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In vitro blood flow model with physiological wall shear stress for hemocompatibility testing—An example of coronary stent testing

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Hemocompatibility of blood contacting medical devices has to be evaluated before their intended application. To assess hemocompatibility, blood flow models are often used and can either consist of in vivo animal models or in vitro blood flow models. Given the disadvantages of animal models, in vitro blood flow models are an attractive alternative. The in vitro blood flow models available nowadays mostly focus on generating continuous flow instead of generating a pulsatile flow with certain wall shear stress, which has shown to be more relevant in maintaining hemostasis. To address this issue, the authors introduce a blood flow model that is able to generate a pulsatile flow and wall shear stress resembling the physiological situation, which the authors have coined the “Haemobile.” The authors have validated the model by performing Doppler flow measurements to calculate velocity profiles and (wall) shear stress profiles. As an example, the authors evaluated the thrombogenicity of two drug eluting stents, one that was already on the market and one that was still under development. After identifying proper conditions resembling the wall shear stress in coronary arteries, the authors compared the stents with each other and often used reference materials. These experiments resulted in high contrast between hemocompatible and incompatible materials, showing the exceptional testing capabilities of the Haemobile. In conclusion, the authors have developed an in vitro blood flow model which is capable of mimicking physiological conditions of blood flow as close as possible. The model is convenient in use and is able to clearly discriminate between hemocompatible and incompatible materials, making it suitable for evaluating the hemocompatible properties of medical devices. © 2016 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/). [http://dx.doi.org/10.1116/1.4958979]

I. INTRODUCTION

Cardiovascular implants are used extensively in the industrialized nations where their inhabitants suffer from cardiovascular diseases. To treat these diseases, a plethora of medical devices has been conceived. However, in one way or the other, these devices have certain drawbacks that limit their use. This can either be their thrombogenicity, or their tendency to result in endothelial dysfunction, inflammation, or thrombocytopenia. Blood contacting devices can activate thrombocytes to adhere, form aggregates or secrete products, activate the coagulation cascade, the kallikrein cascade, the complement cascade, or trigger immunological responses. How and which mechanisms are being activated can depend on material characteristics such as surface roughness, functional groups, chemical potential, charge, or hydrophobicity.

Hemocompatibility of blood contacting medical devices has to be evaluated before their intended application. The international standard ISO-10993, for biological evaluation of medical devices (ISO 10993: Biological evaluation of medical devices—Part 4: Selection of tests for interactions with blood), can be used as a directive to evaluate these hemocompatible characteristics. To assess hemocompatibility, blood flow models are often used and can either consist of in vivo animal models or in vitro blood flow models. Given the disadvantages of animal models, such as higher costs, more variability, more time consuming, and insensitivity due to overwhelming short-term effects of tissue injury, in vitro blood flow models are more attractive. Moreover, blood composition between various species can vary considerably, which can lead to over- or underestimation of human blood reactions to biomaterials, as opposed to in vitro blood flow models that can be used with human blood. Nowadays, several in vitro blood flow models are often used, these being the peristaltic pump model and the Chandler model, or...
their derivatives. These models have a number of disadvantages, such as blood/air interfaces, limited flow, nonpulsatile flow, and higher hemolysis. Kolandaivelu and Edelman have developed a more advanced flow model, introducing pulsatile flow by rotating a test loop around its axis “in a prescribed and controlled fashion to modulate the inertial flow of the contained fluid through transmitted shear forces from the tubing wall, thereby generating relative flow.”

However, all these aforementioned models seem to be mainly developed with the focus on their ability to generate a certain flow rather than a certain wall shear stress, which has shown to be more relevant in maintaining hemostasis. To address this issue, we have amended the model of Kolandaivelu and Edelman, being able to generate a pulsatile flow resembling the physiological situation and to control the wall shear stress. We have coined our model the “Haemobile,” and in a previous report, the model was compared to the Chandler model and peristaltic pump model.

To further characterize the hemodynamics of the model, we measured various flow profiles, created by various settings of the Haemobile, and calculated the corresponding velocity profiles and (wall) shear stress profiles. As an example, we evaluated the thrombogenicity of two drug eluting stents, one that was already on the market and one that was still under development. After identifying proper conditions resembling the wall shear stress in coronary arteries, we compared the stents with each other and to two often used reference materials. These experiments resulted in high contrast between hemocompatible and incompatible materials, showing the exceptional testing capabilities of the Haemobile.

II. MATERIAL AND METHODS

A. Principal of the Haemobile

The principal of generating pulsatile flow inside a test loop is based on angular momentum. Blood inside a polyvinyl chloride test loop containing a unidirectional check valve [Fig. 1(b)] is exposed to an angular acceleration. The check valve functions as a heart valve, i.e., preventing backward flow of blood, and is also necessary to accelerate the blood as well as the test loop. Following acceleration, the test loop is decelerated again till a standstill. The angular momentum of the blood results in blood flowing relatively to the polyvinyl chloride test loop and is not hindered by the valve but only by shear forces. During this period of blood flow, the test loop is returned to its starting position. By repeating the process, a pulsatile flow is created.

B. System design of the Haemobile

The Haemobile consists of a metal enclosure, containing a stepper motor, stepper motor driver, microcontroller, liquid crystal display, and a rotary encoder with a pushbutton [Fig. 1(a)]. On top of the Haemobile, a round plateau was mounted to the axle of the stepper motor; this plateau facilitated fixation of the test loops.

Custom embedded software was developed to control the rotating motion of the plateau carrying the test loops. The algorithm of Atmel was used to calculate the delay between steps of the stepper motor; enabling acceleration/deceleration. The angular acceleration was set at a fixed value of 3600°/s²; this variable was fixed to prevent resonance of the system that occurred at certain accelerations. The angle of rotation and the maximum angular velocity could be adjusted within certain chosen limits, 0°–180° and 360°–1080°/s, respectively. The maximum angular velocity of the clockwise and anticlockwise motion could be adjusted separately. Additionally, a delay between motions (pulses) could be set.

Using the algorithm mentioned above, numerical simulations were performed to calculate the angle, angular velocity, and angular acceleration/deceleration in time.

C. Flow measurements

To validate the function of the device, a single test loop, consisting out of 45 cm medical grade polyvinyl chloride tubing with an internal diameter of 3 or 5 mm and a generic polypropylene ball-valve, was constructed. The test loop was filled with blood mimicking fluid enabling Doppler flow measurements by means of an external flow sensor at 40 samples per second (Transonic HT-110, Transonic Systems, Inc., Ithaca, NY, USA). The angle of rotation was set to 180°, and the angular velocity was varied between 360° and 1080°/s. At each setting, the flow was recorded in LabVIEW (National instruments, Austin, TX, USA) for a period of 20 s, typically capturing around ten motions.

D. Data processing

The raw signals of the velocity were postprocessed in MATLAB (MathWorks, Natick, MA, USA). The flow was recorded for 20 s, and waveforms were smoothed using a Savitzky-Golay filter, which reduced signal noise with minimal influence on peak magnitudes. Subsequently, ensemble averaging was performed to obtain representative velocity...
waveforms. The peaks of the recorded waveform were used to calculate the mean period for the superimposition of the individual cycles; this, in turn, enabled the computation of the ensemble average. From the ensemble average, parameters such as the period, frequency, flow per pulse (area under the curve), mean flow, and the maximum and minimum peak flow could be derived.

E. Velocity profile and shear stress computation

From the ensemble average blood flow, the velocity profiles and the (wall) shear stress were derived using the equations below, based on the work of Womersley. The mathematical derivations of the equations are shown in the Appendix. If a steady pulsatile flow of a viscous fluid in a rigid tube with circular cross-section is assumed, the equation for the velocity profile is given as

\[ u(r, t) = \text{Real} \left\{ \sum_{n=1}^{\infty} \frac{\hat{q}_n}{\pi R^2} \left( \frac{J_0 \left( \frac{a_n}{R} \right)}{J_0 \left( \frac{1}{a_n} \right)} \right) \left( 1 - \frac{2J_1 \left( \frac{a_n}{R} \right)}{\frac{1}{a_n} \left( \frac{1}{a_n} \right) J_0 \left( \frac{1}{a_n} \right) \left( \frac{1}{a_n} \right)} \right) e^{i \omega t} \right\}, \]

where \( r \) is the radial coordinate, \( R \) is the radius of the tube, \( a = R \sqrt{\omega/\nu} \) is the Womersley number, \( J_0 \) is a Bessel function of the first kind of zero order, \( J_1 \) is a Bessel function of the first kind of the first order, \( \omega = 2\pi f \) is the angular frequency, and \( \hat{q} \) are the complex Fourier coefficients of the recorded flow. The shear stress is given as

\[ \tau(r, t) = \text{Real} \left\{ \sum_{n=1}^{\infty} \frac{\mu \hat{q}_n}{\pi R^2} \left( \frac{J_0 \left( \frac{a_n}{R} \right)}{J_0 \left( \frac{1}{a_n} \right)} \right) \left( 1 - \frac{2J_1 \left( \frac{a_n}{R} \right)}{\frac{1}{a_n} \left( \frac{1}{a_n} \right) J_0 \left( \frac{1}{a_n} \right) \left( \frac{1}{a_n} \right)} \right) e^{i \omega t} \right\}. \]

The viscosity (\( \mu \)) and density (\( \rho \)) of the blood mimicking fluid were 3.78 mPa s and 1035 kg/m³, respectively. The wall shear stress is computed for \( r = R \).

F. Hemocompatibility testing

As an example of the hemocompatibility testing capabilities of the Haemobile, the thrombogenicity of two drug eluting coronary stents was evaluated: one predicate stent (in the new ISO 10993-4 standard the term “legally marketed reference device” is used) and a test stent that was still under development. Additionally, low density polyethylene (Goodfellow Cambridge, Ltd., Huntingdon, UK) and medical steel (stainless steel AISI 316L, Goodfellow Cambridge, Ltd., Huntingdon, UK) were tested and served as a low and high reference materials, respectively. Blood of three different donors was used, and the experiments were performed in duplicate per donor; this resulted in six test loops per type of stent or reference material. Venous blood was collected with a 19 gauge butterfly needle from healthy volunteers who received no medication within two weeks prior to blood withdrawal. A whole blood sample (60 ml) was taken using a clinical dose of heparin (1.5 IU/ml, Leo Pharmaceutical Products BV, Weesp, the Netherlands) as anticoagulant. Typical blood activation values immediately after blood withdrawal are given in Table I.

| Variable             | Baseline          | Empty PVC loop (after 1 h at 37°C) |
|----------------------|-------------------|-----------------------------------|
| PLT (10^9/l)         | 251 ± 64          | 189 ± 49                          |
| TXB2 (ng/ml)         | 0.97 ± 1.44       | 26.4 ± 13.0                       |
| C5b-9 (ng/ml)        | 13.7 ± 5.65       | 53.2 ± 47.9                       |
| Elastase (ng/ml)     | 1.95 ± 1.16       | 2.95 ± 1.32                       |
| TAT III (ng/ml)      | 1.35 ± 0.84       | 8.99 ± 8.63                       |
| Free Hb (%)          | 0.10 ± 0.03       | 0.25 ± 0.14                       |

Stents were deployed into medical grade polyvinyl chloride tubing (Nalgene Nunc International Corporation, Rochester, NY, USA) with an internal diameter of 3 mm [Fig. 1(c)]. A typical test loop contained approximately 3 ml of blood. The reference materials, low density polyethylene and medical steel, were cut into 3 × 20 mm segments with a thickness of 0.3 mm and were positioned in separate test loops. The test loops were filled within 20 min from blood withdrawal and circulation was performed for 1 h at 37°C by placing the Haemobile with the test loops in an incubator. The Haemobile was set at an angle of rotation of 180°, a clockwise angular velocity of 720°/s, an anticlockwise angular velocity of 360°/s, an angular acceleration/deceleration of 3600°/s² (fixed setting), and there was no delay between motions. This resulted in a flow profile with a mean flow of 19.4 ml/min and a mean wall shear stress of 0.50 Pa (5 dyn/cm²), corresponding to a typical mean wall shear stress of 0.68 Pa (6.8 dyn/cm²), as observed in coronary arteries.

Following incubation, blood was collected and the materials were gently washed with Tris buffered saline, photographed, and cut into three pieces per sample. One piece was used to quantify platelet adhesion on the washed materials. Platelet adhesion onto the surface of the test materials was measured by means of a colorimetric assay, based on the presence of acid phosphatase in platelets. Briefly, a piece of material was submerged in citrate buffer, containing p-nitrophenyl phosphatase and Triton X-100. Substrate conversion was proportional to the amount of adhered platelets. The optical density of 100 µl substrate was used to compare stents and reference materials with each other.

The second piece of material was used to quantify the amount of fibrin bound to the surface. The samples were submerged in a solution containing fibrin specific horseradish peroxidase-labeled antibodies (Murine MAb against fibrin
neotope beta-chain IgG, Sekisui Diagnostics LLC, Lexington, MA, USA) and were incubated for 1 h at room temperature. Afterward, the samples were rinsed with Tris buffered saline and incubated with O-phenylenediamine substrate solution. Substrate conversion was proportional to the amount of adhered fibrin. The optical density of 100 µl substrate was used to compare stents and reference materials with each other.

The third piece was immediately fixated in 2% glutaraldehyde in cacodylate buffer for visualization by means of scanning electron microscopy. Samples were dried in a series of alcohol with two final steps containing tetramethylsilane. Thereafter, samples were gold/palladium sputtered and visualized using a tabletop scanning electron microscope (Phenom-World BV, Eindhoven, the Netherlands).

III. RESULTS

A. Mechanical validation

System design was first evaluated by running a numerical calculation of the control algorithm of the stepper motor. Figures 2(a)–2(c) show the angle of rotation, angular velocity, and angular acceleration of the plateau of the Haemobile in time. The ensemble average of the corresponding flow measurement of a test loop exposed to this motion is also

![Fig. 2. Mechanical validation of the Haemobile. On the left hand side, simulation of one repetition of the Haemobile with the following settings: angle of rotation = 180°; clockwise angular velocity = 720°/s; anticlockwise angular velocity = 360°/s; angular acceleration/deceleration = 3600°/s² (fixed setting). (a) Angle vs time, (b) angular velocity vs time, and (c) angular acceleration/deceleration vs time. On the right hand side, ensemble average of the flow measurement and calculation of the velocity profile and shear stress of a test loop with an internal diameter of 3 mm and with the same settings as mentioned above. (d) Ensemble average of the measured flow (solid blue line) and typical coronary blood flow (dashed red line) (Ref. 23), (e) velocity across the diameter in time, and (f) shear stress across the diameter in time.](image)
shown [Fig. 2(d)]. From this measurement, the pulsatile nature of the flow is evident. Using the theory of Womersley, the three dimensional velocity profile [Fig. 2(e)] and the (wall) shear stress [Fig. 2(f)] were calculated.

**B. Flow measurements**

The angular velocity was varied from 360°/s to 1080°/s in steps of 90°/s. For each setting of the angular velocity, a flow recording was captured and the three dimensional velocity profile and (wall) shear stress were calculated. This was performed for two types of test loops with either an internal diameter of 3 or 5 mm. Figure 3(a) shows the influence of the angular velocity of the Haemobile on the flow, increasing the angular velocity resulted in increased flow. Similarly, the mean wall shear stress also increased with increasing angular velocity [Fig. 3(b)]. Finally, Fig. 3(c) demonstrates the major influence of the cross sectional area of the test loop on the wall shear stress, as the 3 mm diameter test loop shows larger mean wall shear stress values than the 5 mm diameter test loop.

**C. Hemocompatibility testing**

As an example of the hemocompatibility testing capabilities of the Haemobile, the thrombogenicity of two drug eluting coronary stents was evaluated and compared to two reference materials. Following the circulation experiment, visual inspection of the test materials already revealed large differences, where the test stent and medical steel portrayed clear thrombus formation, and the predicate stent and low density polyethylene were almost “clean” (Fig. 4). Similarly, the number of adhered platelets was larger to the test stent as compared to the predicate stent [Fig. 5(a)]. Platelet adhesion to the predicate stent was comparable to the low reference material, low density polyethylene. Fibrin binding showed similar differences between materials; however, the test stent was even higher than the positive reference material, medical steel [Fig. 5(b)]. Scanning electron microscopy supported the macroscopic and biochemical findings, the test stent and medical steel were covered by extensive thrombus formation, whereas the predicate stent and low density polyethylene showed almost no thrombus formation (Fig. 6).

![Fig. 3. Influence of test loop internal diameter on flow and wall shear stress. (a) Mean flow as a result of the programmed angular velocity. (b) Calculated mean wall shear stress as a result of the programmed angular velocity. (c) Correlation between flow and wall shear stress. Closed and open circles represent an internal diameter of 3 and 5 mm, respectively.](image1)

![Fig. 4. Macroscopic images of tested samples after incubation in Haemobile test loops. (a) Predicate stent. (b) Test stent. (c) Negative reference material, low density polyethylene. (d) Positive reference material, medical steel.](image2)
IV. DISCUSSION

In this study, we have presented our method for evaluating in vitro hemocompatibility. The main innovations of our model are pulsatile flow and controlled (wall) shear stress. The system has a low background for thrombosis and can clearly discriminate between known positive and negative reference materials. Besides, we have shown its usability for hemocompatibility testing by comparing two differently coated drug eluting stents.

The handling and filling of the test loops is very convenient, as the syringe used for blood withdrawal can directly be connected to one side of the unidirectional check valve. In this way, up to 40 test loops can be filled within 20 min from blood withdrawal, complying with the recommendation of using fresh blood for in vitro testing. Choosing tubing with a different diameter for the test loop enables the assessment of medical devices with varying diameters. The limited volume of blood necessary for filling a single test loop enables the use of paired test samples, i.e., using blood from the same donor for test and control samples. This, in turn, minimizes the variance between donors, increasing contrast between hemocompatible and -incompatible materials and thus increasing the reliability of hemocompatibility assessments.

The holy grail of hemocompatibility research remains the creation of a completely hemocompatible surface. To mimic the endothelial lining of the human vasculature may, however, be impossible. Yet, the need for testing the hemocompatible properties of newly developed devices remains. When these properties are evaluated in vitro, the Chandler loop is most often used; however, it has two major drawbacks. First, the air/liquid interface leads to protein denaturation and platelet aggregation. And second, the model does not offer pulsatile flow, and moreover, the flow is limited to a point where the air bubble will circulate with the tubing, while the wall shear stress does not approach physiological values.

Shear induced platelet adhesion and aggregation to collagen occurs within 2 s, where half the number of reacting platelets adheres within 240 ms. Furthermore, platelet adhesion is dependent on the amount of shear, for instance, increasing the shear rate from 50 to 500 s⁻¹ increased platelet adhesion to polyethylene by about eightfold. This shows the importance of shear stress for efficient hemostasis under flow conditions.

![Figure 5](image1)

**Fig. 5.** Platelet adhesion and fibrin binding after incubation in Haemobile test loops. Data are presented as mean ± standard deviation. (a) Platelet adhesion assessed by platelet acid phosphatase acid activity. (b) Fibrin binding assessed by fibrin antibody binding. MS, medical steel; LDPE, low density polyethylene. The Student’s t-test was used to calculate probability values.

![Figure 6](image2)

**Fig. 6.** Scanning electron microscopy images of tested samples after incubation in Haemobile test loops. Magnification 600×. (a) Predicate stent with a clean surface, the white spot in the middle shows a scratch in the surface coating. (b) Test stent with extensive thrombus formation covering the entire surface of the stent. (c) Low density polyethylene showing no blood components on its surface. (d) Medical steel showing extensive thrombus formation.
and in contact of blood with medical devices. Moreover, this emphasizes the need for a test setup which can mimic pulsatile flow, where peak flow, peak shear rate, and peak wall shear stress can determine platelet response, and subsequently, the hemocompatibility test results.

Besides the presentation of our test model, we have shown an example of testing medical devices. In our example, we have tested the thrombogenicity of two drug eluting coronary stents. The test stent was compared to a predicate stent and to positive and negative reference materials. It is clear that the test system has a low background for thrombotic elements nor does it possess a protective endothelial covering. It is compatible in our experience, it cannot secrete potent antithrombotic components nor does it possess a protective endothelial covering. In this frame of reference, it becomes possible to interpret the results of the test stent under development and the predicate stent, where the results were clearly in favor of the predicate stent.

Besides thrombogenicity assays, our model can also be used for other regularly employed assays, as prescribed by the ISO10993-4 standard. These assays evaluate platelet activation by measuring release products in the circulated blood, coagulation markers in the circulated blood, complement activation markers in the circulated blood, a blood count, and finally the degree of hemolysis. Typical blood activation values immediately after blood withdrawal and after 1 h of circulation in an empty control loop are given in Table 1.

| Parameter          | Value |
|--------------------|-------|
| Blood withdrawn    |       |
| After 1 h          |       |

That the endothelial lining of the human vasculature may be impossible to mimic is also valid for our test loops, which consist of polyvinyl chloride. Though being very hemocompatible in our experience, it cannot secrete potent antithrombotic components nor does it possess a protective endothelial glyocalyx that contains proteins that mediate in coagulation, fibrinolysis, and hemostasis.22

In conclusion, we have developed an in vitro blood flow model which is capable of mimicking physiological conditions of blood flow as close as possible. This model is able to clearly discriminate between hemocompatible and incompatible materials, making it suitable for evaluating the hemocompatible properties of medical devices.

**APPENDIX**

When considering a steady pulsatile flow of a viscous fluid in a rigid tube with circular cross-section with radius r, the Navier-Stokes equations in cylindrical coordinates can be reduced to

\[ \rho \frac{D u}{D t} = -\frac{\partial p}{\partial z} + \mu \left( \frac{\partial^2 u}{\partial r^2} + \frac{1}{r} \frac{\partial u}{\partial r} \right). \] (A1)

With \( \rho \) being the density of the fluid, \( \frac{\partial p}{\partial z} \) being the pressure gradient in the longitudinal direction of the tube, \( \mu \) being the viscosity of blood, and \( u \) being the velocity of the fluid at distance \( r \) from the centerline in the longitudinal direction, \( z \). Since the generated blood flow in the Haemobile is harmonic, we can write

\[ u(r, t) = \text{Real} \left( \sum_{i=0}^{\infty} \hat{u}(r) e^{i\omega t} \right), \] (A2)

\[ p(z, t) = \text{Real} \left( \sum_{i=0}^{\infty} \hat{p}(z) e^{i\omega t} \right), \] (A3)

where \( \omega = 2\pi f \) is the angular frequency with \( f \) being the frequency in Hz. Substituting Eq. (A2) into Eq. (A1) and rearranging the equation results in

\[ \frac{\partial^2 \hat{u}}{\partial r^2} + \frac{1}{r} \frac{\partial \hat{u}}{\partial r} - \frac{i\omega}{\nu} \hat{u} = -\frac{\partial \hat{p}}{\partial z}, \] (A4)

where the kinematic viscosity is \( \nu = \mu/\rho \). The velocity profile was first obtained by Womersley.14

\[ \hat{u}(r) = \frac{1}{i\omega \rho} \left( \frac{-\partial \hat{p}}{\partial z} \right) \left( 1 - \frac{J_0 \left( \frac{\pi R^3}{\nu^3} \right)}{J_0 \left( \frac{\pi R^3}{\omega^3} \right)} \right), \] (A5)

Solving the integral

\[ \hat{q} = \int_0^R \hat{u} 2\pi r dr = \int_0^\pi \frac{2\pi r}{i\omega \rho} \left( \frac{-\partial \hat{p}}{\partial z} \right) \left( 1 - \frac{2J_1 \left( \frac{\pi R^3}{\nu^3} \right)}{\pi R^3 J_0 \left( \frac{\pi R^3}{\omega^3} \right)} \right) dr, \] (A6)

where \( J_1 \) is a Bessel function of the first kind and first order. Rearranging the equation

\[ \left( \frac{-\partial \hat{p}}{\partial z} \right) = \frac{i\omega \rho \hat{q}}{\pi R^2 \left( 1 - \frac{2J_1 \left( \frac{\pi R^3}{\nu^3} \right)}{\pi R^3 J_0 \left( \frac{\pi R^3}{\omega^3} \right)} \right)}. \] (A7)

Substituting Eq. (A7) into Eq. (A4) yields

\[ \hat{u}(r, \omega) = \hat{q} \left( \frac{J_0 \left( \frac{\pi R^3}{\nu^3} \right)}{J_0 \left( \frac{\pi R^3}{\omega^3} \right)} \right) \left( 1 - \frac{2J_1 \left( \frac{\pi R^3}{\nu^3} \right)}{\pi R^3 J_0 \left( \frac{\pi R^3}{\omega^3} \right)} \right). \] (A8)
where $\hat{q}$ are the complex Fourier coefficients of the recorded flow. Finally, taking the real part of this complex function gives the velocity

$$u(r, t) = \text{Real} \left( \sum_{a=1}^{\infty} \frac{\hat{q}}{\pi R^2} \left( \frac{J_0 \left( \frac{r}{R} \right)^{3/2}}{1 - J_0(\alpha i^{3/2})} - \frac{2J_1(\alpha i^{3/2})}{\alpha i^{3/2}J_0(\alpha i^{3/2})} \right) \right).$$

(A9)

The shear stress is defined as

$$\tau = -\mu \frac{\partial u}{\partial r}.$$  

(A10)

Substituting Eq. (A8) into Eq. (A10)

$$\dot{\tau}(r, \omega) = -\frac{\mu \hat{q}}{\pi R^2} \left( \frac{J_0 \left( \frac{r}{R} \right)^{3/2}}{1 - J_0(\alpha i^{3/2})} - \frac{2J_1(\alpha i^{3/2})}{\alpha i^{3/2}J_0(\alpha i^{3/2})} \right).$$  

(A11)

Finally

$$\tau(r, t) = \text{Real} \left( \sum_{a=1}^{\infty} -\frac{\mu \hat{q}}{\pi R^2} \left( \frac{J_0 \left( \frac{r}{R} \right)^{3/2}}{1 - J_0(\alpha i^{3/2})} - \frac{2J_1(\alpha i^{3/2})}{\alpha i^{3/2}J_0(\alpha i^{3/2})} \right) e^{i \omega t} \right).$$  

(A12)

where $\hat{q}$ are the complex Fourier coefficients of the recorded flow. Finally, taking the real part of this complex function gives the velocity

$$u(r, t) = \text{Real} \left( \sum_{a=1}^{\infty} \frac{\hat{q}}{\pi R^2} \left( \frac{J_0 \left( \frac{r}{R} \right)^{3/2}}{1 - J_0(\alpha i^{3/2})} - \frac{2J_1(\alpha i^{3/2})}{\alpha i^{3/2}J_0(\alpha i^{3/2})} \right) e^{i \omega t} \right).$$  

(A9)

The shear stress is defined as

$$\tau = -\mu \frac{\partial u}{\partial r}.$$  

(A10)

Substituting Eq. (A8) into Eq. (A10)

$$\dot{\tau}(r, \omega) = -\frac{\mu \hat{q}}{\pi R^2} \left( \frac{J_0 \left( \frac{r}{R} \right)^{3/2}}{1 - J_0(\alpha i^{3/2})} - \frac{2J_1(\alpha i^{3/2})}{\alpha i^{3/2}J_0(\alpha i^{3/2})} \right).$$  

(A11)

Finally

$$\tau(r, t) = \text{Real} \left( \sum_{a=1}^{\infty} -\frac{\mu \hat{q}}{\pi R^2} \left( \frac{J_0 \left( \frac{r}{R} \right)^{3/2}}{1 - J_0(\alpha i^{3/2})} - \frac{2J_1(\alpha i^{3/2})}{\alpha i^{3/2}J_0(\alpha i^{3/2})} \right) e^{i \omega t} \right).$$  

(A12)