mean velocity of the RBC is not observed for any of the \(K_s\) value (see Figure 4.19a and Figure 4.19b).

![Figure 4.19: (a) Variation of the deformation index of the RBC with time for different \(K_s\) values (b) Variation of the mean velocity of the RBC with time for different \(K_s\) values](image)

### 4.8 Summary

A two-dimensional spring network model is used in combination with the SPH method to simulate the motion and deformation of a single RBC in a uniform capillary. From this study, the conclusions below are drawn.

- Due to the existence of the RBC in the blood flow, the velocity profile of the blood flow in a capillary takes a blunt shape, compared with parabolic shape velocity profile in the pure plasma flow.
• At the steady state, RBC reaches a parachute shape and all the particles used to represent the membrane of the RBC move at a unique velocity.

• The size of the RBC affects the amount of deformation of the RBC and the larger RBCs deform more compared with the smaller RBCs.

• The deformation of the RBC decreases for a given pressure, when the inlet diameter of the capillary increases. However, the mean velocity of the RBC increases with inlet diameter of the capillary. On the other hand, the $DI$ of the RBC increases with the inlet pressure of the capillary when the inlet diameter of the capillary increases. Similarly, the mean velocity of the RBC increases with the inlet pressure of the capillary.

• The membrane bending stiffness significantly influences the RBC’s $DI$ and mean velocity. The $DI$ decreases when the membrane bending stiffness increases. RBCs show substantial variation in the deformed RBC’s morphology when the membrane bending stiffness increases.

• Finally, the simulation results reveal that the effects of the spring constant for stretching/compression ($K_l$) and penalty coefficient ($K_s$) on the deformation behaviour of the RBC are negligible.

Furthermore, in this chapter, the study was limited to a single RBC in a uniform capillary. However, to be more realistic, the influence of the other RBCs on the motion and deformation behaviour of RBCs and different geometrical conditions of the capillaries should be considered.
Chapter 5: Deformation Behaviour of a Single Two-Dimensional Red Blood Cell in a Stenosed Capillary

It is important to study the RBCs’ motion and deformation accurately, when they are squeezing through capillaries. Since some diseases such as malaria, cancer and sickle cell anaemia can alter the deformability of the RBCs (Jiang, et al., 2013) they might not be able to deform enough to pass through the narrower capillaries. Further, if the blood vessel is stenosed, there is a high risk of microvascular blockage (Cooke, et al., 2001), which leads to stopping the blood flow in that capillary. In the past, very few studies have been conducted to explore the RBCs’ motion and deformation behaviour in stenosed capillaries. Hosseini et al. (2009) have presented the RBCs’ ability of squeezing through a tiny capillary, whose diameter is smaller than the mean diameter of the RBCs. They used the SPH method and modelled the microchannel, such that it has a larger uniform diameter at the inlet and a smaller uniform diameter at the outlet. Vahidkhah et al. (2012) proposed an immersed boundary–lattice Boltzmann method to investigate the RBCs’ behaviour in a stenosed arteriole. However, they presented more qualitative results.

This chapter provides comprehensive quantitative analysis of the motion and deformation of a single RBC through a stenosed capillary. In detail, Section 5.1 will present the deformation behaviour of a single RBC through a stenosed capillary. Section 5.2 and Section 5.3 will discuss the effect of the membrane bending stiffness of the RBC and the inlet pressure of the capillary on the motion and deformation...
behaviour respectively. After that, the effect of the capillary diameter on the behaviour of the RBC will be discussed in Section 5.4. Finally, the impact of the diameter of the stenosed section on the motion and deformation of the RBC will be explored systematically in Section 5.5. A concise summary is presented in Section 5.6 to close this chapter.

5.1 Deformation behaviour of a single RBC in the stenosed capillary

The motion and deformation of the RBC is examined, when it passes through a stenosed capillary. The capillary wall is constructed by a set of wall particles, as shown in Figure 5.1. The inlet and outlet diameters ($d_i$ and $d_o$ respectively) are set to 9.6 $\mu$m, while the minimum diameter of the stenosed area ($d_c$) is set to 5.6 $\mu$m. The total length of the capillary ($L$) is 40 $\mu$m and the horizontal distance from the RBC’s centre to the inlet boundary and narrowest part of the stenosed section ($l_1$ and $l_2$ in that order) are 4.9 $\mu$m and 15 $\mu$m respectively. The inlet pressure is set to 500 Pa, while the outlet pressure is set to zero. The pressure is then converted into the body forces and applied on the plasma particles. Periodic boundary conditions are applied to the inlet and the outlet of the capillary and repulsive forces are applied to the fluid particles to avoid the penetration of fluid particles through solid walls (Polwaththe-Gallage, et al., 2014). Due to the pressure difference between inlet and outlet of the capillary, the plasma particles start to move and they create an additional pressure on the RBC. Therefore, gradually the RBC begins to accelerate.
When the cell advances in the capillary it deforms from its initial biconcave shape to the parachute shape. During its motion through the stenosed capillary (from $t = 0.2$ ms to $t = 0.375$ ms), it exhibits the maximum deformation when it flows through the stenosed area. However, once it leaves the stenosed section, it recovers its typical deformed parachute shape (see Figure 5.2). The mean velocity of the RBC also reaches its maximum value, when the cell passes through the stenosed area (see Figure 5.3). Since blood flow rate is a constant in a capillary for a given pressure, flow velocity increases, when the flow area reduces. Similarly, the flow velocity of plasma including blood cells increases, when they are flowing though narrower sections of the capillaries. As can be seen in Figure 5.4, the velocity streamlines of the flow at the stenosed area are converged and as a result of this, the velocity at the stenosed area increases.
The motion of the plasma flow without any RBC within the flow is simulated under the same simulation conditions. In that simulation, it is found that the velocity of the plasma flow shows the highest value about 0.13 m/s within the stenosed area.
Chapter 5: Deformation Behaviour of a Single Two-Dimensional Red Blood Cell in a Stenosed Capillary

(see Figure 5.5). However, this value is greater than the peak mean velocity of the RBC. Furthermore, the peak mean velocity of plasma flow is compared with a single RBC when they flow through a uniform capillary and a stenosed capillary (see Figure 5.5). At this time, the flow domain closer to the RBC is analysed and the flow field shows a blunt velocity profile. However, plasma particles located far away from the RBC shows a less blunt parabolic velocity profile. The simulation results reveal that the maximum mean velocity of the RBC in a uniform capillary is significantly greater than that value of the RBC when it flows through a stenosed capillary (see Figure 5.5). When the RBCs or stenosed sections exist in the capillary, they act as barriers to the plasma flow and as a result velocity of the plasma flow reduces. However, plasma will flow at a higher velocity when there are no stenosed areas and also no RBCs in the flow.

![Figure 5.5: Velocity profile of the flow field for different conditions](image)

The energy related with the bending deformation \( (E_b) \) is a direct measurement of the RBC’s bending deformation and it shows a maximum value about \( 2.07 \times 10^{-10} \) J at \( t = 0.275 \) ms, at which time the RBC passes though the stenosed area (see Figure 5.6). During this time period, the energy related to stretching/compression \( (E_l) \) shows a slight increase as well, which contributes to increasing the total energy.
\( (E_{\text{total}}) \) to about \( 2.3 \times 10^{-10} \) J. After approximately 0.45 ms, energy related to bending \( (E_b) \) or stretching/compression \( (E_l) \) does not show any variation with the time. It confirms that the cell has gained a stable shape in terms of energy. For whole simulation time the energy related to the penalty function \( (E_s) \) almost remains at zero, as a result of the incompressibility of the RBC membrane.

The deformation index \( (DI_1) \) is employed to quantify the deformation of the RBC, since it is directly related to the morphology of the RBC. Here, the \( DI_1 \) is the ratio between the measured maximum lengths of the RBC in \( x \)-direction to \( y \)-direction (see Figure 5.7 and Eq. (5.1)). The calculated \( DI_1 \) shows a gradual growth with time and it reaches its maximum value \( (1.31) \) at \( t = 0.275 \) ms (see Figure 5.8). The variation of \( DI_1 \) with time follows a similar trend as change in bending energy, with time up to \( t = 0.45 \) ms. However, the calculated \( DI_1 \) shows a continuous increase even after \( t = 0.45 \) ms. On the other hand, bending energy does not show any significant change with time after \( t = 0.45 \) ms (see Figure 5.6). The deformed shape of the RBC is analysed after \( t = 0.45 \) ms and it is discovered that the deformed RBC shape does not show a fully symmetrical shape. Therefore the deformation index \( (DI_2) \) is modified as given by Eq. (5.2).
Chapter 5: Deformation Behaviour of a Single Two-Dimensional Red Blood Cell in a Stenosed Capillary

\[ DI_i = \frac{\max(l_1, l_2)}{d} \]  

(5.1)

\[ DI_2 = \frac{l_1 + l_2}{2d} \]  

(5.2)

The calculated modified deformation index \((DI_2)\) shows a very similar trend as change in bending energy with time (see Figure 5.6) \(DI_2\) does not change with time, after \(t = 0.45\) ms Therefore, it can be concluded that the modified deformation index predicts the amount of deformation more accurately, compared with the conventional deformation index. For the latter part of the study, the modified deformation index \((DI_2)\) is employed for more practical comparisons.

Figure 5.7: Deformed parachute shape of the RBC

Figure 5.8: Variation of conventional deformation index and modified deformation index with time

Figure 5.8: Variation of conventional deformation index and modified deformation index with time
5.2 Effect of the membrane bending stiffness on the deformation behaviour

![Figure 5.9: Variation of the modified deformation index with RBC’s position for different $K_b$ values]

The effect of the membrane bending stiffness on the deformation behaviour of the RBC is studied. Malaria parasites increase the bending stiffness of the RBCs and this results in decreasing the deformability of the RBCs. It has been found that the bending stiffness of the RBCs increases, when they are infected by the malaria (Hosseini, et al., 2009). Further, it is found that the deformability of the cancer cells are higher than the healthy matured cells (Hou et al., 2009). The $K_b$ value is changed from $K_b = 5 \times 10^{-10}$ Nm ($K_{b1} = K_b$) to $K_{b1} = 1 \times 10^{-10}$ Nm ($K_{b1} = 0.2 K_b$), $25 \times 10^{-10}$ Nm ($K_{b1} = 5 K_b$), $50 \times 10^{-10}$ Nm ($K_{b1} = 10 K_b$), $75 \times 10^{-10}$ Nm ($K_{b1} = 15 K_b$), and $100 \times 10^{-10}$ Nm ($K_{b1} = 20 K_b$); where $K_{b1}$ is the new membrane stiffness of the RBC. All the other parameters are set as described earlier. Simulation results reveal that the $DI_2$ of the RBC decreases with the membrane bending stiffness value (see Figure 5.9). When the membrane bending stiffness is less than its typical value (when $K_b = 0.2 K_b$), the RBC does not show any considerable change in the deformed shape (see Figure 5.10 and Figure 5.11). However, local deformation of the membrane is predicted (see Figure 5.10 at $t = 0.36$ and $0.42$ ms). The deformed
shape of the RBC exhibits a more rounded shape for the higher $K_b$ values when it flows through the narrowest region of the capillary (see Figure 5.12-Figure 5.15). Furthermore, the thickness at the top and bottom is higher.

Figure 5.10: Deformed RBC shape at $t = 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36, 0.42, 0.48, 0.54, 0.60, 0.66$ and $0.72$ ms for $K_b = 1 \times 10^{-10}$ Nm ($K_b = 0.2K_b$)

Figure 5.11: Deformed RBC shape at $t = 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36, 0.42, 0.48, 0.54, 0.60, 0.66$ and $0.72$ ms for $K_b = 5 \times 10^{-10}$ Nm ($K_b = K_b$)

Figure 5.12: Deformed RBC shape at $t = 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36, 0.42, 0.48, 0.54, 0.60, 0.66$ and $0.72$ ms for $K_b = 25 \times 10^{-10}$ Nm ($K_b = 5K_b$)

Figure 5.13: Deformed RBC shape at $t = 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36, 0.42, 0.48, 0.54, 0.60, 0.66, 0.72, 0.78$ and $0.84$ ms for $K_b = 50 \times 10^{-10}$ Nm ($K_b = 10K_b$)
In addition, the RBCs with higher membrane bending stiffness values exhibit a more rounded deformed shape, when they reach further downstream of the capillary (see Figure 5.13-Figure 5.15). The variation of the mean velocity of the RBC does not show any significant change when the $K_b$ value of the RBC is changed (see Figure 5.16). However, the total time to reach the outlet of the capillary for each RBC is calculated and it is observed that the total time taken by the RBC to reach the outlet of the capillary increases with the $K_b$ value (see Figure 5.17). Therefore, when the number of stiffened RBCs (with higher $K_b$ value) in the blood increases, it will slow down the blood flow rate. Furthermore, RBCs demonstrate the same deformation behaviour before they enter into the stenosed region. As it can be seen from Figure 5.10 to Figure 5.15, at $t = 0.24$ ms, the deformed shape of the RBC for all the bending stiffness values are nearly the same and the mean velocity of the of the RBC are also approximately the same. However, after the stenosed area they
show significant variation in the deformed shape and the variation in the mean velocity of the cells is higher (see Figure 5.16).

![Graph showing RBC's mean velocity vs position for different K values](image)

Figure 5.16: Variation of the RBC’s mean velocity with its position for different $K_b$ values

![Graph showing elapsed time vs membrane bending stiffness](image)

Figure 5.17: Variation of the elapsed time to reach the capillary outlet for different RBC’s membrane $K_b$ values

### 5.3 Effect of the inlet pressure on the deformation behaviour

At this time, the effect of the inlet pressure on the motion and deformation behaviour of the RBC is investigated. Five different inlet pressure values, 100 Pa, 200 Pa, 300 Pa, 400 Pa and 500 Pa, are employed for the capillary. All the other parameters are set as described earlier. The mean velocity of the RBC increases
almost proportionally with the inlet pressure and it shows its maximum value when the RBC passes through the narrowest section of the capillary (see Figure 5.18). Further, the modified deformation Index \((D_{I2})\) of the RBC is compared for above mentioned inlet pressure values. However, the results do not show any significant variation or describable trend as can be seen in Figure 5.19. However, the RBC’s membrane bending energy for each case is analysed and it is found that the RBC’s membrane bending energy increases with the inlet pressure of the capillary (see Figure 5.20).

It can be clearly seen from Figure 5.20 that the bending deformation of the RBC initiates quickly and is faster when the inlet pressure is higher. When the RBC passes through the stenosed section of the capillary with the inlet pressure of 500 Pa, blood cell gain the maximum bending energy. Moreover, after leaving the stenosed area, the bending energy of the RBC is still higher compared with the other RBCs that move though the capillaries with lower inlet pressures (i.e. 100 Pa, 200 Pa, 300 Pa and 400 Pa). Since the membrane bending energy is a direct measurement to quantify the amount of bending deformation of an RBC, it can be stated that the bending deformation of the RBC increases with the capillary inlet pressure.
Therefore, the DI is not a fine indicator to measure and compare the amount of bending deformation of the RBCs. This measurement does not exhibit the exact nature of the deformed RBC, especially when they show asymmetric behaviours.

Figure 5.19: Variation of modified deformation index with RBC’s position for different inlet pressures

Figure 5.20: Variation of the RBC’s membrane bending energy with RBC’s position for different inlet pressures
5.4 Effect of the capillary diameter on the deformation behaviour

The effect of the capillary diameter on the motion and deformation behaviour is investigated. In this study, seven different capillary inlet diameter ($d_i$) values are employed: 8.0 $\mu$m, 7.0 $\mu$m, 6.0 $\mu$m, 5.4 $\mu$m, 4.8 $\mu$m, 4.2 $\mu$m and 4.0 $\mu$m for the capillary (see Figure 5.21). The capillary outlet diameter value is set the same as the inlet diameter ($d_o$) while the minimum diameter of the stenosed section of the capillary ($d_c$) is kept as 5.6 $\mu$m for all the simulations. The inlet and outlet pressures are set to 400 Pa and zero respectively, while all the other parameters are unchanged.

![Figure 5.21: Capillary geometry for different diameter values when diameter in the stenosed section is 5.6 $\mu$m](image)

The variation of the deformation indices ($DI$s) for different capillary diameters demonstrates the same trend as $DI$s are maximised when the RBC passes through the stenosed section of the capillary. When RBCs flow through narrower capillaries, they initiate to deform quickly compared to the wider capillaries (see the Figure 5.22 $x = 0$ to $x = 4 \mu$m). However, when the cells (RBCs, in narrower capillaries) pass through the stenosed area, they show smaller $DI$s. This confirms that even though the minimum diameter of each stenosed section is the same, the overall diameter will have an impact on the deformation of the RBC. Interestingly, when the RBCs advance further and when they leave the stenosed region, the $DI$ of the RBCs in narrower capillaries is higher compared to the wider capillaries.
Furthermore, examination shows that the maximum $DI$ of the RBC increases with the capillary diameter, when it passes through the stenosed section and it reaches a peak steady value about 1.51 (see Figure 5.23), when the capillary diameter is $7.0 \mu m$. 

Figure 5.22: Variation of modifies deformation index with RBC’s position for stenosed capillaries with different diameters

Figure 5.23: Variation of maximum deformation index in the stenosed region for the capillaries with different diameters
The mean velocity of the RBC shows its highest value about 0.085 m/s when the RBC moves though the stenosed section of the capillary with \( d_l = d_0 = 8.0 \mu m \). However, mean velocities do not show significant variation, before and after the stenosed area (see Figure 5.24). Especially, after the stenosed section, the mean velocities of the RBC are almost the same.

![Figure 5.24: Variation of RBC’s mean velocity with RBC’s position for stenosed capillaries with different diameters](image)

5.5 Effect of the diameter at stenosed region on the deformation behaviour

The effect of the minimum diameter of the stenosed section \( (d_c) \) on the deformation behaviour of RBC is studied. Six \( d_c \) values (see Figure 5.25) are used; 7.2 µm, 6.4 µm, 5.6 µm, 4.8 µm, 4.0 µm and 3.2 µm for the stenosed diameter of the capillary while maintaining other parameters constant.
Simulation results reveal that the RBC in the capillary with the stenosed diameter of 7.2 μm (which is the least stenosed) shows almost fully symmetrical deformed shapes throughout its whole motion in the capillary (see Figure 5.26). Interestingly, RBCs, moving in stenosed capillaries with minimum stenosed diameter of 6.4 μm and 5.6 μm, (see Figure 5.27 and Figure 5.28) demonstrate more asymmetric shape, when they get closer to the outlet. However, the RBC in the capillary with stenosed diameter of 4.8 μm shows a fully symmetrical deformed shape except in the stenosed region (see Figure 5.29). Further simulations reveal that an RBC can even squeeze through a stenosed capillary with the minimum diameter of 3.2 μm. The RBC shows an extreme deformation and it produces a very complicated asymmetric deformed shape as shown in Figure 5.30 and Figure 5.31, when it leaves the stenosed area. Further, it exhibits a slipper-like shape after leaving the stenosed area. However, the shape would continue to evolve and attain more symmetrical parachute shape, if the simulations were carried out in a longer capillary. Moreover, RBCs exhibit different deformed shapes with lack of up/down symmetry (see Figure 5.28 and Figure 5.31 near downstream). This deformed shapes mainly depend on the flow velocity,
geometry of the flow channel and the membrane bending stiffness ($K_b$) of the RBCs (Kaoui, et al., 2009).

Figure 5.26: Deformed RBC shape at $t = 0, 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32, 0.36, 0.40, 0.44, 0.48$ and $0.52$ ms for the stenosed diameter of $7.2 \, \mu m$

Figure 5.27: Deformed RBC shape at $t = 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55$ and $0.60$ ms for the stenosed diameter of $6.4 \, \mu m$

Figure 5.28: Deformed RBC shape at $t = 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36, 0.42, 0.48, 0.54, 0.60, 0.66$ and $0.72$ ms for the stenosed diameter of $5.6 \, \mu m$

Figure 5.29: Deformed RBC shape at $t = 0, 0.08, 0.16, 0.24, 0.32, 0.40, 0.48, 0.56, 0.64, 0.72, 0.80, 0.88$ and $0.96$ ms for the stenosed diameter of $4.8 \, \mu m$

Figure 5.30: Deformed RBC shape at $t = 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3$ and $1.4$ ms for the stenosed diameter of $4.0 \, \mu m$
The mean velocity of the RBC reduces with the severity of the stenosis. For example, when the stenosed diameter is high (3.2 µm), which is a highly stenosed capillary, the RBC gains the lowest mean velocity (see Figure 5.32). Moreover, all the RBCs have reached almost the same DI after the stenosed area (see Figure 5.33). However, the DI of RBC in the capillary with stenosed diameter of 3.2 µm exhibits an increase when it advances after the stenosed area. This might be due to the asymmetrical shape of the deformed RBC.

Figure 5.32: Variation of RBC’s mean velocity with RBC’s position for stenosed capillaries with different stenosed diameters
5.6 Summary

A two-dimensional spring network model is used in combination with the SPH method to simulate the motion and deformation of a single RBC in a stenosed capillary. From this study, the below conclusions are drawn.

- The overall blood flow rate in capillaries reduces when the capillary is stenosed and RBC moves at its highest mean velocity when it passes through the narrowest section of the capillary.

- When the RBCs are passing through the stenosed sections of the capillary the RBC membrane is not only bending, but also stretching.

- The deformation index is not a sensible measurement to accurately quantify the amount of deformation of an RBC, when they show asymmetric behaviours in the blood flow.

- The membrane bending stiffness has a direct impact on the RBC’s mean velocity and mean velocity of the RBC decreases when the...
membrane bending stiffness increases. RBCs show significant variation in the deformed shape when their membrane bending stiffness is changed.

- The deformation of the RBC increases for a given pressure, when the inlet diameter of the capillary increases. Similarly, the mean velocity of the RBC increases with the inlet diameter of the capillary.

- RBCs can even pass through the stenosed capillaries with minimum diameter of 3.2 µm and they show highly asymmetrical deformed shapes when they are flowing through narrower sections. It is also revealed that further reduction of the stenosis blocks the RBC.

Furthermore, in this study, a number of asymmetrical behaviours of the RBC is observed. Therefore, it is essential to carry out a study on a three-dimensional RBC model to investigate more precise behaviours of the RBCs. In a following chapters, (i.e. Chapter 7) the behaviour of the three-dimensional RBCs will be comprehensively reviewed.
Chapter 6: Deformation Behaviour of Multiple 2D Red Blood Cells in Capillaries

This chapter details the qualitative and quantitative analysis of the motion and deformation of multiple RBCs in a uniform capillary under different conditions. Although in blood there are a number of RBCs and blood is continuously flowing through the blood vessels, in order to clearly model the interactions between RBCs, only two RBCs are considered in this study. The effect of the initial distance between two RBCs, membrane bending stiffness of one RBC and undeformed diameter of one RBC on the motion and deformation of both RBCs in a uniform capillary is studied. Finally, the deformation behaviour of two RBCs in a stenosed capillary is also examined in this chapter.

In detail, Section 6.1 will present how the deformation behaviour of two RBCs are changed due to the hydrodynamics interaction between two RBCs, and Section 6.2 explains the effect of the number of RBCs in a given capillary on the motion and deformation of the RBCs. The effect of the distance between two RBCs on the deformation behaviour of the two RBCs will be discussed in Section 6.3. Section 6.4 and Section 6.5 will present the effect of the membrane bending stiffness and undeformed diameter of one RBC on the motion and deformation of both RBCs. After that, the deformation behaviour of two RBCs in a stenosed capillary will be discussed in Section 6.6. Finally, a concise summary is presented in Section 6.7 to close this chapter.
6.1 Motion and deformation of two RBCs in an uniform capillary

Motion and deformation of multiple RBCs in a uniform capillary is studied. The behaviour of two RBCs in a uniform capillary is compared with the behaviour of a single RBC in the same capillary (see Figure 6.1). The total length \( L \) and the diameter \( D \) of the capillary are set to 50 \( \mu m \) and 9.6 \( \mu m \) respectively. The inlet pressure is set to 512.5 Pa, while the outlet pressure is set to zero. The pressure is then converted into the body forces, which are applied on the plasma particles. In this study, in order to avoid the cell to cell contact Lennard-Jones (LJ) type repulsive forces repulsive forces are applied pair-wisely (Polwaththe-Gallage, et al., 2014).

Due to the pressure gradient imposed into the capillary, plasma particles and RBCs start to move. For convenience, the right-side cell is defined as the leading RBC (1\textsuperscript{st} RBC) and the left-side RBC is defined as the trailing RBC (2\textsuperscript{nd} RBC). The horizontal distance from the inlet boundary to the trailing RBC’s centre; \( l_1 \) is set to 3 \( \mu m \) and the distance between two RBCs’ centres; \( l_2 \) is set to 5 \( \mu m \).

![Figure 6.1: Initial particle configuration of the flow field with two RBCs](image-url)
When two cells move in the capillary, they start to deform from their initial biconcave shape to the parachute shape. However, the leading RBC exhibits a larger deformation compared to the trailing RBC (see Figure 6.2 and Figure 6.3). The calculated $DI$ of the leading RBC is even greater than that of the single RBC flowing through the same capillary under the same pressure gradient (see Figure 6.2). On the other hand, the trailing RBC shows less deformation compared to the leading RBC and also its $DI$ is less than that of the single RBC flowing alone in the same capillary (see Figure 6.2).

This phenomenon occurs due to the hydrodynamic interaction between two RBCs (Pozrikidis, 2005a; Shi et al., 2013). It can be seen from Figure 6.3 that the flow streamlines are disturbed due to the presence of two RBCs. The flow streamlines (in the $x$-direction) follow the capillary wall and are parallel to each other near the inlet boundary (see Figure 6.3) as a result of the applied pressure at the inlet of the capillary. However, when they reach the trailing RBC, the flow streamlines diverge from each other. According to the Bernoulli's principle, the pressure behind the trailing RBC increases (see Figure 6.4), when the flow...
streamlines depart from each other. The departed flow streamlines flow almost parallel to each other again after the trailing RBC and they converge immediately after the leading RBC. The converged streamlines cause the creation of a low pressure region right after the leading RBC (see Figure 6.4). This pressure variation is not enormous to clearly demonstrate in a pressure field (see Figure 6.4). However, it serves to create a difference in the DI of two RBCs.

Figure 6.3: Velocity streamlines of the whole flow field, at \( t = 0.080 \) ms when two RBCs flow in a uniform capillary

Figure 6.4: Pressure (P) variation of the flow field adjacent to two RBCs, at \( t = 0.080 \) ms when two RBCs flow in a uniform capillary

When the leading RBC flows in the \( x \)-direction (see Figure 6.3), it is not influenced by any other cell. Furthermore, as explained earlier, the pressure of the flow domain in the right-side of the leading RBC is lower compared to the left-side
of the cell. Therefore, the leading RBC is subjected to a higher deformation. On the other hand, the leading RBC acts as an obstacle to the trailing RBC and the deformation of the trailing RBC is affected by the presence of the leading RBC. Furthermore, the trailing RBC seeks to drive the plasma particles between the trailing and leading RBCs and the pressure in the region between two RBCs rises. Therefore, an additional pressure is applied by the plasma particles on the left-side of the leading RBC. That pressure causes further deformation of the leading RBC (see Figure 6.2). Meanwhile, the increased pressure in the flow region between two cells causes application of an additional pressure on the right-side of the trailing RBC. It results in reducing the deformation of the trailing RBC.

The mean velocities of two RBCs are slightly lower than that of a single RBC flow alone in the same capillary under the same pressure gradient (see Figure 6.5). Two RBCs act as an obstruction to the plasma flow and cause reduction of the flow velocity of the whole flow field. As a result of this reduction, the mean flow velocities of two RBCs reduce. As can be seen in Figure 6.5, the mean velocity of the leading RBC is slightly higher than that of the trailing RBC. Therefore, it can be predicted that the overall blood flow rate reduces slightly with the increased number of RBCs in the capillary due to the presence of more obstructions in the flow field.
This slight variation of the velocities of the two RBCs happens to be due to the
difference in the deformation of two RBCs. Since the deformation of the leading
RBC is greater than that of the trailing RBC, the leading RBC follows the flow
streamlines and makes fewer disturbances to the flow field. Therefore, the leading
RBC’s mean velocity is slightly higher than the mean velocity of the trailing RBC. It
is clear that the $DI$ of an RBC depends on the number of RBCs in the capillary and
that the deformation of the RBCs varies even in the same capillary under the same
pressure gradient. Even though the mean velocities of two RBCs show slight
deviation, the amount of deformation of two RBCs is considerable. In the following
sections, the effects of the additional RBC in the capillary on the deformation of both
RBCs are comprehensively studied.

### 6.2 Effect of the number of RBCs in the capillary

![Graph showing variation of deformation index of RBCs](image)
The effect of the number of RBCs on the deformation behaviour of the RBCs is studied. The inlet pressure is set to 512.5 Pa, while the outlet pressure is set to zero. In this study, the number of RBCs in the capillary is changed from one to two and three. Here, $l_1$ is set to 3 $\mu$m and the distance between consecutive two RBCs is set to 5 $\mu$m.

Simulation results reveal that when the number of RBCs in the capillary increases from one to two, the $DI$ of the leading RBC increases and the $DI$ of the trailing RBC decreases, compared to the single RBC case (see Figure 6.6). Moreover, when the number of RBC increases to three, the leading RBC shows a similar behaviour as the leading RBC in two RBCs’ case. However, the $DI$ of the trailing RBC further reduces compared with the value of the trailing RBC of the two RBCs’ case (see Figure 6.6). The middle RBC of three RBCs takes an in-between value for the deformation index at the steady state and it is very close to the $DI$ of the single RBC condition (see Figure 6.6). Since blood continuously flows within the cardiovascular network, there is no leading or trailing RBC in the blood flow. Therefore, it can be concluded that all the RBCs would reach the same $DI$ value, if there was a continuous flow of RBCs in a capillary.

Figure 6.7: Variation of the mean velocity of the RBCs, for different numbers of RBCs in the capillary
Moreover, the mean velocities of the RBCs show slight variations from each other when the number of RBCs in the capillary changes. When there is only one RBC in the capillary, that RBC gains the highest mean velocity (see Figure 6.7). The mean velocity of the RBCs decreases when the number of RBCs in the capillary increases (see Figure 6.7). As can be seen in Figure 6.7, the mean velocity of the trailing RBC of three RBCs is the lowest among all the RBCs’ mean velocities.

In addition, the mean velocity of an RBC affects the DI of the RBC and it has been found that the deformation of the RBCs increases when the mean velocities of the RBCs increase (Shi, et al., 2012). As can be seen in Figure 6.6, the DI of the leading RBC of three RBCs is slightly lower that that value of the leading RBC of two RBCs. This slight drop in the DI occurs due to the reduction in the mean velocity of the leading RBC of three RBCs compared to that value of the leading RBC of two RBCs.

In other words, when the number of RBCs in the capillary increases, the mean velocities of the RBCs decrease (see Figure 6.7) and as a result, the DIs of the RBCs decrease. Therefore, it is not possible to compare the DIs of the RBCs even in the same capillary, when the number of RBCs changes. In order to clearly demonstrate the interactions between RBCs, only two RBCs are considered and thereby the effects of the changes in mean velocities of the RBCs (due to the change in number of RBCs) are eliminated.
6.3 **Effect of the distance between two RBCs**

The effect of the initial distance between two RBCs with similar properties on their deformation behaviour is studied (see Figure 6.8). Here, $l_1$ is set to 3 $\mu$m and $l_2$ is varied to 3, 4, 5, 6 and 7 $\mu$m. When $l_2$ is equal or less than 2 $\mu$m, two RBCs are overlapped. Therefore, in this study, the minimum value used for $l_2$ is 3 $\mu$m. The inlet pressure is set to 512.5 Pa, while the outlet pressure is set to zero. The total length ($L$) and the diameter ($D$) of the capillary are set to 50 $\mu$m and 9.6 $\mu$m respectively. All the other simulation parameters are kept constant (see Table 3.1). The $DI$ and mean velocities of two RBCs are analysed for each case.

Simulation results reveal that when two RBCs are closer to each other at the beginning of the simulations (at $t = 0$), the difference between their $DIs$ is higher at the steady state (see Figure 6.8 and Figure 6.10). On the other hand, the $DIs$ of the two cells do not show a considerable difference, when the initial distance between two RBCs is higher. As can be seen in Figure 6.8a when the initial distance between two RBC is 3 $\mu$m, there is a noteworthy difference in the deformed shapes of two RBCs, starting from $t = 0.08$ ms. The difference in the deformed shape increases with time and at $t = 0.40$ ms, it is very significant (see Figure 6.8a). However, when the initial distance between two RBC is 7 $\mu$m, there is not any considerable difference in the deformed shapes of two RBCs (see Figure 6.8e) at any time. The analysis of the pressure fields for each case suggests that the pressure difference ($\Delta P$) between left and right had sides of the trailing RBC increase with the initial distance between two RBCs (see Figure 6.9). Furthermore, it can be seen from Figure 6.9 that the pressure difference for the leading RBC generally decreases when the initial distance between two RBCs increases. Therefore, the pressure difference ($\Delta P$) curves for leading and trailing RBCs approach each other when the initial distance between
two RBCs increases. As a result, the two RBCs attain a similar deformed shape, when the initial distance of two RBCs increases.

Figure 6.8: Deformed shapes of two RBCs at \( t = 0, 0.08, 0.16, 0.24, 0.32, \) and \( 0.40 \) ms, when the initial distance between two RBCs is (a) 3 \( \mu \)m, (b) 4 \( \mu \)m, (c) 5 \( \mu \)m, (d) 6 \( \mu \)m and (e) 7 \( \mu \)m; red-colour represents the trailing RBC and blue-colour represents the leading RBC.
Figure 6.9: The variation of the pressure difference (ΔP) between left and right hand sides of two RBCs with initial distance between two RBCs at $t = 0.30$ ms

Figure 6.10 shows the variation of the $DI$ of two RBCs with time. The curves suggest that the RBCs reach a steady deformed shape at about $t = 0.60$ ms. The leading RBC experiences the maximum deformation when the distance between two RBCs is 3 μm. On the other hand, the trailing RBC exhibits the minimum $DI$ when the distance between two RBCs is 3 μm. The deformation of this RBC occurs slowly as the gradient of the curve is lesser, compared with the other curves in Figure 6.10.

Figure 6.10: Variation of the $DI$ of the leading and trailing RBCs with time; when the two RBCs are initially separated by a distance of $l$
Although the deformed shapes of the leading RBC and trailing RBC are different from each other, the mean velocities of two RBCs do not show significant deviation. However, there is a slight deviation of velocities of two RBCs. As can be seen in Figure 6.11, the mean velocity of the leading RBC is slightly greater than that of the trailing RBC. Since the leading RBC’s deformation is higher, compared with the trailing RBC, the leading RBC makes fewer disturbances to the flow stream lines and thus gains a slightly higher mean velocity as explained earlier. Due to the difference in mean velocities of two RBCs, they depart from each other. The distance between two RBCs increases rapidly when the initial distance is \(3 \mu m\), and it reaches about \(4.5 \mu m\) at \(t = 0.4\) ms (see Figure 6.12). Conversely, the distance between two RBCs is increased by only \(0.5 \mu m\) after \(0.4\) ms when the initial distance between two RBCs is \(7 \mu m\) (see Figure 6.12). It can also be seen from Figure 6.12 that the gradient of the curves decrease with the distance between two RBCs.

![Figure 6.11: Variation of the mean velocity of the 1st RBC (leading RBC) 2nd RBC (trailing RBC) and with time; when the two RBCs are initially separated by a distance of \(l\)](image)

In other words, when two RBCs are closer, the distance between two RBCs increases quickly (Figure 6.12). As a result, the hydrodynamic effect from one RBC on the other reduces. From Figure 6.10, it is evident that two RBCs in a given capillary tend to attain the same deformed shape when the distance between two RBCs increases...
(due to the weakening of the hydrodynamic interaction between two RBCs). When the hydrodynamic interaction between two RBCs is extremely weakened, two RBCs will not depart from each other. Furthermore, blood continuously flows through the cardiovascular network. According to the simulation results, it is very hard to observe two RBCs flowing very closer to each other in the cardiovascular network due to the hydrodynamic interaction between them.

![Graph showing the variation of the distance between two RBCs with time for different initial distances](image)

**Figure 6.12:** Variation of the distance between two RBCs with time; for different initial distances \( l \) between two RBCs

### 6.4 Effect of membrane bending stiffness

The effect of the membrane bending stiffness of one RBC on the deformation behaviour of both RBCs is studied and presented in Figure 6.13. It has been found from the literature that the membrane deformability of the RBCs drops by more than ten times, compared with healthy RBCs, when RBCs are infected by a plasmodium parasite in malaria (Fedosov et al., 2011). Furthermore, it is found that the deformability of the cancer cells is higher than the healthy matured cells (Hou, et al., 2009). In order to investigate the effects of an infected RBC on the deformation behaviour of both RBCs, the membrane bending stiffness of one RBC is changed, as described in the following sections. In the 1st study, the membrane bending stiffness of the leading RBC is changed from \( K_b \) to 0.01 \( K_b \), 0.2 \( K_b \), 10 \( K_b \) and 50 \( K_b \) while
maintaining the membrane bending stiffness of the trailing RBC to $K_b$ ($5 \times 10^{-10}$ Nm).

Here, $l_1$ and $l_2$ are set to $3 \mu m$ and $5 \mu m$ respectively. The inlet pressure is set to $512.5$ Pa, while the outlet pressure is set to zero. All the other simulation parameters are kept constant (see Table 3.1).

![Figure 6.13: Variation of the DI of the leading and trailing RBCs with time; for different $K_b$ values of the leading RBC with fixed $K_b$ value of $5 \times 10^{-10}$ Nm for the trailing RBC](image)

![Figure 6.14: Deformed shapes of the leading and trailing RBCs at $t = 0.40$ ms with fixed $K_b$ value of $5 \times 10^{-10}$ Nm for the trailing RBC and the leading RBC’s $K_b$ value is (a) $0.01 K_b$ (b) $K_b$ (c) $50K_b$](image)

It is clear that, if the membrane bending stiffness of an RBC increases, the $DI$ of that RBC decreases. However, it can be seen that the changes in the membrane bending stiffness of the leading RBC affects the deformation of both RBCs (see Figure 6.13 and Figure 6.14). When the membrane bending stiffness of the leading RBC is ten times greater than that of the trailing RBC, initially, the leading RBC deforms quickly (gradient of the curve is higher). However, after 0.50 ms, both RBCs show almost the same deformed shape. The leading RBC with the membrane
stiffness of $50K_b$ shows the least bending deformation and its deformation occurs slowly (gradient of the curve is less). In this case, the trailing RBC shows the second least deformation (see Figure 6.13) as its deformation is influenced by the leading RBC. On the other hand, the bending deformation of the leading RBC increases when the membrane bending stiffness of the leading RBC decreases to $0.2K_b$. However, the trailing RBC does not show any significant change in the deformed shape. Furthermore, when the leading RBC’s membrane stiffness is decreased by a hundred times, no considerable difference in the deformation of either leading RBC or trailing RBC is observed compared with the previous case ($0.2K_b$).

![Figure 6.15](image)

Figure 6.15: Variation of the DI of the leading and trailing RBCs with time; for different $K_b$ values of the trailing RBC with fixed $K_b$ value of $5 \times 10^{-10}$ Nm for the leading RBC

![Figure 6.16](image)

Figure 6.16: Deformed shapes of the leading and trailing RBCs at $t = 0.40$ ms with fixed $K_b$ value of $5 \times 10^{-10}$ Nm for the leading RBC and the trailing RBC’s $K_b$ value is (a) $0.01K_b$, (b) $K_b$, (c) $50K_b$

In the 2nd study, the membrane bending stiffness of the trailing RBC is changed from $K_b$ to $0.001K_b$, $0.01K_b$, $10K_b$ and $50K_b$ while maintaining the
membrane bending stiffness of the leading RBC to $K_b$ ($5 \times 10^{10}$ Nm). All the other simulation parameters are kept constant (see Table 3.1). Simulation results reveal that the deformation behaviour of the leading RBC is not affected by the increase in the membrane bending stiffness of the trailing RBC (see Figure 6.15). Again, no significant variation in the deformation behaviour of the leading RBC is observed when the membrane bending stiffness of the trailing RBC is reduced.

The mean velocities of both RBCs reduce when the membrane bending stiffness of the leading RBC increases (see Figure 6.17 and Figure 6.18). However, the mean velocities of the RBCs do not show noticeable change with the decrease in the membrane bending stiffness in either leading RBC or trailing RBC (see Figure 6.17 and Figure 6.18). On the other hand, the existence of the trailing RBC with higher membrane bending stiffness reduces the mean velocity of the leading RBC slightly, while the mean velocity of the trailing RBC reduces considerably (see Figure 6.18). Therefore, if the membrane stiffness of an RBC increases in the blood flow, it affects the mean velocities of other RBCs as well as the overall blood flow rate.

![Figure 6.17: Variation of the mean velocity of the leading and trailing RBCs with time; for different $K_b$ values of the leading RBC ($K_{b1}$) with fixed $K_b$ value of $5 \times 10^{10}$ Nm for the trailing RBC](image)
6.5 Effect of the undeformed diameter of the RBC

In the above simulations the undeformed diameter of the RBC was set to 7.64 µm. However, it is an average value for a healthy RBC at rest. In general, the diameter of a healthy RBC varies between 6-8 µm (Dupire, et al., 2012). In this study, the effect of the initial undeformed diameter of one RBC on the deformation behaviour of both RBCs is studied (see Figure 6.19-Figure 6.23). In order to change the initial undeformed diameter of the RBC, the radius of the initial circle (see Section 3.2) is changed to 3.3 µm, 2.8 µm, 2.25 µm and 1.95 µm. Thereby the RBCs with initial diameters of 9.03 µm, 7.64 µm, 6.16 µm, and 5.29 µm are generated and are used for the following simulations. Although the diameter of healthy RBCs varies between 6-8 µm, RBCs with the diameters of 9.03 µm and 5.29 µm are chosen for the simulations, in order to clearly grasp the effect of the undeformed diameter of one RBC on the deformation behaviour of both RBCs. In the 1st study, the initial undeformed diameter of the leading RBC is changed from 7.64 µm to 9.03 µm, 6.16 µm, and 5.29 µm while keeping the initial undeformed diameter of the trailing RBC to 7.64 µm. In this study, \( l_1 \) and \( l_2 \) are set to 3 µm and 5 µm respectively. The inlet pressure is set to 512.5 Pa, while the outlet pressure is set to zero. All the other simulation parameters are kept constant (see Table 3.1).
It can be seen from Figure 6.19 that the change in the initial diameter of the leading RBC affects the $DI$ of the trailing RBC. The increase of the initial diameter of the leading RBC causes an increase in the $DI$ of the leading RBC and it decreases the $DI$ of the trailing RBC (see Figure 6.19). Since the leading RBC has a larger cross sectional area, it deforms more compared to the RBCs having smaller cross sectional areas and at the same time, a larger RBC distorts the plasma flow more. As a result, the hydrodynamic interaction between two cells is greater and it directly affects the $DIs$ of both RBCs (see Section 6.1). Furthermore, the mean flow velocities of both RBCs reduce (see Figure 6.20) due to the existence of the larger leading RBC and this also affects the $DI$ of the trailing RBC indirectly, which significantly reduces it.

Figure 6.19: Variation of the $DI$ of the leading and trailing RBCs with time; for different initial undeformed diameter values ($d_{RBC1}$) of leading RBC with fixed initial undeformed diameter of 7.64 $\mu$m for the trailing RBC.
On the other hand, when the initial diameter of the leading RBC is 6.16 $\mu$m, due to the decrease in the initial undeformed diameter of the leading RBC, its $DI$ reduces. Furthermore, less cross sectional area of the leading RBC makes fewer disturbances on the plasma flow and thereby the hydrodynamic interaction between two cells becomes weaker. Therefore, the two RBCs show almost similar $DI$ at their steady state. Furthermore, when the initial diameter of the leading RBC is decreased to 5.29 $\mu$m, due to the further decrease in the initial undeformed diameter of the leading RBC, its $DI$ further reduces. Thus, the cross sectional area of the leading RBC does not create a significant disturbance on the plasma flow and the hydrodynamic interaction between two cells becomes even weaker. Therefore, as explained in Section 6.1, the trailing RBC (which has the larger undeformed initial diameter) shows higher $DI$, while the leading RBC (which has the smaller undeformed initial diameter) shows lesser deformation.
explained earlier, the RBCs experiencing higher $\Delta P$ exhibit larger $DI$s when the initial undeformed diameter of the leading RBC increases. Therefore, as it can be seen from Figure 6.21 that the $\Delta P$ for the trailing RBC generally decreases when the initial undeformed diameter of the leading RBC increases. Therefore, as explained earlier, the RBCs experiencing higher $\Delta P$ exhibit larger $DI$s and the RBCs experiencing smaller $\Delta P$ exhibit lower $DI$s (Figure 6.19).

![Graph showing the variation of pressure difference ($\Delta P$) between left and right hand side of two RBCs with different initial undeformed diameter values of leading RBC ($d_{RBC1}$) at $t = 0.20$ ms](image)

**Figure 6.21:** The variation of the pressure difference ($\Delta P$) between left and right hand side of two RBCs with different initial undeformed diameter values of leading RBC ($d_{RBC1}$) at $t = 0.20$ ms.

The analysis of the pressure fields in this case suggest that the pressure difference ($\Delta P$) between left and right hand sides of the leading RBC increases with the initial undeformed diameter of the leading RBC (see Figure 6.21). Furthermore, it can be seen from Figure 6.21 that the $\Delta P$ for the trailing RBC generally decreases when the initial undeformed diameter of the leading RBC increases. Therefore, as explained earlier, the RBCs experiencing higher $\Delta P$ exhibit larger $DI$s and the RBCs experiencing smaller $\Delta P$ exhibit lower $DI$s (Figure 6.19).

![Graph showing the variation of $DI$ of the leading and trailing RBCs with time; for different initial undeformed diameter values ($d_{RBC2}$) of the trailing RBC with fixed initial undeformed diameter of 7.64 $\mu$m for the leading RBC](image)

**Figure 6.22:** Variation of the $DI$ of the leading and trailing RBCs with time; for different initial undeformed diameter values ($d_{RBC2}$) of the trailing RBC with fixed initial undeformed diameter of 7.64 $\mu$m for the leading RBC.
In the 2nd study, the initial undeformed diameter of the trailing RBC is changed from 7.64 µm to 9.03 µm, 6.16 µm, and 5.29 µm while keeping the initial undeformed diameter of the leading RBC to 7.64 µm. Simulation results reveal that the deformation behaviour of the leading RBC is not greatly affected by the increase in the initial undeformed diameter of the trailing RBC (see Figure 6.22). Moreover, there is no significant variation observed in the deformation behaviour when the initial undeformed diameter of the trailing RBC is reduced. However, when the initial undeformed diameter of the trailing RBC is 5.29 µm, the DI of the leading RBC slightly reduces.

![Diagram showing the variation of RBC's mean velocity with time](image)

Figure 6.23: Variation of the mean velocity of the 1st RBC (leading RBC) 2nd RBC (trailing RBC) with time; for different initial undeformed diameter values of 1st RBC

The mean velocity of the leading RBC at the steady state is not significantly affected by the decrease in the initial undeformed diameter of the trailing RBC. However, it can be seen from Figure 6.23 that the mean velocity of the trailing RBC reduces slightly, when the initial diameter of the trailing RBC is increased up to 9.03 µm. In this case, two RBCs take up a higher volumetric ratio in the problem domain due to the higher undeformed diameters (cross sectional area) of the trailing RBC. Therefore, compared with the other cases, disturbance on the plasma flow is higher and as a result, a slight drop in the mean velocities of the RBCs is expected.
Figure 6.24: The variation of the pressure difference ($\Delta P$) between left and right hand side of two RBCs with different initial undeformed diameter values of trailing RBC ($d_{RBC2}$) at $t = 0.20$ ms

Here, the analysis of pressure fields shows that the pressure difference ($\Delta P$) between left and right hand sides of the leading RBC remains almost constant when the initial undeformed diameter of the trailing RBC increases (see Figure 6.24). Therefore, the leading RBCs do not show considerable variation of $DI$ at the steady state when the initial undeformed diameter of the trailing RBC increases (see Figure 6.22). However, it can be seen from Figure 6.24 that the $\Delta P$ for the trailing RBC increases when the initial undeformed diameter of the trailing RBC increases. As can be seen from Figure 6.22 and Figure 6.24, the $DIs$ of the trailing RBCs change, according to the $\Delta P$.

### 6.6 Deformation of two RBCs in a stenosed capillary

There is a high risk of microvascular blockage in the blood vessels with a stenosed section (Cooke, et al., 2001). In order to predict the behaviour of the RBCs through these sections, the motion and deformation of two RBCs in a stenosed capillary is studied and presented in this section. The behaviour of two RBCs in the stenosed capillary is compared with the behaviour of a single RBC in the same
capillary. The total length \( (L) \) and the diameter \( (D) \) of the capillary are set to 60 \( \mu m \) and 9.6 \( \mu m \) respectively, while the minimum diameter of the stenosed area \( (d) \) is set to 5.6 \( \mu m \). The inlet pressure is set to 615 Pa, while the outlet pressure is set to zero. The horizontal distance from the inlet boundary to the trailing RBC’s centre; \( l_1 \) is set to 3 \( \mu m \) and the distance between two RBCs’ centres; \( l_2 \) is set to 5 \( \mu m \). In order to compare the behaviour of two RBCs under the same simulation conditions, a single RBC is used with \( l_1 = 8 \mu m \) (case 1; see Figure 6.25a) and \( l_1 = 3 \mu m \) (case 2; see Figure 6.25b) to individually simulate the behaviour of the leading and trailing RBCs respectively.

Similar to the previous simulations, due to the applied pressure at the inlet, plasma particles and RBCs start to move. The leading RBC of two RBCs shows a higher DI compared to that of the trailing RBC when it moves through the narrowest section in the capillary (see Figure 6.26). The results in Figure 6.26 reveal that the DI of the leading RBC of two RBCs is even greater than that of the single RBC in case 1 (where \( l_1 = 8 \mu m \); Figure 6.25b). Furthermore, the DI of the trailing RBC is less than that of the single RBC in case 2 (where \( l_1 = 3 \mu m \); Figure 6.25 c). This increase in the DI of the leading RBC and decrease in the DI of the trailing RBC of two RBCs occur due to the hydrodynamic interactions between two RBCs, compared with the single RBC cases (Figure 6.25b and Figure 6.25c). These simulation results further reveal that, when there is a single RBC in the stenosed capillary, the maximum DI of the RBC increases, as the initial distance of the RBC from the narrowest section of the capillary increases (see Figure 6.26).
Figure 6.25: Initial particle configuration of the flow filed when (a) Two RBCs in the stenosed capillary, (b) Case 1: A single RBC in the stenosed capillary with $l_1 = 8 \, \mu m$ (c) Case 2: A single RBC in the stenosed capillary with $l_1 = 3 \, \mu m$
Interestingly, as shown in Figure 6.27, the mean velocities of two RBCs are noticeably lower than the single RBC situations (case 1; Figure 6.25b and case 2; Figure 6.25 c). Therefore, two RBCs take a longer time to reach the stenosed section of the capillary and the outlet of the flow domain. It can be seen from Figure 6.27 that initially, two RBCs move almost at the same mean velocity. Since the blood flow rate is constant in a capillary for a given pressure, flow velocity increases, when the flow area reduces in the stenosed section. Similarly, when the leading RBC is moving through the stenosed section of the capillary, its mean velocity increases significantly. During this time period, the leading RBC slightly blocks the plasma flow through the stenosed section in the capillary and as a result, a small reduction of the trailing RBC’s mean velocity can be seen (see Figure 6.27). The trailing RBC also reaches its maximum mean velocity when it passes though the stenosed section. However, at that time no noticeable change in the mean velocity of the leading RBC can be observed, since the leading RBC is already downstream of the capillary. Finally, when both RBCs exit from the stenosed section, the mean velocities of the RBCs increase and they reach a similar velocity with a very slight reduction.
compared to that of the single RBC cases (case 1; Figure 6.25b and case 2; Figure 6.25c).

6.7 Summary

A two-dimensional DEM model based on a spring network is successfully used in combination with the SPH method, to study the interaction between two RBCs and its effect on their motion and deformation. From this study, the below conclusions may be drawn.

- Due to the hydrodynamic interaction between two RBCs, the leading RBC is always subjected to a higher deformation compared to the trailing RBC. The leading RBC’s \( DI \) is greater than that of a single RBC flow in the same capillary under the same conditions. The trailing RBC’s \( DI \) is less than that of a single RBC flow in the same capillary under the same conditions.

- The distance between two RBCs makes a significant impact on the motion and deformation of two RBCs. When two RBCs are moving closer to each other, the hydrodynamic interaction between the two RBCs is higher and the

![Figure 6.27: Variation of the mean velocity of the RBCs with time; when two RBCs are present in a stenosed capillary and a single RBC is present in the same capillary at different positions](image)

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relative velocity of the two RBCs is also higher, which increases the distance between them.

- When the leading RBC becomes stiffer as a result of infection by a disease like malaria, it directly affects the motion and deformation of the trailing RBC. However, the motion and deformation of the leading RBC is not greatly influenced by the properties of the trailing RBC.
- RBCs with larger undeformed diameters slower down the mean velocity of the RBCs as well as the blood flow rate.
- When the capillary has a stenosed section, the mean velocity of both RBCs decrease significantly before the stenosed section. However, after the stenosed section they gain their velocity back, similar to the uniform capillary situation.

Furthermore, it is expected to extend the study to three-dimensional RBCs, to capture more realistic motions and deformations of the RBCs.
Chapter 7: Deformation Properties of Three-Dimensional Red Blood Cells in Capillaries

Generally, the motion and deformation of the RBCs are highly three-dimensional, as they exhibit three-dimensional deformations in the blood vessels (Secomb et al., 2007). However, most of the developed numerical models are two-dimensional and are unable to capture the three-dimensional nature of the RBC motion and deformation. The developed three-dimensional model (see Section 3.2) is employed to investigate the three-dimensional behaviour of RBCs in capillaries. The deformation behaviour of a single RBC and multiple RBCs in stenosed and narrow capillaries are studied and the results are presented in this chapter.

In detail, Section 7.1 will present the deformation behaviour of a single three-dimensional RBC in a stenosed capillary and Section 7.2 explains the simulation results obtained by introducing multiple RBCs in stenosed capillaries. The effect of the membrane bending stiffness of five identical RBCs on their deformation behaviour will be discussed in Section 7.3. Section 7.4 will present the behaviour of three identical RBCs in capillaries with narrow sections, which are narrower than the RBCs’ mean diameter at rest. After that, the critical diameter of a stenosed capillary, which would prevent the motion of blood through that capillary, will be discussed in Section 7.5. Finally, a concise summary is presented in Section 7.6 to close this chapter.
7.1 Deformation behaviour of the RBC through a stenosed capillary

The motion and deformation of a three-dimensional RBC is analysed when the RBC passes through a stenosed capillary. A three-dimensional stenosed capillary is generated using a set of wall particles as shown in Figure 7.1, such that the minimum distance between two neighbouring particles is equal to 0.4 $\mu$m. The inlet diameter, outlet diameter and the diameter at the stenosed section of the capillary are set to 10.0 $\mu$m, 10.0 $\mu$m and 6.8 $\mu$m respectively while the overall length of the capillary is set to 31.6 $\mu$m. The energy constants of the RBC membrane and the other simulation parameters are set as in Table 3.2.

![Figure 7.1: Geometry of the stenosed capillary](image)

The inlet pressure of the capillary is set to 150 Pa and the outlet pressure is set to zero. Lenard-Jones type repulsive forces are introduced to the fluid particles to avoid any penetration of fluid particles through the capillary wall and RBC membrane (Polwaththe-Gallage et al., 2012). Due to the applied pressure at the inlet, plasma particles start to move and that motion creates an additional pressure on the
RBC. Therefore, due to the flow pressure applied by the plasma, the RBC begins to flow with plasma. The RBC deforms gradually as it moves through the capillary and the largest deformation of the RBC is observed when the RBC flows through the stenosed section of the capillary. The typical parachute shape is observed, before and after the stenosed section, while the bullet-like shape is observed when the RBC passes through the narrowest section of the capillary (see Figure 7.2).

![Figure 7.2: Deformed shape of the RBC when they flow in a stenosed capillary with the stenosed diameter of 6.8 µm at (a) t = 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ms](image)

Once the RBC passes the stenosed section of the capillary, it gains the parachute shape again, as shown in Figure 7.2. The bending and stretching/compression energy increases rapidly when the RBC moves through the stenosed section of the capillary. During that time, the total energy of the RBC membrane reaches the highest value. Even though the stretching/compression energy contributes to the growth of total energy, bending energy is the most significant element, contributing to total energy (See Figure 7.3). At $t = 0$ s all the energies are zero except bending energy, since the RBC membrane is not flat (angle between two neighbouring triangles is not zero) at the rest, as can be seen in Figure 3.20. However, the growth in the bending energy is greater than that of the stretching/compression energy. Furthermore, it can be seen from Figure 7.3 that, before RBC enters the stenosed section of the capillary (at $t = 0.0005$ s), the stretching/compression energy does not show any significant growth but the bending energy shows a considerable increase with time. Therefore, it can be concluded that the deformation of the RBC basically occurs as a result of the bending of the cell and
not due to the stretching/compression. Furthermore, energy values related with the volume constraint, local and total area do not show any substantial change throughout the RBC’s whole motion and this implies that the RBC is incompressible and its volume and the surface membrane area do not change at all during its motion through the capillaries.

![Energy Variation](image)

**Figure 7.3**: Variation of the different energies of the RBC

The RBC exhibits an asymmetric deformed shape along the y-axis just after the stenosed section. In this section, the major lengths of the RBC in x- and in z-directions (major lengths) are measured separately in order to calculate the $DI$ of the RBC. The $DI$ of the RBC is then calculated by dividing the measured major lengths by the minor length of the RBC (length in y-direction). Figure 7.4 shows that the calculated two $DI$ values exhibit a slight mismatch with each other just after peak value (just after passing the stenosed area). However, two curves converge on each other when the RBC approaches the downstream of the capillary. Few previous studies have reported this kind of asymmetric deformed shapes of the RBC (Kaoui, et al., 2009). When the RBC passes through the stenosed section, the bullet-like deformed RBC flows at the highest velocity (see Figure 7.5), similar to the two-dimensional simulations (Polwaththe-Gallage et al., 2015).
7.2 Deformation behaviour of multiple RBCs through a stenosed capillary

In this study, five identical RBCs are used to simulate the motion and deformation behaviour of the RBCs in a stenosed capillary. For convenience, the RBC closest to the stenosed section is defined as the 1st RBC (the leading RBC) and the RBC closest to the inlet boundary is defined as the 5th RBC (the last RBC); in
that order other RBCs are numbered. The inlet and outlet diameters ($d_i$ and $d_o$ respectively) are set to 10.0 $\mu$m, while the minimum diameter of the stenosed area ($d_c$) is set to 6.8 $\mu$m. The total length of the capillary ($L$) is 57.2 $\mu$m and the horizontal distance from the 5th RBC’s centre to the inlet boundary and from the 1st RBC to the narrowest part of the stenosed section ($l_1$ and $l_6$ in that order) are 3.4 $\mu$m and 10.6 $\mu$m respectively. The distances between two consecutive RBCs ($l_2$, $l_3$, $l_4$, and $l_5$) are set to 4 $\mu$m. The inlet pressure is set to 1000 Pa, while the outlet pressure is set to zero.

Figure 7.6: Geometry of the stenosed capillary

Due to the pressure gradient in the capillary, RBCs initiate to flow with plasma. The RBCs exhibit more or less deformations before entering the stenosed section of the capillary (see Figure 7.7b; at $t = 0.01$ ms). However, at this stage, the 1st RBC shows more deformation compared with the following RBCs, due to the hydrodynamic interaction (Pozrikidis, 2005a; Shi, et al., 2013) between RBCs (see Section 6.1 for more details). The 1st RBC moves though the stenosed section at about $t = 0.02$ ms and it experiences severe deformation during that time (see Figure 7.7c). When $t = 0.025$ ms, the 1st RBC exits from the stenosed section and it recovers typical deformed shape, a parachute shape. When $t = 0.025$ ms, the 2nd RBC moves through the stenosed section and similar to the 1st RBC, the 2nd RBC also
undergoes a large deformation. Similar to the 1\textsuperscript{st} and 2\textsuperscript{nd} RBCs, all the RBCs experience severe deformation, when they pass through the narrowest section of the capillary and they recover their typical deformed parachute shape after the stenosed section. Therefore, same kind of behaviour would be expected for the all the RBCs, if they flow further after the stenosed section of the capillary.
Figure 7.7: Deformation of five RBCs when they flow in a stenosed capillary with the stenosed diameter of 4.4 µm at (a) $t = 0$ ms, (b) $t = 0.1$ ms, (c) $t = 0.2$ ms, (d) $t = 0.3$ ms, (e) $t = 0.4$ ms and (f) $t = 0.47$ ms.

Figure 7.8 shows the variation of the DI of five RBCs with time. As expected, due to the hydrodynamic interaction between RBCs, the 1$^{\text{st}}$ RBC shows the maximum $DI$ (when $t = 0.018$ ms) and the $DIs$ of the following RBCs are lesser than that of the 1$^{\text{st}}$ RBC when they pass through the stenosed section.

Due to the hydrodynamic interaction between RBCs, it is expected to observe a lower $DI$ for the 3$^{\text{rd}}$ RBC compared to that value of the 2$^{\text{nd}}$ RBC during their motion through the stenosed section of the capillary. However, the maximum $DI$ of the 3$^{\text{rd}}$ RBC does increase compared to the value of the 2$^{\text{nd}}$ RBC. Similarly, the 3$^{\text{rd}}$, 4$^{\text{th}}$ and 5$^{\text{th}}$ RBC shows higher $DI$ compared with their preceding RBC in the stenosed section (see Figure 7.8). It is not possible to explain this phenomenon with the aid of the hydrodynamic interaction between RBCs and further studies have to be done to describe this behaviour. Moreover, the $DI$ of the 1$^{\text{st}}$ RBC reduces significantly when it exits the stenosed section (see Figure 7.8; after $t = 0.018$ ms). However, the $DI$
gradually increases again with time (see Figure 7.8) and the 1\textsuperscript{st} RBC shows higher \textit{DI} compared with the other RBCs due to the hydrodynamic interaction between RBCs.

![Figure 7.8: Variation of the deformation index of five RBCs with time when they flow in a stenosed capillary with the stenosed diameter of 6.8 \(\mu\)m](image)

Rapid growths in the mean velocities of the RBCs are observed when they are flowing through the stenosed section of the capillary (see Figure 7.9). However, the 1\textsuperscript{st} RBC flows at a slower mean velocity during its motion through the stenosed section, compared with the other RBCs. On the other hand 5\textsuperscript{th} RBC flows at the
highest mean velocity compared with that value of the other RBCs (see Figure 7.9). When the 1st RBC exits the stenosed section of the capillary the number of obstacles in the flow field before the stenosed region reduces by one. Therefore the mean velocity of the flow increases and so does mean velocity of the 2nd RBC. Similarly, the mean velocity of the other RBCs increase compared with that value of the preceding RBC when it passes through the stenosed section.

In order to explain the increase in the maximum $DI$ of the RBC compared with the preceding RBC’s maximum $DI$ when it passes through the stenosed section, further simulations are conducted. In this study, a single RBC is employed with the different horizontal distances from the RBC’s centre to the inlet boundary ($l_1$). Thereby the horizontal distances from the RBC’s centre to the stenosed section ($l_2$) is changed. Three cases are studied with $l_1 = 3.4 \mu m$, 11.4 $\mu m$ and 19.4 $\mu m$. A capillary with the stenosed diameter of 6.56 $\mu m$ is used and all the other simulation conditions are kept the same as described earlier.

The change in the $DI$ of the RBCs with time for the three different cases is analysed. The simulation results show that the RBC, initially positioned closer to the stenosed section ($l_1 = 3.4 \mu m$), experiences less deformation compared to the other RBCs, initially set farther away from the stenosed section (see Figure 7.11). As can be seen in Figure 7.11, the RBC relevant to $l_1 = 3.4 \mu m$ (initially set farther from the stenosed section) undergoes a considerable deformation before it enters to the

![Figure 7.10: Geometry of the stenosed capillary with a single RBC](image)

Figure 7.10: Geometry of the stenosed capillary with a single RBC
stenosed section. Moreover, this RBC is subjected to a further deformation while it is passing though the stenosed section and it shows the maximum $DI$ among three RBC (initially set at $l_1 = 3.4 \mu m$, $11.4 \mu m$ and $19.4 \mu m$). On the other hand, the RBC set closer to the stenosed section does not have enough time to deform before entering to the stenosed section. The deformation of this RBC mainly occurs when it passes through the stenosed region. Therefore, it can be concluded that the RBCs set closer to the stenosed section experience less deformation compared with the deformation of the RBCs set farther away from the stenosed section.

![Figure 7.11: Variation of the deformation index of three RBCs with time when the initial position of the RBC is changed in the capillary with the stenosed diameter of 5.2 $\mu m$](image)

In reality, the blood continuously flows and RBCs exhibit deformation (deformed shapes) at all times. However, for the numerical simulations, an initial condition ($t = 0$) should be assumed. For simplicity, in this study it is assumed that the RBC begins with its typical biconcave shape and there is no deformation at $t = 0$. For the above three cases, if the horizontal distance from the RBC’s centre to the stenosed section ($l_2$) increases considerably, the maximum $DI$ of all the RBC would have been the same, when it passes through the stenosed section. However, it is computationally very expensive to increase the horizontal distance from the RBC’s mass centre to the stenosed section ($l_2$), since it boosts the problem domain.
significantly. The lift in the problem domain would take a longer time to solve the problem and it would be very inefficient. Therefore, the problem domain is controlled for the conditions, as explained in earlier sections.

The variation of the maximum $DI$ of five RBC, when they pass through the stenosed capillary, can be explained (see Figure 7.8) using the above argument. The $1^{st}$ RBC of five RBCs shows the highest maximum $DI$ when it passes through the stenosed section, due to the hydrodynamic interaction between the RBCs. The maximum $DI$ of the $2^{nd}$ RBC is lower than the value of the $1^{st}$ RBC, as expected again due to the hydrodynamic interaction between RBCs. However, the maximum $DI$ of the $3^{rd}$ RBC shows greater value, compared with that value of the $2^{nd}$ RBC. This phenomenon happens due to the difference in the horizontal distance from the RBC’s mass centre to the stenosed section ($l_2$). Initially (at $t = 0$), the $3^{rd}$ RBC is set farther away from the stenosed section compared to the $2^{nd}$ RBC and the $3^{rd}$ RBC takes a longer time to enter the stenosed section of the capillary. During that time, the RBC experiences a significant deformation. The increase in the $DI$ of the $3^{rd}$ RBC just before entering into the stenosed section of the capillary is higher than the value of the $2^{nd}$ RBC. The $DI$ of both RBCs increases significantly, while they pass through the stenosed region. However, due to the change in $DIs$ of the $2^{nd}$ and $3^{rd}$ RBCs just before entering the stenosed section of the capillary, the $3^{rd}$ RBC exhibits a higher maximum $DI$ when it passes through the stenosed section compared with the value of the $2^{nd}$ RBC. Similarly, trailing RBCs exhibit higher maximum $DI$ compared with their preceding RBCs (except the $1^{st}$ RBC). Therefore, it can be concluded that the initial horizontal distance from the RBC’s centre to the stenosed section ($l_2$) is a crucial parameter in numerical simulations, when the simulations are carried out to capture the behaviour of RBCs in a stenosed capillary. This parameter has to be chosen properly, to obtain
reliable enough results without affecting the computation cost much. However, this study was limited to the conditions discussed for Figure 7.7.

7.3 Deformation behaviour of the RBCs with different bending stiffness values in a stenosed capillary

In this study, the membrane bending stiffness of the RBCs on their motion and deformation is studied. Similar to the previous study, five identical RBCs are used to simulate the motion and deformation behaviour of the RBCs in a stenosed capillary. A capillary with a total length of the capillary ($L$) of 57.2 $\mu m$ is used for this study. The inlet ($d_i$) and outlet diameters ($d_o$) of the capillary are set to 10.0 $\mu m$, while the minimum diameter of the stenosed area ($d_c$) is set to 5.2 $\mu m$. The inlet pressure is set to 500 Pa, while the outlet pressure is set to zero. The membrane bending stiffness of all the RBCs is changed from $K_b$ ($1 \times 10^{-10}$ N) to 0.1 $K_b$, 10 $K_b$ and 20 $K_b$, 30 $K_b$, and 40 $K_b$. The five identical RBCs and all the other simulation parameters are set as described earlier.

Simulation results reveal that the RBCs show nearly fully symmetrical deformed shapes (see Figure 7.12a) throughout their motion in the stenosed capillary when the membrane bending stiffness of the RBCs is decreased by 10 times (0.1 $K_b$). However, they show local uneven deformation with wrinkles on the membranes (see Figure 7.12a and Figure 7.13) when they pass through the stenosed region and just after the stenosed section. On the other hand, the five RBCs with higher bending stiffness values do not show any wrinkle on the deformed membrane. However, the 1st RBC of the five RBCs with bending stiffness of 10 $K_b$ show more or less asymmetric behaviour when it reaches the downstream of the capillary (see Figure 7.12c at $t = 0.144$ ms). The asymmetric behaviour of that RBC is clearly
evident when the RBCs have higher bending stiffness values; 20 $K_b$, 30 $K_b$ and 40 $K_b$ (see Figure 7.12d, Figure 7.12e and Figure 7.12f at $t = 0.144$ ms). Interestingly, the RBCs with the highest bending stiffness values (30 $K_b$ and 40 $K_b$) do not show observable deformation during their motion before the stenosed (see Figure 7.12e and Figure 7.12f at $t = 0.048$ ms). However, they deform into bullet-like shapes when they pass through the stenosed region and the deformed RBCs are not symmetrical in shape (see Figure 7.12e and Figure 7.12f at $t = 0.096$ ms).

Furthermore, the RBCs show rolling motions after the stenosed region of the capillary (see Figure 7.12e and Figure 7.12f at $t = 0.144$ ms). Additionally, the RBC with the highest bending stiffness value (40 $K_b$) exhibits complicated asymmetric deformed shapes when it passes through the narrowest section of the capillary.

(a) $t = 0.048$ ms  
(b) $t = 0.048$ ms  
(c) $t = 0.048$ ms  
(d) $t = 0.048$ ms  
(e) $t = 0.048$ ms
The RBCs are subjected to the largest deformation when they pass through the stenosed section of the capillary, and as a result of the deformation, the total membrane energy of the RBCs reaches its maximum value when they pass through the stenosed section of the capillary. The average total membrane energy of one RBC is calculated when it passes through the stenosed section and is plotted against the membrane bending stiffness value of the RBC, as shown in Figure 7.14. It can be seen that the total membrane energy of one RBC increases significantly with the membrane bending stiffness of the RBCs. The RBCs with higher membrane energies are very unstable and then there is a possibility of rupture in the RBC membrane due to higher unstable membrane energies (Omori et al., 2012)
It is not possible to employ the DI to compare the amount of deformation, when RBCs show highly asymmetrical three-dimensional deformed shapes. Furthermore, the average total membrane energy does not reflect the amount of bending or the DI of the RBCs, since the higher membrane bending stiffness values always contribute to higher total membrane energy of the RBCs. Therefore, the average membrane energy ($E_b$) is normalised using membrane bending stiffness ($K_b$) as,

$$\frac{E_b}{K_b} = \frac{1}{2} \sum_{n=1}^{NB} L_n \tan^2 \left( \theta_n - \frac{\theta_{n,0}}{2} \right)$$ (7.1)

In this study, $E_b / K_b$ is employed to compare the amount of deformation of the RBCs. It can be seen that the RBC with the lowest membrane stiffness ($0.1 K_b$) shows a very high $E_b / K_b$ value compared with the value of the RBCs with the typical membrane bending stiffness ($K_b$). The $E_b / K_b$ decreases substantially when the membrane bending stiffness of the RBCs increases from $K_b$ to $10 K_b$ (see Figure 7.15). Moreover, $E_b / K_b$ further decrease gradually with the membrane bending stiffness of the RBC when the membrane bending stiffness of the RBCs increases from $10K_b$ to $50 K_b$ (see Figure 7.15). However, that value reaches more or
less steady value, when the membrane bending stiffness increases from $50 \, K_b$ to $100 \, K_b$. It can be concluded that, irrespective of the membrane bending stiffness of the RBCs, RBCs deform into a certain amount in order to pass through the stenosed section of the capillary.

![Graph](image)

Figure 7.15: Variation of the deformation index of three RBCs with time when the initial position of the RBC is changed in the capillary with the stenosed diameter of $5.2 \, \mu m$

### 7.4 Deformation behaviour in narrow capillaries

It is generally known that the diameters of the capillaries vary between 5-10 $\mu m$ (Boryczko et al., 2003). In this section, the motion and deformation of three RBCs are investigated in the capillaries with narrower sections, which are narrower than the diameter of the RBCs at rest. Three RBCs with identical properties are employed for this simulation in a capillary with the total length ($L$) of $60.0 \, \mu m$. The diameter ($D_c$) and the length ($l$) of the narrow section of the capillary are set to $6.0 \, \mu m$ and $21.2 \, \mu m$ respectively. The inlet ($d_i$) and outlet ($d_i$) diameters of the capillary are set to $10.0 \, \mu m$, while the inlet and outlet pressures are set to $1000 \, Pa$ and zero respectively.
Simulation results show that three RBCs initially deform into parachute shapes before entering the narrow section of the capillary. Interestingly, they exhibit bullet-like shapes when they are flowing through the narrow section of the capillary (see Figure 7.17), while the bullet-like deformed shapes of the RBCs remain unchanged during their whole motion in the narrower section of the capillary. During that time, the $DIs$ of the three RBCs are similar to each other (see Figure 7.17 and Figure 7.18). However, after the narrow section, the 1$^{\text{st}}$ RBC exhibits a more deformed shape with a higher $DI$ while the 3$^{\text{rd}}$ RBC shows a rounder shape with a lower $DI$. On the other hand, the 2$^{\text{nd}}$ RBC takes an intermediate $DI$ compared with that values of the 1$^{\text{st}}$ and the 3$^{\text{rd}}$ RBCs. This difference in the $DIs$ of the RBCs occurs due to the hydrodynamic interaction between the RBCs.
The mean velocities of the three RBCs gradually increase before the narrow section of the capillary (see Figure 7.19). Three RBCs reach maximum steady mean velocities, when they flow through the narrow section. However, the mean velocities of the three RBCs drop back to lower values after the narrow section of the capillary (see Figure 7.19). Initially, the RBC’s mean velocity curves show very high fluctuations and this reflects the nature of instability in the RBCs under these conditions.
simulation conditions. However, as can be seen from Figure 7.19, the mean velocities of the three RBCs show stable values with fewer fluctuations, when they leave the narrow section of the capillary.

Figure 7.18: Variation of the DI of three RBCs with time when they flow in a capillary with a narrow section

Figure 7.19: Variation of the mean velocity of three RBCs with their position in the capillary when they flow in a capillary with a narrow section
7.5 Critical diameter of the stenosed section to stop the motion of blood flow

The critical stenosed diameter of a capillary is investigated to halt the blood flow in the capillary with the aid of five identical RBCs. In this study, the diameter of the stenosed section is changed to 8.4, 7.6, 6.8, 6.0, 5.2, 4.4, 3.6 and 2.8 µm in the capillary. The inlet \((d_i)\) and outlet diameters \((d_o)\) of the capillary are set to 10.0 µm, while the total length of the capillary \((L)\) is set to 57.2 µm. The inlet and outlet pressures are set to 1000 Pa and zero respectively.

The time taken by the 1st RBC to reach the outlet of the capillary is measured for each case. Any motion of the five RBCs is not observed when the stenosed diameter of the capillary is 3.6 µm and 2.8 µm. However, the five RBCs start to flow very slowly in the capillary when the stenosed diameter of the capillary is 4.4 µm. As can be seen in Figure 7.20, the time taken by the 1st RBC to reach the outlet of the capillary is about 1.3 ms and that is the slowest among all the other cases. Furthermore, when the stenosed diameter of the capillary is changed from 4.4 µm to 5.2 µm, the time taken by the 1st RBC to reach the outlet of the capillary reduces

![Figure 7.20: Variation of the time taken by the 1st RBC to reach the outlet of the capillary with different stenosed diameter values, when the inlet and outlet pressure are 1000 Pa and 0 respectively](image-url)
significantly. Further increase in the stenosed diameter of the capillary reduces the elapsed time more. However, as can be seen from Figure 7.20, the time taken by the 1st RBC to reach the outlet of the capillary reaches a nearly stable value when the stenosed diameter of the capillary increases further. With the aid of a Matlab curve fitting tool, the stenosed diameter of the capillary is predicted when the elapsed time tends to infinity. It is found from Figure 7.20 that the time taken by the 1st RBC to reach the outlet of the capillary tends to infinity when the diameter of the stenosed section of the capillary is 4.004 µm. Hence, it can be concluded that the five RBCs do not show any motion in a stenosed capillary with inlet pressure of 1000 Pa and outlet pressure of zero when the minimum diameter of the stenosed section is 4.004 µm. Therefore, 4.004 µm is the critical diameter for the stenosed capillary, which stops the blood flow for the above inlet and outlet pressures.

![Figure 7.21: Variation of the time taken by the 1st RBC to reach the outlet of the capillary with different stenosed diameter values, for different inlet pressure values while the outlet pressure is zero](image)

In the same way, the critical diameter of the stenosed section of the capillary, which stops the blood flow, is found for different inlet pressure values. In this study, the inlet pressure of the stenosed capillary is changed to 500, 1000, 1500, 2000 and 2500 Pa while keeping the outlet pressure of the capillary to zero. All the other
parameters are kept constant. Similar to the previous case, the inlet pressure is set to 1000 Pa. The time taken by the 1st RBC to reach the outlet of the capillary increases when the stenosed diameter of the capillary decreases. However, when the inlet pressure of the capillary increases, the critical diameter for the stenosed capillary, which stops the blood flow, do not show significant variation (see Figure 7.21 and Table 7.1). On the other hand, when the inlet pressure of the capillary is deceased up to 500 Pa, the critical diameter for the stenosed capillary (which stops the blood flow) shows considerable increase (see Figure 7.21 and Table 7.1).

<table>
<thead>
<tr>
<th>Inlet Pressure of the stenosed capillary (Pa)</th>
<th>Pressure gradient (MPa/m)</th>
<th>Critical diameter of the stenosed section(µm)</th>
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</thead>
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<td>4.472</td>
</tr>
<tr>
<td>1000</td>
<td>17.4825</td>
<td>4.004</td>
</tr>
<tr>
<td>1500</td>
<td>26.2237</td>
<td>3.985</td>
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<tr>
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<td>43.7062</td>
<td>3.989</td>
</tr>
</tbody>
</table>

### 7.6 Summary

A three-dimensional DEM model based on a spring network is used in combination with the SPH method to model the motion and deformation of RBCs on capillaries. From this study, the conclusions below are drawn.

- The RBCs experience a larger deformation and they exhibit bullet-like shapes when they pass through the stenosed section of the capillary. However, once they move out from the capillary section, RBCs recover their typical deformed parachute shape.
• Energy curves prove that the deformation of the RBCs occurs not only due to bending but also due to the RBC’s membrane stretching when they pass through the stenosed section of the capillary.

• In numerical simulation, initial setting of the RBCs directly affected the deformation behaviour of the RBCs. The lengths of the capillaries should be long enough to obtain reliable enough results without overly affecting the computation cost.

• When the membrane bending stiffness of the RBCs increase, like in malaria infected RBCs, they show highly asymmetrical deformed shapes and rolling motions. On the other hand the RBCs with lower membrane bending stiffness values exhibit wrinkles on the membrane when they are deforming.

• Irrespective of the membrane bending stiffness of the RBCs, RBCs deform into a certain amount in order to pass through the stenosed section of the capillary.

• The RBCs exhibit bullet-like shapes when they flow through the capillaries with narrower sections, which are narrower than the diameter of the RBCs at rest. However, they show parachute shapes when the diameter of the section, in where RBCs are moving, increases.

• There is a certain critical diameter for a given stenosed capillary and for a given pressure gradient, which completely stops the motion of blood with RBCs, leading to microvascular blockages.
Chapter 8: Conclusions and Recommendations

In this research, the meshfree particle approach is employed to investigate the motion and deformation properties of the RBCs in capillaries. The viscoelastic membrane of the RBC is modelled by the discrete element method (DEM), based on an elastic spring network. The haemoglobin in the RBC and the plasma in the blood are modelled as SPH particles. In this chapter, an overall summary is provided in section 8.1. The limitations of this research are clarified in Section 8.2 and suggestions and recommendations for future research are discussed in Section 8.3.

8.1 Research summary

A number of models have been developed to describe the RBC behaviour in microvessels. However, it has been found that the meshfree particle methods have several advantages over the conventional grid-based methods in modelling the behaviour of RBCs. In particular, SPH, as one of the popular and well-established meshfree particle approaches, has been proposed by researchers and has received widespread attention to solve micro-scale hydrodynamic problems. Furthermore, it is found that the minimum energy approach provides a more realistic initial geometrical shape for the RBC membrane that is closer to the matured healthy RBCs’ shape. Therefore, in this research, a coupled SPH-DEM model was developed to investigate the behaviour of RBCs’ capillaries. Conclusions from this research can be summarised as below:

- Behaviour of a single two-dimensional red blood cell in a uniform capillary
Although in blood there are a large number of RBCs, at the beginning of this research, a single two-dimensional RBC in a uniform capillary is considered to analyse the behaviour of the RBC. From the simulations, it was found that due to the existence of the RBC in the blood flow, the velocity profile of the blood flow in a capillary takes a blunt shape, compared with a parabolic shape velocity profile in the pure plasma flow. Furthermore, at the steady state, the RBC reached a parachute shape and all the particles used to represent the membrane of the RBC moved at a unique velocity. In addition, it was found that the size of the RBC affects the amount of deformation of the RBC and the larger RBCs deform more, compared with the smaller RBCs. Moreover, simulation results were supportive in proving that the deformation of the RBC decreases for a given pressure, when the inlet diameter of the capillary increases. However, the mean velocity of the RBC increased with inlet diameter of the capillary when the inlet diameter of the capillary increased. On the other hand, the DI of the RBC increased with the inlet pressure of the capillary. Similarly, the mean velocity of the RBC increased with the inlet pressure of the capillary. Finally, it was found that only the membranes’ bending stiffness significantly influences the RBC’s DI and mean velocity. This is mainly due to the discoidal biconcave shapes of the RBCs and the blunt parabolic shape of the plasma flow in smaller blood vessels. The DI decreased when the membrane bending stiffness increased. RBCs showed substantial variation in the deformed RBC’s morphology when the membrane bending stiffness increased.

- Behaviour of a single two-dimensional red blood cell in a stenosed capillary

From the simulations of a single two-dimensional red blood cell in a stenosed capillary, it was found that the overall blood flow rate in capillaries reduces when the
capillary is stenosed and RBC moves at its highest mean velocity when it passes through the narrowest section of the capillary. Furthermore, when the RBCs were passing through the stenosed sections of the capillary, the RBC membrane was not only bending, but also stretching. In addition, it was found that the deformation index is not a sensible measurement to accurately quantify the amount of deformation of an RBC, when they show asymmetric behaviours in the blood flow. Moreover, simulation results were supportive in proving the RBCs’ ability of passing through the stenosed capillaries with minimum diameter of 3.2µm, and they showed highly asymmetrical deformed shapes when they were flowing through narrower sections. It is also revealed that further reduction of the stenosis blocks the RBC.

- Behaviour of multiple two-dimensional red blood cells in capillaries

From the results obtained from the simulations of multiple red blood cells in capillaries, it was revealed that the deformation of the RBCs changes due to the hydrodynamic interaction. In addition, it was found that the mean velocity of the RBCs reduces with the number of RBCs in the capillary and it indirectly affects the deformation behaviour of the RBCs. Therefore, in order to clearly demonstrate the interactions between RBCs, only two RBCs are considered and thereby the effects of the changes in mean velocities of the RBCs (due to the change in number of RBCs) are eliminated. Simulation results revealed that due to the hydrodynamic interaction between two RBCs, the leading RBC is always subjected to a higher deformation compared to the trailing RBC. Moreover, the leading RBC showed a greater $DI$ than that of a single RBC flow in the same capillary under the same conditions. The trailing RBC’s $DI$ was less than that of a single RBC flow in the same capillary under the same conditions. In addition, it was found that the distance between two RBCs makes a significant impact on the motion and deformation of two RBCs.
When two RBCs were moving closer to each other, the hydrodynamic interaction between the two RBCs was higher and the relative velocity of the two RBCs was also higher, which caused to increase the distance between them. Moreover, the simulation results supported proof that when the leading RBC becomes stiffer as a result of infection by a disease like malaria, it directly affects the motion and deformation of the trailing RBC. However, the motion and deformation of the leading RBC was not greatly influenced by the properties of the trailing RBC. Finally, it was found that when the capillary has a stenosed section, the mean velocity of both RBCs decreases significantly before the stenosed section. However, after the stenosed section they gained their velocity back similar to the uniform capillary situation.

- Deformation property of three-dimensional red blood cells in capillaries

The simulations of three-dimensional RBCs showed that the RBCs experience a larger deformation and they exhibit bullet-like shapes when they pass through the stenosed section of the capillary. However, once they moved out from the capillary section, the RBC recovers its typical deformed parachute shape. In addition, it was found that in numerical simulation, initial settings of the RBCs directly affect the deformation behaviour of the RBCs. The lengths of the capillaries in the model should be long enough to obtain reliable enough results without affecting the computation cost much. Moreover, simulation results supported proof that when the membrane bending stiffness of the RBCs increases, like in malaria infected RBCs, they show highly asymmetrical deformed shapes and rolling motions. On the other hand, the RBCs with lower membrane bending stiffness values exhibit wrinkles on the membrane when they deform. Further simulations revealed that irrespective of
the membrane bending stiffness of the RBCs, RBCs deform a certain amount in order to pass through the stenosed section of the capillary. Furthermore, the simulation results exposed that the RBCs exhibit bullet-like shapes when they flow through the capillaries with narrower sections, which are narrower than the diameter of the RBCs at rest. However, they show parachute shapes when the diameter of the section, in where the RBCs are moving, increases. Finally, it was found that there is a certain critical diameter for a given stenosed capillary and for a given pressure gradient, which completely stops the motion of blood with RBCs, which leads to microvascular blockages.

Major conclusions drawn from this research

- The deformation index is not a sensible measurement to accurately quantify the amount of deformation of an RBC, when they show asymmetric behaviours in the blood flow.

- The RBCs become stiffer when they are infected by malaria and they exhibit different deformed shapes. The mean velocity of the infected RBCs decreases as the RBCs become stiffer and it leads to slow down the blood flow rate in the cardiovascular network.

- There is a certain critical diameter for a given stenosed capillary and for a given pressure gradient, which completely stops the motion of blood with RBCs.

Overall, the simulation results obtained in this research could be used to diagnose the infected RBCs and predict the stiffness of the infected or abnormal RBCs. In addition to that, the simulation results obtained in this research could be
used to design a blood filter to filter out the infected RBCs and the findings of this research could be used to predict the motion and deformation behaviour of malaria infected RBCs at different stages.

8.2 Limitations

Though this study has provided a comprehensive knowledge on the behaviour of RBCs in capillaries, there are still several limitations that need to be clarified.

- **Lack of experiments:** In this research it was found that there is a critical diameter for a stenosed capillary, which can halt the blood flow in that capillary. However, there is no experimental evidence to prove that phenomena. In order to verify the results obtained from the developed model, more experiments are needed to be carried out.

- **Initial configuration of the problem domain:** It was found that in numerical simulation, initial setting of the RBCs directly affects the deformation behaviour of the RBCs. The lengths of the capillaries should be long enough to obtain reliable enough results without affecting the computation cost. In order to examine the behaviour of the RBCs in the long run, longer channels should be considered. Furthermore, in this research it was assumed that the initial shape of the RBCs is biconcave (at $t = 0$) and they are set in vertical directions, However, when the RBCs are entering into the capillaries, they could have uneven orientations with complicated deformed shapes. Therefore, in order to investigate the behaviour of RBCs more accurately, these conditions need to be considered.
• **Influence of other blood cells:** In this study, it was hypothesised that the behaviour of the RBC is not affected by either white blood cells or platelets and because of that reason, 99% of the blood cells are RBC. However, to be more precise, the influences of the white blood cells and platelets need to be considered and they have to be modelled in the problem domain.

• **Mass transfer through the cell membrane:** When the RBCs squeeze through the capillaries, the exchange of oxygen (O\textsubscript{2}) and carbon dioxide (CO\textsubscript{2}) takes place through the thin wall of the capillary. However, this research did not focus on the mass transfer through the RBC membrane.

• **Changes in RBCs’ properties with time:** It has been found that the biconcave shape of the RBCs remains only over its life span of 120 days. The morphology and the properties of the RBCs change when the RBCs get older. However, in this study, only the matured RBCs with their characteristic properties are considered to investigate the behaviour in capillaries.

• **Flexible nature of the blood vessels:** It has been found that the blood vessels are not rigid tubes (Connes et al., 2013) and their diameter may react to the high pressures and flow conditions. However, in this study all the blood vessels are considered to be rigid and the flexibility or elasticity nature of the blood vessels were ignored.
8.3 Future direction

To overcome the above mentioned limitations, several suggestions and recommendations are proposed.

It is evident that the advancement of computational science allows us to develop numerical models to simulate and predict complicated biomechanical problems. Though the model provides predictions on behaviour of RBCs in different capillaries under different flow conditions, more experiments are intended to be carried out in order to prove the predicted results. The necessity of longer capillaries were evident during the simulations, however, growth in the problem domain comes at a huge computational cost. Therefore, it is encouraging to develop new numerical schemes and algorithms to obtain the simulation results in longer capillaries without affecting computational cost much.

It would be interesting to develop a model to investigate the behaviour of RBCs, when all the RBCs white blood cells and platelets are present in the blood flow. A similar approach (DEM model based on the spring networks) could be used to model the white blood cells and platelets, however, with different properties. In addition, this approach could be improved to simulate the mass transfer through the RBC membrane and capillary walls. Again, it would be easier to employ a similar meshfree approach to model the mass transfer phenomena through solid walls.

Finally, development of a model to predict the changes in the behaviour of RBCs with their age would be more appropriate to predict the ageing effect on the motion and deformation of the RBCs. On top of that, the flexible nature of the capillary wall could be modelled using a DEM model based on a spring network.


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