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論文内容要旨

Over the years, various experimental methods have been applied in an effort to understand the blood flow behavior in microcirculation. Most of our current knowledge in microcirculation is based on macroscopic flow phenomena such as Fahraeus effect and Fahraeus-Linqvist effect. The development of optical experimental techniques has contributed to obtain explanations on the way the blood flows through microvessels. Although the past results have been encouraging, detailed studies on the flow properties of blood in the microcirculation has been limited by several technical factors such as poor spatial resolution and difficulty to obtain quantitative detailed measurements at such small scales. Therefore, there is still a lack of knowledge on the microscale flow behavior of blood cells through microvessels.

In recent years, due to advances in computers, optics, and digital image processing techniques, it has become possible to combine a particle image velocimetry (PIV) system with a conventional microscope. As a result, this combination, known as a micro-PIV, has greatly increased the resolution of the conventional PIV. Although the conventional micro-PIV technique has proven to be useful in measuring the flow behavior in microfluidics devices, the entire flow field is illuminated and consequently the out-of-focus emitted light can result in high levels of background noise, which degrades the measured velocity fields. More recently, considerable progress in the

development of confocal microscopy and the advantages of this technique over conventional microscopy have led to a new technique known as confocal micro-PIV. This technique combines the conventional PIV system with a spinning disk confocal microscope (SDCM), which has the ability to obtain in-focus images with an optical thickness less than 1 μm (optical sectioning effect) and also to improve the particle image contrast, definition, and spatial resolution. This emerging technique has been successfully applied to measure homogenous fluids, however the question whether a confocal micro-PIV system is a suitable technique to study the blood flow behavior through microchannels remains.

In our work, a confocal micro-PIV system and also a confocal micro-particle tracking velocimetry (PTV) system are used, for the first time, to investigate *in vitro* blood flow through microchannels. By using these systems we have aimed to obtain further insights into the complex flow properties of blood in the microcirculation with the expectation that a better understanding on the blood flow phenomena will make a contribution to the prevention, diagnosis, and treatment of vascular diseases.

The validity of our confocal micro-PIV system is performed by comparing the experimental results of pure water seeded with tracer particles with an established analytical solution for a steady flow in a long straight microchannel. Good agreement was obtained, especially around the centre of the microchannel, with errors on the order of 5% or less. Furthermore, we have also demonstrated, for the first time, the ability of the confocal micro-PIV to generate 3D velocity profiles of a blood cell suspension fluid (~ 4% haematocrit (Hct)).

The confocal system is used to determine both ensemble and instantaneous velocity profiles for *in vitro* blood (haematocrit up to 17%), flowing through a 100-μm square glass microchannel at a constant flow rate and low Reynolds number (Re = 0.025). It was observed small fluctuations in the instantaneous velocity profiles which were found to be closely related to the increase with the Hct. Although the micro-PIV community tend to ignore these fluctuations, this study shows that the root mean square (RMS) values increase with the haematocrit implying that the presence of RBCs within the plasma flow strongly influences the measurements of the instantaneous velocity fields. Consequently, information provided by instantaneous velocities should be taken into account. Furthermore, by measuring the velocity profiles *in vitro* blood (20% Hct) in a rectangular (300 μm wide, 45 μm deep)

polydimethysiloxane (PDMS) microchannel, small fluctuations were also found in the velocity profiles. Therefore, our results clearly show evidence that the encountered fluctuations are closely related to the motion and interaction of RBCs when flowing in a crowded environment.

We show that the confocal micro-PIV system is able to measure with good accuracy the blood plasma flow with Hct up to 9%, in a 100 μm square microchannel. However, for Hct bigger than 9%, the light absorbed and scattered by the RBCs contributes to diminish the concentration of tracer particles in the acquired confocal images. Hence, a novel approach was implemented to the confocal system in order to obtain more reliable quantitative measurements on the motion of blood cells at high suspensions of RBCs. By using labeled RBCs and Dextran-40, a confocal micro-PTV system was employed, for the first time, in an effort to track individual tracer cells at high concentration suspensions of RBCs. The ability of the confocal system to generate thin in-focus planes has allowed both qualitative and quantitative measurements in flowing blood at concentrated suspensions (up to 35% Hct) of cell-cell hydrodynamic interaction, RBC orientation and RBC radial dispersion at different depths. Such data is thought to be extremely relevant to elucidate the blood transport mechanisms and associated diseases such as thrombosis and atherosclerosis.

By using the confocal micro-PTV system, the RBCs radial dispersion coefficient (D_{yy}) was measured in the middle plane of a 50μm and 100 μm glass capillaries in both diluted and concentrated suspensions (Hcts up to 35%) at low Reynolds numbers (Re from 0.003 to 0.005). There is evidence that the RBCs D_{yy} tends to increase with the Hct but, at Hct of about 25%, it tends to level off. This finding suggests that, at moderate Hcts, the development of the plasma layer and the consequent decrease of the local cell density, surrounding the RBCs, may enhance the radial dispersion of RBCs. In addition, we have also found that D_{yy} is greatest at radial positions between 0.4R to 0.8R, whereas at locations adjacent to wall (0.8R to 1R) and around the middle of the capillary (0R to 0.2R) the paths of the tracer RBCs tend to exhibit lower radial displacements. Furthermore, our results also provide evidence that RBCs D_{yy} tends to decrease with the diameter. This phenomenon is believed to be due to Hct reduction with the diameter (Faharaeus effect) and also to geometry constrictions which limit the amplitude of the RBCs radial displacements. Hence, this finding seems to indicate that the reduction of RBC radial dispersion may be linked to the decrease in apparent viscosity with decreasing diameter (Faharaeus-Lindqvist effect).

The work reported in this thesis represents the first application of a confocal micro-PIV/PTV system to study the blood flow behavior through microchannels. The confocal system proves to be able to eliminate the problems and concerns of the experimental techniques used in the past and provide additional detailed description on the RBC motion not obtainable by other conventional methods. Finally, the research carried out throughout this thesis provides the basis not only to obtain further insights on the blood mass transport mechanisms under both physiological and pathological conditions but also to improve the existing theories, models, and computer simulations on the blood flow at both micro and macroscale levels.

論文審査結果の要旨

人体の微小循環は，物質輸送や血圧の調節など，生命現象の基礎を担っている．しかしながら，微小循環の血流の定量的な計測は非常に難しく，血液の流動状態の詳細な検討は十分にされていないのが現状である．本論文は，共焦点マイクロ PIV-PTV システムを用い，微小流路内の血流動態を解析したもので，全編 8 章からなっている．

第 1 章は序論である．

第 2 章では，PIV および PTV 計測の基本原理について共焦点マイクロ PIV では原理的に，従来手法に比べ空間・時間分解能が大きく改善されることを示している．また，両者の比較実験も行い，実際にどの程度分解能が改善されるかを示している．

第 3 章では，生理食塩水と血液を用い，正方形ガラス管内の血流の 3 次元速度分布を，共焦点マイクロ PIV により計測している．生理食塩水の実験結果は，ニュートン流体に対する理論解と良い一致を見せ，計測結果の妥当性が確認されている．また，生理食塩水と血液の結果を比較した結果，血液の流れには細かな時間変動成分が存在することが示されている．微小流路内の血流においては，平均流量が一定であっても，血球スケールでは流れは強い非定常性を示すことを明らかにしている．

第 4 章では，ヘマトクリット（赤血球の体積分率）を最大 17%まで増加させ，流れの非定常成分に及ぼすヘマトクリットの影響を共焦点マイクロ PIV により調べている．その結果として，ヘマトクリットを増加させることにより赤血球間の流体力学的な干渉が増加し，流れの非定常成分が増加することを明らかにしている．

第 5 章では，流路の材料としてガラスではなく Polydimethysiloxane(PDMS)を用い，血流の共焦点マイクロ PIV 計測を行なっている．そして，ガラスに比べ PDMS では複雑な形状の流路を作成しやすい点など，PDMS 流路を用いることの利点をまとめている．

第 6 章では，染色された赤血球を用いることで，流れ中の 1 つ 1 つの赤血球の軌跡や運動状態を，共焦点マイクロ PTV により調べている．PIV ではなく PTV を用いることで，より高ヘマトクリットの血液を用いることが可能となり，壁面近傍だけでなく管路中央付近の赤血球の挙動も観察できることを示している．その結果として，流れ中の赤血球は周囲の血球と干渉しながら回転や移動をしており，非定常性の強い挙動を示すことを明らかにしている．

第 7 章では，共焦点マイクロ PTV を用いて赤血球の軌跡を計測し，赤血球の自己拡散係数を計算している．赤血球の自己拡散係数は，流れによって血球成分がどの程度攪拌されるかを示す指標であり，血液の連続体モデルを構築する上で重要な物理量であることを述べている．ヘマトクリットを変化させて赤血球の自己拡散係数を調べた結果，拡散係数はヘマトクリットの増加と共に増加するものの，ヘマトクリット 25%程度以上では増加率は鈍くなることが明らかにされている．また，管直径や半径位置が自己拡散係数に及ぼす影響も明らかにされている．

第 8 章は結論である．

以上要するに，本論文は共焦点マイクロ PIV-PTV システムを用い，微小流路内の血流動態に関する新知見を数多く提供したものであり，生体機械工学の発展に寄与するところが少なくない．

よって，本論文は博士(工学)の学位論文として合格と認める．