

Ghost Cells for Mechanical Circulatory Support In Vitro Testing: A Novel Large Volume Production

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The aim of this work is to establish a large volume batch production system to produce sufficient volumes of ghost cells to facilitate hemolysis testing of mechanical circulatory support devices. A volume of more than 405 mL with a hematocrit of at least 28% is required to perform in vitro hemolysis testing of mechanical circulatory support devices according to international standards. The established ghost cell production method performed at the institute is limited to 3.1 mL of concentrated cells, that is, cells with 100% hematocrit, due to predominantly manual process steps. Through semi-automation of the existing method by using the large volume batch production system, productivity is increased 60-fold to 188 mL while almost doubling process efficiency to 23.5%. Time-consuming manual work such as pipetting is now supported by sensor-based process engineering. With the help of the large volume batch production system, the objective of producing large quantities of ghost cells is successfully achieved. Thus, this work lays the foundation for spatially resolved hemolysis evaluation of mechanical circulatory support devices in combination with the small-scale fluorescent hemolysis detection method.

1. Introduction

Remaining challenges in the development of blood-circulating devices, such as mechanical circulatory support (MCS) devices including ventricular assist devices, oxygenators, and heart valve

prostheses, are the minimization of flow-induced hemolysis and thrombus formation. Detailed knowledge of spatially resolved blood flow velocities within these devices is crucial for patient safety during clinical applications and constitutes a cost-saving potential during device development.

So far, the most common method used to evaluate the hemolytic potential of MCS devices is in vitro hemolysis testing. The amount of free plasma hemoglobin is usually assessed by manual, discontinuous blood sample photometry during the in vitro test. This method lacks spatial resolution within the investigated device, since hemolysis is determined as an averaged value for the entire test circuit. Additionally, hemolysis can be computed using numerical in silico calculations; however, these simulation models lack validation. In order to validate the numerical models directly, data from in vitro tests is mandatory.^[1]

Apart from hemolysis evaluation, the visualization of blood flow velocities is of

great interest. Different methods that have been used so far to visualize blood flow velocities include double slit photometry,^[2–5] laser doppler anemometry,^[6,7] and particle image velocimetry (PIV).^[8–11] In particular, PIV has become the most commonly used method, due to its accuracy and the ability to resolve the instantaneous flow field in a complete plane. In combination with blood, however, the penetration depth of the PIV laser is severely limited by the opaqueness of whole blood, making measurements on device level impossible. Ghost cells (GCs) may provide a solution to these problems.

GCs are red blood cells (RBCs) with less intracellular hemoglobin, which increases their transparency. Modifying the RBCs of whole blood to transform them into GCs results in an almost ideal blood substitute as the suspension becomes transparent while it retains its initial blood-like properties (e.g. the non-Newtonian behavior).^[12–14] Recently, the so-called fluorescent hemolysis detection method (FHDM), which is mainly based on PIV was tested in a proof-of-concept.^[15] By replacing the majority of the hemoglobin with a dicitratocalcat(II)-complex $[\text{Ca}(\text{C}_6\text{H}_5\text{O}_7)_2]^{4-}$ and suspending an extracellular fluorescent calcium indicator in the artificial plasma, the feasibility of spatially resolved hemolysis detection was demonstrated. No fluorescent signal can be detected as long as an intact membrane prohibits

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DOI: 10.1002/biot.201900239

the binding of the indicator and calcium. Once the membrane tears, the binding takes place, resulting in a detectable fluorescent signal.

FHDM in combination with GCs promises a significantly increased measuring depth compared to that of PIV, thus enabling spatially resolved hemolysis detection while testing MCS devices in vitro and is therefore of great interest. However, a comparably large volume of GC suspension (>405 mL) is necessary for MCS device testing performed in accordance with the American Society for Testing and Materials (ASTM) F1841-97 (2017) standard. So far, only small quantities of GCs were produced in time-consuming procedures and were mainly used to study protein or membrane properties on a small scale.^[16] Thus, there is an increasing interest in effective large-scale GC production.

One of the core problems in the production process is the separation of cells and surrounding liquid medium. A large volume of hypotonic medium compared to the initial RBC volume is necessary to reduce the intracellular hemoglobin concentration efficiently. The removed hemoglobin from the cell interior leads directly to higher optical GC transparency. After a first lysis, GC transparency increases 28 times, after the second 52 times, and after the third 58 times compared to that of RBCs, thus providing the basis for PIV measurements.^[14]

Currently, there exist two methods for extracting the GCs from the large volume of hypotonic medium. A method that uses a hemocompatible ceramic cross-flow filter to retain the cells and allow excess fluid to pass through.^[14] One disadvantage of this method is filter clotting. The more common method for extracting cells is based on centrifugation. However, the main issue of centrifugation is the extensive manual effort required to remove the supernatant after each run, leading to only small quantities of GCs per cycle.

As both available production methods lack efficiency, the focus of this study was on establishing an alternative production process that is able to generate the volume of GCs required according to the ASTM F1841-97 (2017) standard in a reasonable amount of time while maintaining the physiological properties of blood.

2. Experimental Section

The GC production process presented in this study is based on methods for GC production that have been described previously.^[14,17,18] In these procedures, porcine RBCs (Figure 1A) are suspended in a hypotonic hemolysis solution (HS) for osmotic swelling (Figure 1B,C). During this controlled lysis, hemoglobin leaves the cells (Figure 1C). The osmotic equilibrium is restored by adding a hypertonic reversal solution (RS) (Figure 1D). In order to increase transparency, the controlled lysis can be repeated several times. Finally, the GCs are resealed in a heating chamber to achieve impermeability again (Figure 1E).

The HS is a buffer solution consisting of potassium dihydrogen phosphate (KH_2PO_4 , Merck, Germany) and disodium hydrogen phosphate dihydrate (Na_2HPO_4 , Roth, Germany). Whereas KH_2PO_4 acts as an acid, Na_2HPO_4 acts as a base. Calibrated on a pH-value of 6.8 at 0 °C and 40 mOsmol L^{-1} osmolarity, concentrations of $c(\text{KH}_2\text{PO}_4) = 1.3375 \text{ g L}^{-1}$ and

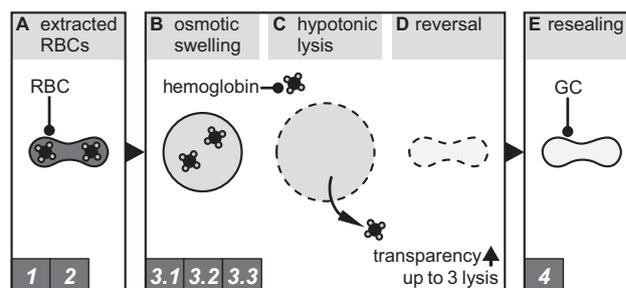


Figure 1. Schematic depiction of the transformation process from A) red blood cell (RBC) to E) ghost cell (GC). Steps B) to D) could be repeated up to three times to increase the cells' transparency. In the lower left corner, the corresponding numbers of the process steps are displayed for classification into the entire process shown in Figure 2.

$c(\text{Na}_2\text{HPO}_4) = 1.2071 \text{ g L}^{-1}$ solved in distilled water were needed, as adapted from refs. [14,18–20].

Since plasma osmolarity differs between humans and different pig races, the isotonic surrounding was adapted to pigs at 340 mOsmol L^{-1} . The RS restored this isotonic surrounding after hypotonic lysis. Due to the mixing ratio, an osmolarity of 1660 mOsmol L^{-1} was required for the RS to restore the osmotic balance. Therefore, 48.5052 g L^{-1} of NaCl was solved in distilled water. An isotonic saline solution, which was also adapted to porcine plasma osmolarity was used as washing solution (WS). For this purpose, 9.9348 g L^{-1} NaCl (VWR Chemicals, Belgium) was solved in distilled water.

The process steps of the large volume batch production system (LVBPS) are schematically depicted in Figure 2. Numbers 1–4 correspond to the steps of general GC production shown in Figure 1. Steps 5–7 describe additional steps for blood substitute production, including the isotonic WS.

To allow for a large volume GC production, process steps 2–7 were semi-automatized by means of the modules described in the following (Figure 3).

The LVBPS is based on a commercial high-volume centrifuge (Sorvall BIOS 16, Thermo Electron LED GmbH, Germany) with a capacity of 15528 mL in maximum, divided into eight single containers.

A suction unit removed the supernatant to extract the cells and automated steps 2, 3.3, 5, and 6. The unit consisted of eight arms, which carried a pipe each. The pipe was concentric to the container's neck and a conically pointed cylinder was attached to the underside, which dipped into the concentrated cells without stirring them up. A purge unit allowed for simultaneous filling of all eight centrifuge containers with either HS, RS, or WS for the lysis process; the associated reversal; and the final cell wash in steps 3.1, 3.2, or 6, respectively.

The sensor unit included high-resolution color and transparency sensors (based on TCS34725FN, ams AG, Austria) and allowed for the identification of differences between RBCs, blood plasma, GCs, and eluted hemoglobin in the supernatant, which was essential for steps 2, 3.3, 5, and 6 during cell extraction.

The valve unit consisted of eight hose pinch valves that functioned as actuators operating on each centrifuge container individually during emptying and filling in steps 2, 3.3, 5, and 6. The supernatant was pumped out until the sensor unit detects RBCs or GCs. The sensor was calibrated to a transparency threshold

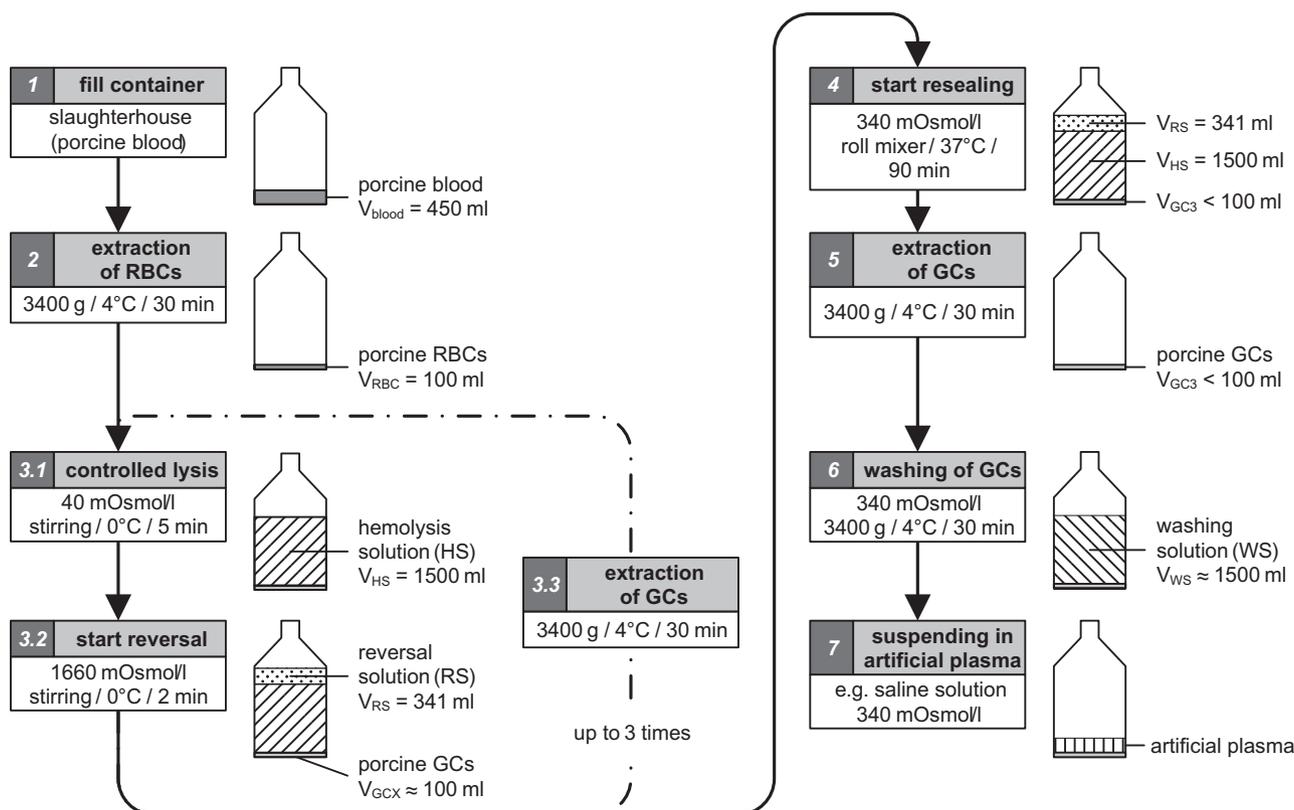


Figure 2. Flow chart of the production process with the main parameters of each step. The corresponding number of the steps are displayed in the lower left corner of Figure 1, which is decisive for classification into the particular category of the process, namely the transformation of red blood cells (RBCs) to ghost cells (GCs), by using the following abbreviations in the figure: GCx, ghost cells with x numbers of lysis; HS, hemolysis solution; RS, reversal solution; WS, washing solution.

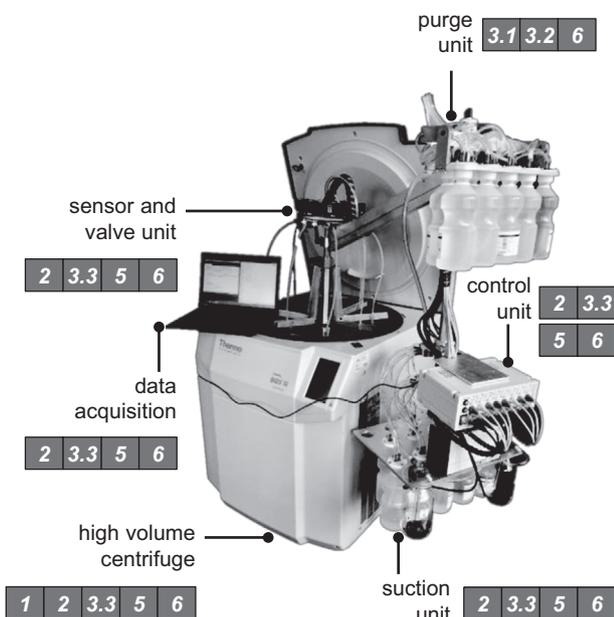


Figure 3. The large volume batch production system with the developed modules; numbers refer to the process steps.

value via pre-tests. As soon as cells passed the hose, the measured transparency decreased. Once the value dropped below the threshold, the valve received a closing-signal.

A control unit evaluated the sensor data and triggered the operation of the hose pinch valves during steps 2, 3.3, 5, and 6. The decisive difference between RBCs with reduced intracellular hemoglobin and the liquid supernatant with eluted hemoglobin lies in the transparency of solid cells compared to the liquid phase. Hence, transparency-sensitive sensors were used to measure the color and transparency change in the fluid inside the tubes and detect the phase separation between supernatant and cells. The supernatant was pumped out through this tube by the suction unit and the hose pinch valves closed as soon as RBCs or GCs were detected in the flow, thus effectively separating supernatant and cells. The values of color and transparency variation measured by the sensors, coded with the current time, were recorded on the SD-card for later evaluation and process control.

The whole system was mounted on a wheeled stand for easy manual positioning in the centrifuge after each centrifugation cycle. The sensor and the valve unit were attached to a height-adjustable cantilever and constantly held in a horizontal position with parallel motion kinematics. As a counterbalance, the suction unit and the vacuum pump were both mounted on the opposite side of the cantilever.

In the following section, individual process steps adapted to the large volume production are discussed in more detail (compare Figure 2).

2.1. Steps 1 and 2

The first two steps correspond to the general protocol for small volume GC production. Porcine blood was collected from the cervical artery of slaughterhouse animals and immediately anticoagulated with 3.8% sodium citrate (Eifelfango GmbH & Co. KG, Germany) in a 1:9 ratio of sodium citrate to blood. Following gentle mixing, blood was filtrated through a nylon tissue and filled into the centrifuge containers (Figure 2, step 1). After centrifugation at $3400 \times g$ and 4°C for 30 min, the newly designed suction unit removed the supernatant including the buffy coat containing white blood cells and platelets (Figure 3, step 2).

2.2. Steps 3.1, 3.2, and 3.3

After precooling the centrifuge to 4°C , each container was filled with HS by the purge unit to start osmotic swelling. The ratio of the remaining volume of concentrated RBCs to HS in step 3.1 and GCs to HS in steps 3.2 and 3.3 was 1:15. To achieve homogenous mixing, the containers in the centrifuge were rotated around their bearing axis for 5 min. The RS pre-cooled to 4°C was then added by the purge unit to restore the osmotic equilibrium in a 3:10 ratio of RBC to RS volume while container rotation was ongoing (Figure 2, step 3.2). Subsequently, GCs were centrifuged with the same parameters as in step 2 and the supernatant was removed by the suction unit once again (Figure 3, step 3.3). To maximize cell transparency, controlled lysis from steps 3.1 to 3.3 was repeated up to three times. Typical dimensions of fluid-conducting cavities in MCS devices were about 10 mm in diameter and sufficient transparency was achieved after three lyses, as described in ref. [14].

2.3. Steps 4 to 7

In the fourth step, the cells were resealed in a heating chamber for at least 90 min at 37°C under continuous rotation. In the next step, GCs were centrifuged with the same parameters as in step 2 and the supernatant was removed by using the suction unit. In step 6, the extracted GCs were flushed with WS, followed by centrifugation and removal of the supernatant from the containers. In the last step, cells were suspended in saline as artificial plasma, whereby the desired hematocrit value could be adjusted.

2.4. Transparency and Rheology

After each lysis, the transparency of the GCs was measured three times per hematocrit ($n = 3$) in a photometer (Ultrospec 2100, Biochrom US; Holliston, MA, USA). As a reference sample, distilled water was measured in a same type of cuvette.

The dynamic viscosity was measured for GCs after one lysis with a tempered cone plate rheometer (Anton Paar MCR502 Cone: CP60-1/TG with Plate: C-PTD200; Anton Paar GmbH, Graz, Austria). The cones' diameter was 59.983 mm, the angle was 0.981° , and the gap size was 0.121 mm. The calculated sample volume is 0.974 mL. To determine the dynamic viscosity, 20 measuring points were recorded in logarithmic subdivisions

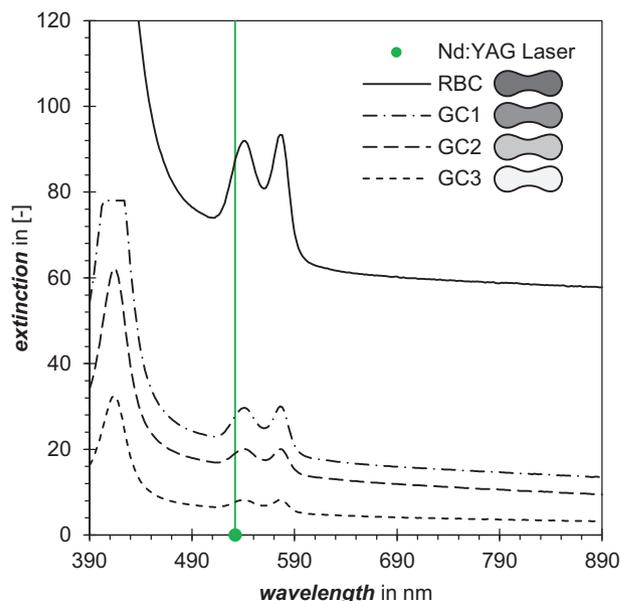


Figure 4. The graphs depict the extinction of light due to washed red blood cells (RBCs), single- (GC1), double- (GC2), and triple-lysed ghost cells (GC3) suspended in washing solution with a hematocrit of 30% ($n = 3$).

from 5 s^{-1} to 2590 s^{-1} of each suspension. The measurement was performed three times per cell type ($n = 3$). Single-lysed GCs were compared with RBCs suspended in saline solution. The hematocrit was adjusted in a range between 40.0% and 41.9%. The temperature during the measurement was controlled to 37°C . The viscosity measurements of the GCs produced using the established method were also performed with a cone plate rheometer (Physica MCR301, AntonPaar GmbH, Austria). The diameter of this measuring system was 49.945 mm, the angle 0.467° , and the gap size 0.054 mm. The sample volume amounted to 0.420 mL.

3. Results

The higher transparency of the GCs compared to RBCs is the basis of the new fluorescent hemolysis detection method (FHDM). **Figure 4** depicts the light extinction due to RBCs suspended in saline solution as compared to single-, double-, and triple-lysed GCs suspended in WS over the spectrum of visible light. Since a hematocrit between 28% and 32% is required for standard hemolysis tests, **Figure 4** provides extinction at a set hematocrit of 30%. Averaged over all measured wavelengths, the single-lysed GCs are 3.650 ± 0.403 (mean \pm standard deviation) times as transparent, double-lysed GCs 5.026 ± 0.564 times as transparent, and triple-lysed GCs 14.020 ± 2.384 times as transparent as RBCs. The vertical green line marks the wavelength of the PIV laser at 532 nm, which is used for the FHDM method. While single-lysed GCs at exactly this wavelength are 3.148 times as transparent as RBCs, the transparency is increased by a further lysis by 4.585 times and by a third lysis by 11.364 times compared to RBCs.

In **Figure 5**, the dynamic viscosity of single-lysed GCs is compared to that of RBCs and plotted above the shear rate. **Figure 5B** shows the x -axis in logarithmic scaling for shear rates smaller

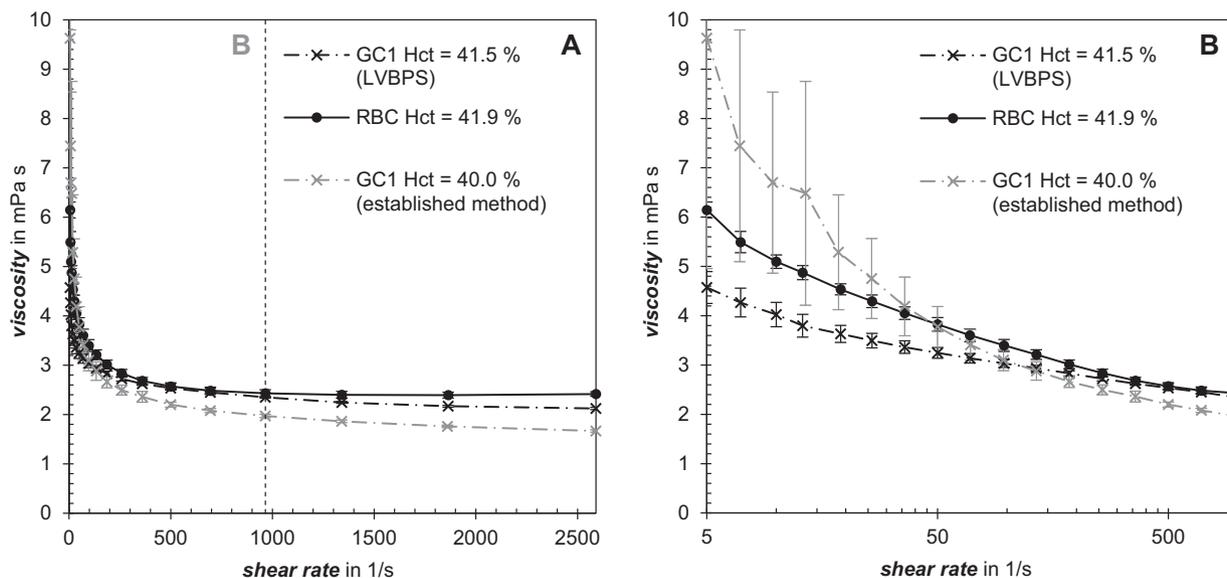


Figure 5. The graph provides the dynamic viscosity as a function of the shear rate. Single-lysed ghost cells (GC1) are compared with red blood cells (RBCs) suspended in saline solution. The hematocrit is 41.5% for GC1—produced with the large volume batch production system—and 41.9% for RBCs. The viscosity was measured at 37 °C with a repetition of three times ($n = 3$).

than 965 s^{-1} to be able to visualize the differences at low shear rates.

By implementing a sensor-based suction unit in a large centrifuge, the production of up to 188 mL of concentrated GCs with three lyses was achieved (Figure 6). Significance of mean values

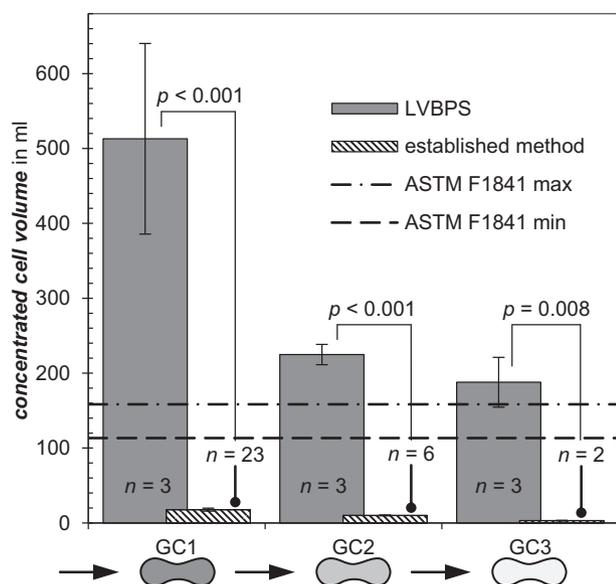


Figure 6. The bar chart shows the volume of different ghost cell (GC) types that can be produced with the large volume batch production system, distinguishing between single-lysed ghost cells (GC1), double-lysed ghost cells (GC2), and triple-lysed ghost cells (GC3). The achievable volume was converted to a hematocrit of 100% for improved comparability and labeled as “concentrated cells”. The dot-dash line shows the upper limit for tests according to the American Society for Testing and Materials (ASTM) F1841-97 (2017) and the dashed line indicates the lower limit. Both limits were also converted to a hematocrit of 100% for comparability.

within each lysis step was calculated by means of t -test for independent samples (SPSS Statistics 24, IBM).

With the established method, it was possible to produce only a small volume of GCs with three lyses so far (3.1 mL), as demonstrated by the crosshatched bars (Figure 6). However, with the new system, the volume increased significantly ($p < 0.01$), as shown by the solid grey bars (Figure 6). Owing to the new LVBPS, the volume of GCs was enlarged even after the third lysis by approximately 60 times. In addition, the automation of several process steps allows the production to be performed within 11 h as compared to approximately 10 h for small volumes.

For the purposes of this study, “efficiency” was defined as the volume ratio of GCs obtained normalized to the GCs of the previous lysis or the original RBC volume, when single-lysed GCs were produced. The volume of the cells is always converted to a hematocrit of 100%. The system achieves an average efficiency of 64.12% after the first lysis, 43.83% from the first to the second, and 83.54% from the second to the third lysis. Finally, an average volume of 187.85 mL concentrated GCs was produced after three lyses. In comparison, the established method achieved an average of only 3.1 mL concentrated GCs, within three lyses in the same time range.^[14]

When comparing the production by the established method to the large-batch production in terms of volume increase, we found that the p -value is less than 0.05 and thus significant for all GC types.

4. Discussion

The overall hemolysis rate in cardiovascular devices is currently based on discontinuous blood sample photometry lacking spatial resolution. When comparing different studies on hemolysis, no consistency can be seen, let alone transparent reporting.^[21] Till now, it is inevitable to manufacture prototypes during the

development of MCS devices, evaluate them in blood tests, and incorporate the findings in design changes. The process is repeated for the optimized prototype. This iterative procedure is cost-intensive and time-consuming.

The FHDM allows for continuous and spatial hemolysis detection by means of calcium-loaded GCs, resulting in the identification of hemolysis hotspots. Not only does this procedure save costs, but it also increases patient safety and care in subsequent clinical studies and therapy. The main limitation of using FHDM on MCS device tests is the small production scale of only 3.1 mL per day.

The main problems of GC production can be attributed to two limiting factors: The first concerns the volume to be handled and the second, the time required for the production process. Both aspects are addressed with the LVBPS by means of the reduced manual effort based on semi-automation. An automatized suction unit in combination with high-resolution sensors was set up, which replaces the former recurring manual and time-consuming step of supernatant removal. By dealing with all eight centrifuge containers simultaneously, the process is further accelerated.

In order to use GCs for cardiovascular device testing, a volume of 405 mL of transparent GCs with a hematocrit between 28% and 32% is required.^[22] Within this work, a centrifuge in combination with a newly designed LVBPS, including a suction unit and additional process engineering, allows for a standardized and reproducible production of up to 671 mL of transparent GC suspension with a hematocrit of 28%. This is in accordance with the volume of 405 mL–495 mL required for MCS device testing by the ASTM F1841-97 (2017) (Figure 6).^[22] Furthermore, this volume of GC suspension is sufficient for other kinds of device testing in flow loops or mock circulation loops, such as durability testing.

A key requirement for standard blood testing is the ability to map the dynamic viscosity of blood when using a substitute. Blood is characterized by its non-Newtonian viscosity. An exponential decrease in viscosity is characteristic for higher shear rates. As shown in Figure 5A, there is a deviation of the dynamic viscosity in the lower shear rate range of less than 360 s^{-1} between GCs and RBCs. The logarithmic plot shown in Figure 5B emphasizes the existing deviation in the interval of low shear rates, which is crucial for clearly demonstrating the limitations of this blood substitute. In MCS devices like blood pumps, such low shear rates occur only at the wall regions for a very low volume fraction. Such fractions are of less relevance to a hemolysis test than they would, for example, be to the high-speed rotating impeller of a centrifugal blood pump. The larger error bars in the single-lysed GC measurement of the established production method indicate how sensitive the measuring device is in the interval of such low shear rates. While the measurements of the established method were still carried out with an older and smaller model of the measuring device, the RBCs and GCs, produced using the LVBPS, could be measured in a new device with a more stable measuring setup. The difference in viscosity between the cells produced with the LVBPS and the cells produced with the established method is probably caused by the hematocrit level that is lower by 1.0%–1.5% in the latter. One advantage of the current measurement was that the GCs were made from exactly

the same porcine blood as the RBCs measured, that is, the same slaughter day, the same transport distance to the institute, etc. As transparency is mandatory for optical measurement methods like FHDM or PIV, the GCs' transparency was increased by a second and third lysis^[13,14] (compare Figure 4). Double-lysed GCs are on average 1.378 ± 0.044 times as transparent as single-lysed GCs and the triple-lysed GCs are on average even 2.772 ± 0.184 times as transparent as double-lysed GCs. The GCs provide significantly higher transparency than RBCs ($p < 0001$ for single-, double-, and triple-lysed GCs) with each further lysis in the wavelength range from 390 nm to 890 nm.

Even though more cells are lost during the second lysis, the required volume given by the ASTM F1841-97 (2017) is still achieved after a third lysis.

Compared to previously presented methods,^[14,17,18] not substantially more time is required by using the LVBPS to increase GC volume by 60 times. With a larger volume to handle, normally the time during centrifugation also increases. Preliminary tests have shown that the time of 40 min per centrifugation can be maintained if the g -force is increased from $1500 \times g$ to $3400 \times g$.

A larger centrifuge leads directly to a larger quantity of GCs. Both the established and the new method require the same time and a single employee. A standardization of the production volume to man hours leads to the same increase in production for both methods. However, the LVBPS leads to higher production efficiency through the implemented automation (see Figure 6). Using the established method, 3.1 mL GCs after three lyses could be obtained from 25 mL RBCs. By using the LVBPS, 187.85 mL GCs after three lyses from initially 800 mL RBCs can be produced in the same time. Therefore, a noticeable increase in efficiency from 12.4% to 23.5% was achieved by GCs subjected to three lyses. The loss of cells during the first lysis is notably higher than during the second and third lyses. This result is also reflected in the calculated efficiency from lysis to lysis. The only way to further increase efficiency while maintaining centrifugation as a method of cell separation is to optimize the suction unit. This part is currently undergoing further optimization in the redesign process. However, the increase in efficiency can mainly be attributed to the initially larger input volume of cells. Without the LVBPS, the manual handling of these fluid quantities would be inconceivable.

The established manual production method starts with 10 Falcon tubes of 50 mL each. After each lysis step, the yielded cells are collected in a smaller number of Falcon tubes. A loss of cells occurs with each container change. With the LVBPS it is no longer necessary to change containers from lysis to lysis. In all eight containers, the entire process runs simultaneously from start to finish without container changes, resulting in increased efficiency. The LVBPS solves the recurring problem of extracting a small number of cells from a large volume of fluid. Nevertheless, there are still some limitations that need to be addressed in the future. The suction unit as actuator on the centrifuge containers is currently not capable of automatically determining the volume of the remaining GC suspension and thus calculating the required volume of the added solution. Adding a load cell to each centrifuge container as well as implementing a calculation module within the control unit will reduce process time. To

further increase the transparency of the resulting GC solution, the centrifuge containers also need to be washed out with the added solutions. Currently, an apparent volume of cells remains sticking to the bottom of the container after, for example, HS is filled in. Consequently, these cells are excluded from the lysis process and finally reduce transparency. In future work, the usability due to higher automation will be improved by additional technical measures, for example, temperature monitoring for complete production documentation.

Furthermore, the maximum capacity of the used centrifuge limits the achievable volume of GCs. Compared to previous studies, we used one of the largest centrifuges available on the market. Nevertheless, due to the required mixing ratios of the additional solutions and the maximum filling level of the containers, the process is limited to 800 mL of initial concentrated RBCs, which results in a maximum of possible 187.85 mL concentrated GCs after three lyses.

Compared to the non-continuous process with subsequent supernatant removal used in this study, a proof-of-principle study was carried out to extract cells from a suspension via a hemocompatible ceramic filter as a continuous process.^[14] A main disadvantage was the significantly decreasing filter coefficient for non-washed RBCs due to the presence of platelets. However, the main problem of this method is filter clotting, which greatly reduces efficiency. The ceramic filter can be burnt out to remove the platelets, which takes time and is not practical during GC production. Furthermore, a filter pore size of 200 nm was required, because of the high RBCs deformability. An external air pressure of 6 bar was applied, pressing the suspension through the filter which resulted in high shear rates on the RBCs possibly causing pre-stress. Although such a continuous process offers more possibilities for automation, the proposed method in this study ensures defined shear and pressure conditions for RBCs as well as steady results for each lysis.

Another method for GC production by means of an ionic medium is described in ref. [23]. The osmotic gradient needed for cell swelling is imprinted by using glycerol. The advantage of this method is fewer changes in the ionic conditions, which are sensitive to the membrane's structure and function. However, washing the cells with a large volume and subsequent centrifugation are also necessary. As a future possibility, the LVBPS could be used with the ionic medium without major changes to the system.

In conclusion, the produced volume of GCs provides the basis for continuous and spatially resolved hemolysis detection in cardiovascular devices, leading to a better understanding of mechanically induced hemolysis hotspots, which cannot be assessed with current methods. Thus, the hemolysis data may provide valuable information regarding the development and optimization of medical devices at an early stage. On the one hand, experimental data can be used for direct modifications in device design and on the other hand for validation of numerical hemolysis-models.^[1] Moreover, the increased available volume of GC solution within one day enables PIV measurements inside flow channels up to 23 mm in diameter regarding flow field visualization.^[14] Following the improvements in the design of the devices will directly lead to better patient care and fewer device-associated problems.

Acknowledgements

This project was funded by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft) - 321130633.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.S. drafted the article, wrote the publication, developed, engineered the apparatus, designed, and conducted experiments, and analyzed the data; J.C.C. evaluated the data, did critical revision of article, and interpreted the data; M.F.M. evaluated the data, did critical revision of the article, and interpreted the results; D.F. did the electrical design and data recording; T.S.-R., U.S., and J.A. annotated the manuscript and did the critical revision of the article.

Keywords

automation, fluorescent hemolysis detection method, ghost cells, hemolysis, spatially resolved hemolysis detection

Received: May 29, 2018
Revised: December 10, 2019
Published online:

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