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KUNSTBLUT - Artificial Blood for Experimental Flow Visualization in Intracranial Aneurysms

Christina Maria Winkler[1,2], Gesine Hentschel[1,2], Marc Müller[1], Philipp Berg[3,4], Péter Kováts[5], Katharina Zähringer[5], Florian Rummel[6], Birgit Glasmacher[1,2].
[1] Institut für Mehrphasenprozesse, Fakultät für Maschinenbau, Leibniz Universität Hannover, Garbsen, Deutschland
[2] NIFE – Niedersächsisches Zentrum für Biomedizintechnik, Implantatforschung und Entwicklung, Medizinische Hochschule Hannover, Deutschland
[3] Forschungscampus STIMULATE, Otto-von-Guericke-Universität Magdeburg, Deutschland
[4] Lehrstuhl für Medizinische Telematik und Medizintechnik, Otto-von-Guericke-Universität Magdeburg, Deutschland
[5] Lehrstuhl für Strömungsmechanik & Strömungstechnik, Otto-von-Guericke-Universität Magdeburg, Deutschland
[6] NETZSCH-Gerätebau GmbH, Selb, Deutschland
e-Mail: winkler@imp.uni-hannover.de

Summary:
Multiphase blood substitute fluids are currently under development for quantifying (pathological) hemodynamics in intracranial aneurysms using particle image velocimetry (PIV). The challenge lies in replicating the thin elastic 7.5 µm red blood cells (erythrocytes). These were reproduced by hydrogel beads. Various blood substitute fluids could be successfully produced, including artificial plasma from a glycerol-water mixture, mixed with spherical and disc-shaped agarose erythrocytes and disc-shaped NOA60 erythrocytes. Artificial erythrocytes, so far up to 29 µm in diameter, were synthesized using microfluidic methods. PIV seeding particles were integrated into the beads to make them visible with optical measurement systems such as PIV and particle tracking velocimetry (PTV).

Keywords: hemodynamics, intracranial aneurysms, microfluidics, hydrogels, particle image velocimetry (PIV).

1. Introduction

Investigating hemodynamics within intracranial aneurysms requires a meticulous understanding of flow dynamics. Here, computational fluid dynamic (CFD) models are a powerful approach [1]. However, it is paramount to validate these models against experimental in vitro flow tests. Among these experimental techniques, particle image velocimetry (PIV) stands out as a non-intrusive optical method for analyzing flow patterns and velocity profiles at high spatial and temporal resolution. This study explores the challenges and advancements of PIV measurements in the context of blood flow dynamics within intracranial aneurysms.
PIV introduces seeding particles into the flow fluid, which are illuminated by lasers. By capturing the scattered light with high-resolution camera systems, researchers can discern particle motion and calculate crucial parameters such as flow velocity and direction [2]. However, traditional PIV techniques face a significant hurdle in capturing flow dynamics within aneurysms due to the opacity of blood. In addition, the multiphase nature of blood (blood plasma with red blood cells) is often neglected [3].
Recognizing these challenges, the scientific community has sought innovative solutions, particularly in the development of one-phase PIV-compatible blood substitute fluids. These fluids aim to mimic the multiphase nature of blood while ensuring optical transparency and rheological similarity [4]. While some strides have been made in measuring near-wall regions using µ-PIV and fluorescence tagging, visualizing three-dimensional flows within central vessel regions requires the use of suitable blood substitutes.
Previous research highlights the lack of commercially available multiphase blood substitute fluids, which has led to fundamental inquiries into their development These substitutes must not only adjust properties such as the index of refraction (IOR) and viscosity, but also replicate the non-Newtonian behavior inherent in blood flow. However, challenges persist including mismatches in IOR and issues with artificial erythrocytes materials, hindering the accurate emulation of blood's properties.
The primary objective of this study is to present a novel approach to creating a multiphase blood substitute fluid tailored for PIV measurements. This involves creating artificial plasma

with PIV-compatible properties and developing hydrogel beads that mimic human erythrocytes with a diameter of approx. 7.5 µm (see Fig. 1). By exploring various manufacturing methods and materials, we aim to use the microfluidic technique to produce micro beads made of hydrogels. This material was chosen due to its elastic and near-transparent properties, which are essential for mimicking erythrocytes.

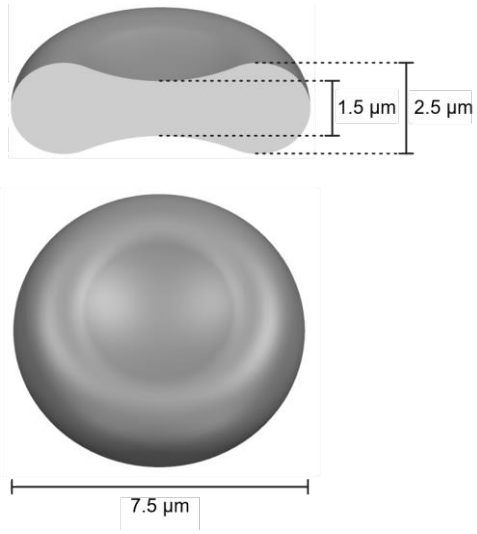

Fig. 1. Schematic representation of a biconcave erythrocyte with a diameter of 7.5 µm and an inner height of 1.5 and outer of 2.5 µm [5].

Our work aims to address the critical need for accurate blood flow visualization within intracranial aneurysms through a systematic approach that encompasses plasma formulation and bead synthesis. By addressing the limitations of current blood substitute fluids, we aim to advance scientific understanding and medical technology in this vital field.

2. Methods

2.1 Single-phase blood substitute

A 36% (v/v) glycerol aqueous solution was used to simulate whole blood. It was prepared at room temperature [6]. The one-phase substitute fluid was intended to exhibit rheological properties comparable to human whole blood with a dynamic viscosity of $\eta = 4.70 \pm 0.30$ mPa·s in big arteries [7].

2.2 Multiphase blood substitutes

The multiphase blood substitutes consist of a glycerol water mixture (plasma) and hydrogel beads (artificial erythrocytes). The beads were produced with microfluidic systems using olive oil as the continuous phase and a hydrogel/polymer solution as dispersive phase (refer Fig. 2). Two different materials were selected for this study: the biological hydrogel agarose (0.3% w/w), which polymerizes at temperatures below 40°C, and the photopolymer NOA60, which undergoes UV-polymerization. The seeding particles (polyamide spericals, LaVision GmbH, diameter 20 µm, 0.2% w/w) were included in the beads by mixing them in the hydrogel/polymer solution before flowing in the microfluidic channel. The produced beads were separated from the oil phase via sedimentation. Thereby the oil with the beads was placed above the glycerol-water mixture, while the beads sank from the oil directly in the aqueous solution. The resulting blood substitute liquids were used for further measurements such as PIV or rheological investigations.

Spherical artificial erythrocytes

Silicone-based microfluidic devices were fabricated using the micro-wire casting method with Sylgard 184. The method involved casting round wires with a diameter of 400 µm with silicone, which were then eliminated after curing. Microfluidic systems with T-junctions were designed (refer Fig. 2). The study investigated the effect of varying flow rates (20 to 90 ml/h) of the continuous and disperse phases on the size and mono-dispersity of the agarose beads.

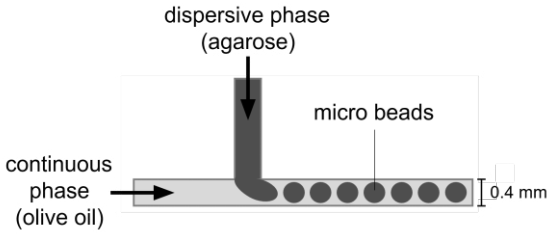

Fig. 2. Schematic representation of the microfluidics set up with agarose as disperse phase and olive oil as continuous phase.

In a further step, the durability and storage of the agarose beads in the 36% (v/v) glycerol-water mixture was analyzed. Weight measurements were taken intermittently within 48 hours and 25 days.

Disc-shaped artificial erythrocytes

The bead shape was influenced by a flat design of the microfluidic channels with a height of 0.5 mm and a width of 4 mm (refer Fig.3) [8]. The manufacturing method was casting with silicone (see above) using a cut aluminum sheet instead of a round wire.

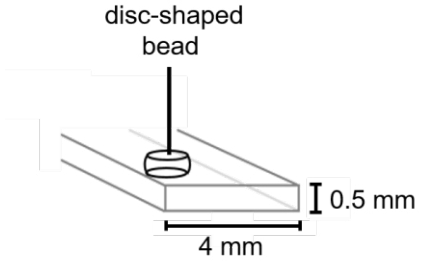

Fig. 3. Schematic representation of the microfluidic channel design to produce disc-shaped beads with channel width >> channel height (adapted to [8]).

Disc-shaped agarose and NOA60 beads were produced. The influence of the flow rates of continuous and disperse phases on the bead diameter was analyzed.

2.3 Optical velocity measurements

PIV measurements were performed with a Nd:YAG Laser (λ=532 nm), a CCD camera (LaVision GmbH) and an in-house manufactured flow channel including a silicone tube lying in a 5% (v/v) glycerol-water mixture for IOR matching. The multiphase blood substitute fluid with spherical agarose beads was passed through a 4 mm silicone tube.
In the second step, the shadow-imaging method was applied. This method differs from PIV in that the artificial erythrocytes are back-illuminated by a LED, which creates shadows on the camera images. These shadows were then tracked by a particle tracking velocimetry (PTV) algorithm to analyze the movement and behavior of the beads.

3. Results

3.1 Spherical artificial erythrocytes

The results confirmed successful integration of PIV seeding particles into the hydrogel beads, as observed in microscopic images. Nearly every bead contained at least one embedded PIV seeding particle. Fig. 4 illustrates examples of integrated particles.

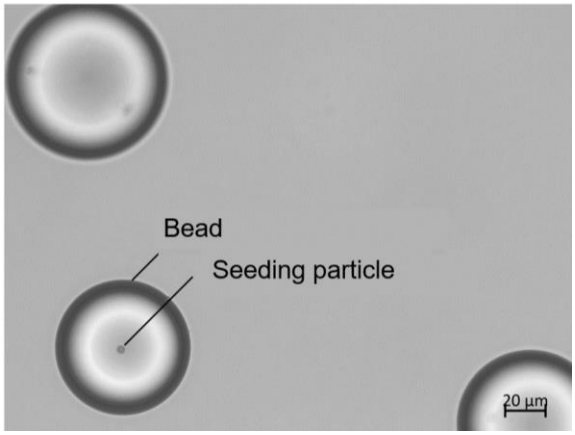

Fig. 4. Light microscopic image of spherical agarose beads including PIV seeding particles.

The experimental results show that the chosen flow rates had an impact on bead size and stability. Fig. 5 illustrates the influence of flow rate on bead diameters. Increasing the flow rate from 20 to 70 ml/h resulted in a continuous decrease in bead diameter from 45 ± 4 µm to 29 ± 2.8 µm. At a flow rate of 90 ml/h, the average bead diameter increased again, reaching 70 µm. This increase was attributed to leakage from the microfluidic systems.
The agarose beads remained intact for 24 hours when stored at room temperature. However, when consistently stored at 4°C, their durability and storage duration were extended to 24 days.

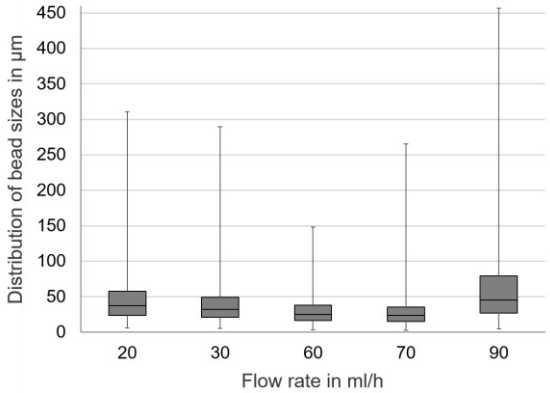

Fig.5. Influence of flow rate on bead diameter. With increasing flow rate from 20 to 70 ml/h, the bead diameter decreased and increased at 90 ml/h again. n=900, n=300 at 90 ml/h.

3.2 Disc-shaped artificial erythrocytes

Our experiments have shown that disc-shaped beads can be synthesized by using very flat and wide microfluidic channels. The agarose discs had a relatively large diameter of approximately 800 µm, compared to spherical beads with diameters of up to 29 µm. The majority of stable agarose discs were produced at a flow rate of 7.2 ml/h (cf. Fig. 6 top).

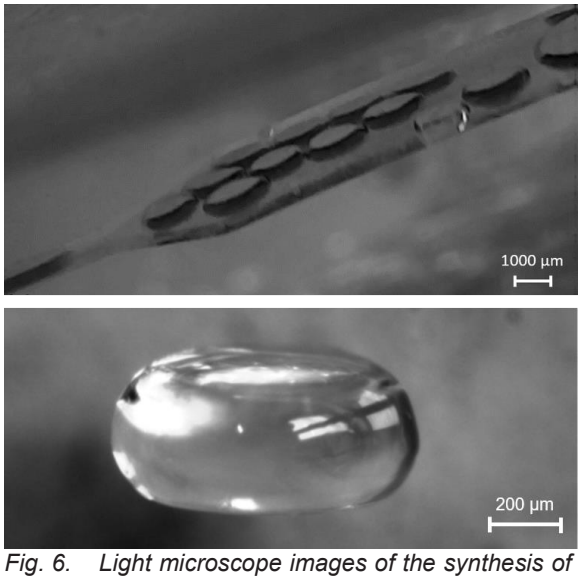

Fig. 6. Light microscope images of the synthesis of disc-shaped agarose beads in microfluidics (top) and a disc-shaped NOA60 bead (bottom).

The effect of flow rate on the proportion of stable NOA60 discs was examined. The results showed that the highest proportion of stable NOA60 discs was achieved at higher flow rates, ranging from 1.2 to 2.4 ml/h (cf. Fig. 6 bottom). The highest proportion 94% was observed at a flow rate of 2 ml/h with a corresponding diameter of 800 µm.

3.3 Optical velocity measurements

The velocity of the multiphase blood substitutes could not be measured via PIV, since the scattered light intensity was too low, due to strong absorption in the different media and very low particle size. Nevertheless, the agarose beads could be successfully tracked using shadow images. Images showed that the beads were formed into large agglomerates and could not be tracked individually as depicted in Fig. 7.

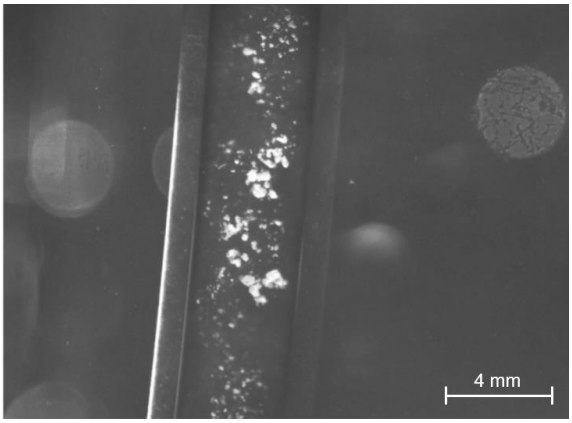

Fig. 7. Snapshot image of a silicone tube which is perfused with the blood substitute fluid (agarose beads and 36% (v/v) glycerol-water mixture).

4. Discussion

The optimization of bead size is a critical aspect of producing erythrocyte-like beads that mimic the dimensions of erythrocytes. In our study, we aimed to approximate the diameter of erythrocytes with 7.5 µm. Results showed that we could produce agarose beads of approx. 29 µm and NOA60 beads of approx. 800 µm. A reduction in bead diameter is essential for the flow behavior of the beads.

The use of biological hydrogels such as agarose showed a shorter storage time and thus a shorter usability of the blood substitute fluids than the use of the photopolymer NOA60. However, only very large bead diameters could be produced with the photopolymer. Synthetic hydrogels such as poly-2-hydroxyethyl methacrylate (pHEMA) or poly-sodium arcylate-co-arcylamide (pSAAm) could provide a promising solution.

The integration of PIV seeding particles into hydrogel beads was a significant advance in our research. In further experiments, staining the beads with fluorescent dyes may be another way to optically track the beads via PIV.

5. Conclusion

In summary, this study is a significant advancement in the development of multiphase blood substitute fluids designed for optical velocity measurement methods like PIV or PTV, specifically in the analysis of hemodynamics in intracranial aneurysms. By successfully synthesizing artificial plasma containing various erythrocyte-like beads, including spherical and disc-shaped agarose erythrocytes, and disc-shaped NOA60 erythrocytes, we have demonstrated the feasibility of mimicking multiphase blood properties for optical velocity measurements.

6. Acknowledgments

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