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INNOVATION

An approach to measuring RBC haemolysis and profiling RBC mechanical fragility

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ABSTRACT

Red blood cells (RBC) can be damaged by medical products, from storage or from disease. Haemolysis (cell rupture and haemoglobin release) is often a key indicator, with mechanical fragility (MF) offering the potential to assess sub-haemolytic damage as well. This article reports on a unique approach to measuring haemolysis, without the need for centrifugation or other sample separation. It also reports on employing that in measuring blood fragility (susceptibility to haemolysis) under shear stress, utilising an electromagnet to cause a bead to oscillate within a cartridge that contains the sample. Cycling between stressing and optical measurement of induced haemolysis at progressively increasing durations of stress provides a fragility profile. Sub-system-level testing shows high accuracy for the haemolysis measurements and fair consistency for MF profiling. Improving accuracy and precision of profiling is a current focus and a fully integrated and automated version of this system is under development.

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1. Introduction

Red blood cell (RBC, erythrocyte) haemolysis is the release of haemoglobin (Hb) and other intracellular components from erythrocytes to the surrounding medium following rupture of the cell membrane. Such extracellular Hb has been associated with adverse reactions and impaired clinical outcomes in patients.[1] Haemolysis can occur *in vivo* due to pathological conditions, transfusions or blood interaction with drugs or implanted devices. Haemolysis also occurs *in vitro* from storage of blood or RBC concentrates or from interaction of a patient's blood with *ex vivo* medical devices. *In vitro* testing of haemolysis is common in haemocompatibility testing for safety assessment of drugs and devices and, thus, plays a critical role in their research and development.

Haemolysis is typically measured by determining the amount of free haemoglobin released into the surrounding media in relation to the total Hb concentration in a given sample, corrected for sample haematocrit.[2] Such determination ordinarily involves separation of intact RBC, often using centrifugation, followed by measurement of free Hb in the supernatant according to one of the many methods available. Such methods are commonly based on optical analysis (either direct optical or added-chemical techniques), with spectrophotometric analysis post-separation being both precise and accurate.[3]

Beyond actual haemolysis (whether present initially or induced via testing), membranes of non-haemolysed cells can suffer damage or disruption that would make them more susceptible for later rupture, such as from subsequent physiological mechanical stress. This potential susceptibility to haemolysis—known as mechanical fragility (MF)—is a property of importance in various areas where RBC damage/health is of interest.[4–6] Typical testing for it entails subjecting a sample to controlled mechanical stress using one of various approaches,[5] prior to measuring the resultant haemolysis. Some of the common ones include use of an orifice or annular gap,[7] couette-type shearing devices [8] or bead(s) moving through the sample.[6] Generally a single index value is obtained, although profiling of MF in two and three dimensions has been suggested—with multiple index values obtainable—for more comprehensive characterisation.[9] Within MF, use of "low-energy" stresses (such as from typical bead oscillation) notably give MF results that more directly reflect RBC membrane mechanics, compared to "high-energy" stresses (such as from sonication).[10]

Here we report, first, on a system that utilises a spectrally based approach to measuring haemolysis that does not require centrifugation or other separation of the sample. Next, we report on a customised device for stress application to samples via bead

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oscillation, which allows controlled variation of multiple stress parameters and which can be used in conjunction with the haemolysis detector to profile RBC fragility. Then, we describe the integration of these in a unified system, aspects of which are patent-pending.[11]

2. Prototyping materials

Current optical components (BluLoop multi-LED light source, USB STS spectrophotometer) were purchased from Ocean Optics (Dunedin, FL). The compressible chambers in the sample-holding cartridges utilised a medical-quality Tygon® ND100-65 tubing (Saint-Gobain; Sterling Heights, MI). Prototype cartridge body exterior structures were made via printing, stereolithography and recently custom machining which gives the most robust result. The electromagnetic bead milling actuator was adapted from a vibrational component obtained from a commercial supplier to unrelated industries (Guitammer; Westerville, OH). The sampling/pinching assembly was custom assembled. Components specific to the integrated system such as the rail carrier and the overall device casing were largely custom machined—except for standard electrical components such as the stepper motors and fans.

3. Haemolysis measurement

The haemolysis detection set-up comprises a light source, a sampling assembly and a spectrophotometer. Illumination from an LED light source is delivered to the sample by a top fibre-optic cable. Light passing through the sample chamber is collected by a bottom fibre-optic cable and delivered to the spectrophotometer. Figure 1 shows the sampling assembly, along with a cartridge that holds the sample in a flexible tubing segment with end-caps. A micromanipulator is manually operated to bring the upper (downward-facing) fibre-optic down to pinch the tube—causing it to be temporarily squeezed against a lower (upward-facing) fibre-optic to achieve a sample thickness suitable for the optical reading. The tubing constituting the flexible sample chamber was selected to have optical transparency as well as a balance of softness (for easy compression) and resilience (for recovery of original shape with minimal fatiguing). Alternatively, the sample chamber could be rigid with a pre-determined thickness—particularly if only haemolysis measurement alone were of interest.

When exposed to air, haemoglobin is predominantly in OxyHb form and direct optical techniques for its measurement in cell-free solutions are well established. Those can utilise either interference-free absorbance in the VIS range [12] or absorbance at the Soret band at 415 nm, typically with Allen correction to mitigate the impact of interfering agents like albumin or lipids (Harboe assay.[13,14]). Both approaches, however, are suitable for use only in a cell-free environment and are a challenge to implement for haemolysis measurements when cell separation is impractical. Method implemented in this work utilises elements of both above methods and is based on a patented technique.[15]

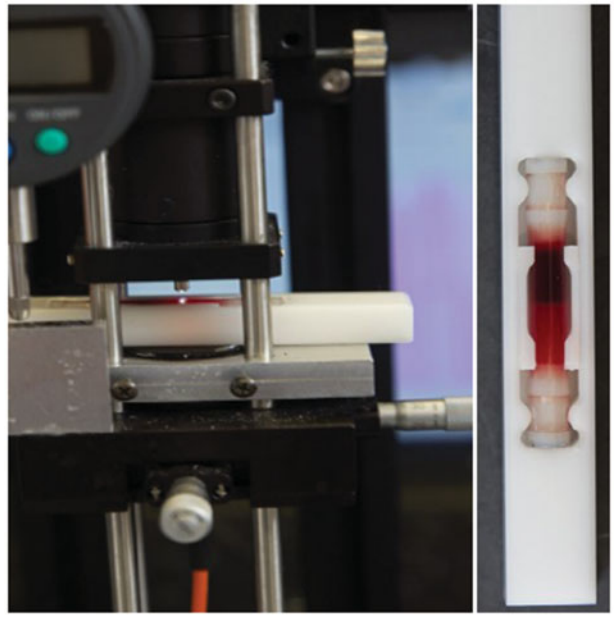

Figure 1. Optical detection unit (left) and a flexible sample-holding cartridge (right). Cartridge consists of an interior flexible tube and a rigid outer holder. Tube optionally contains a bead that can be used for mixing sample or, when oscillated vigorously as part of a fragility test (discussed later), induces mechanical stress to haemolyse the sample.

In the present method, the percentage of haemolysis present is determined by exploiting the "flattening" effect of absorbance spectra in the 390–460 nm range (due to non-homogeneity of RBC samples, as previously described.[16,17]). For a sub-millimetre-thick intrinsically non-homogeneous RBC sample, spectrum-flattening effects would be very pronounced in the high-absorbing Soret region, without affecting lower absorbance in the VIS spectral range. Thus, the composite spectrum of partially haemolysed blood would in the near-UV range present an "apparent" Soret band combining a flattened spectrum for the Hb within cells and a non-flattened spectrum for the extracellular or cell-free Hb and in the VIS range present a spectrum for the combined non-flattened absorbance of the total Hb in the sample. The flattened Hb spectrum in the near-UV region at the Soret band can then be contrasted with the non-perturbed spectrum in the visible region. At different levels of haemolysis and,

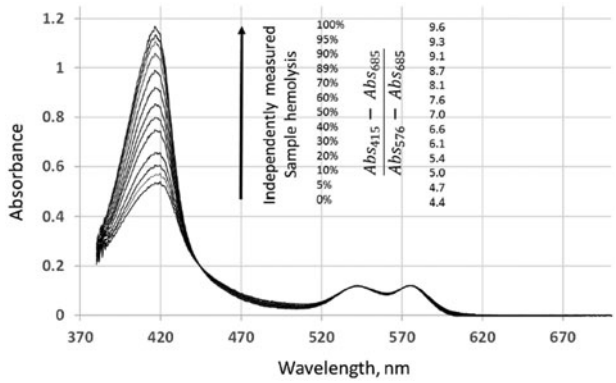

Figure 2. Changes in absorption spectrum of an RBC sample upon haemolysis. RBC were diluted to 0.5 g dL^{-1} haemoglobin (~1.5% haematocrit) concentration with AS3 blood storage solution. Samples of leuko-reduced packed RBC in AS3 storage solution, typically 15–20 g dl^{-1} Hb, were received from the University of Michigan Hospital Blood Bank and were used prior to expiration. Initial total haemoglobin concentration and, thus, haematocrit, was determined using the HemoCue 201 system, with successive changes due to progressive haemolysis estimated through iterative extrapolation based on previous haematocrit level (although high dilution reduces the impact of the correction). Presented are spectra (corresponding $(A_{415}–A_{685})/(A_{576}–A_{685})$ ratios) after the baseline correction with absorption at 685 nm taken as zero and with absorbance at 576 nm normalised to 0.12 au based on extrapolation from two spectra—one slightly higher and another slightly lower than 0.12 au.

thus, different relative fractions of cell-free Hb, there would be changes to the ratio between the apparent Soret peak and the visible spectrum band's maxima. Those would be proportional to the cell-free Hb fraction (see Figure 2), which, compared to total Hb concentration (measured independently or at 100% haemolysis), would determine the fraction of haemolysed cells.

The light source utilises a 420 nm and a 5500 K white LED, with the 420 nm LED (405–435 nm range) providing sufficient illumination at 415 nm, the wavelength of an oxygenated haemoglobin (oxyHb) Soret band maximum and the white LED suitable for detection absorbance at 576 nm (a visible-range oxyHb maximum) and 685 nm (an oxyHb absorbance minimum, used as a baseline reference point). The Ocean Optics USB spectrophotometer with a 10 μm slit provides spectral selectivity to within 1 nm in the 200–800 nm spectral range. Figure 3 illustrates the current accuracy of the present optical approach, which allows measurement to be performed without sample separation, as compared to a conventional optical technique based on supernatant analysis following centrifugation to separate the sample. The percentage of induced haemolysis in the sample is highly correlated with the

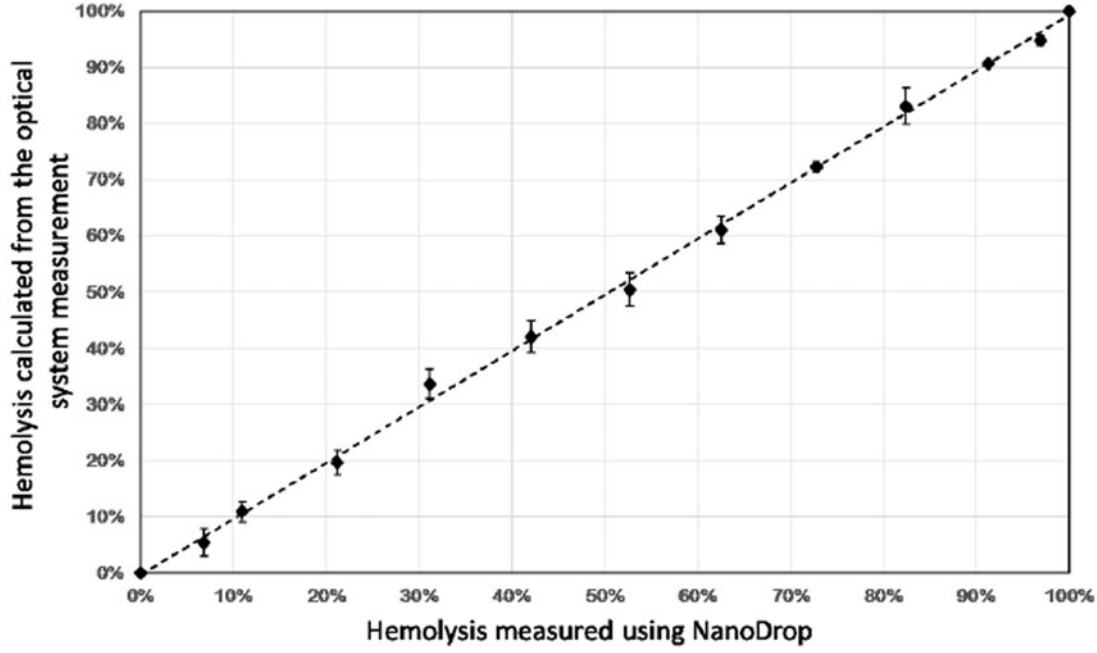

Figure 3. Representative accuracy of detection system's individual haemolysis measurements. The figure shows the correlation between haemolysis measured using the described optical system and haemolysis of the same samples measured using a NanoDrop® N1000 spectrophotometer (Thermo Scientific; Waltham, MA). For NanoDrop® measurement, the sample was prepared by centrifugation twice for 2 min at 5000 rpm in an Eppendorf 5417C centrifuge (Hauppauge, NY) with supernatant obtained after each centrifugation step and haemolysis calculated based on changes in $(A_{576}–A_{685})$ absorbance. Calculation of haemolysis based on the described optical system (without sample separation) was performed from $(A_{415}–A_{685})/(A_{576}–A_{685})$ ratios through extrapolation from ratios at 0 and 100% haemolysis. Error bars are based on SD. All haemolysis values were corrected for haematocrit using the Sowemino-Coker correction [2]. RBC samples were as described in Figure 2.

$(A_{415}–A_{685})/A_{576}–A_{685})$ ratio, which is determined using the technique described above.

Note that optical probing requires diluting a sample of RBC to a Hb concentration range set to ensure that Hb absorbance upon chamber compression is within the dynamic range of the spectrophotometer. For the described configuration, a suitable Hb concentration range was between 0.4–0.7 g dl^{-1}. Selecting compression such that absorbance at 576 nm (corrected for baseline absorbance at 685 nm) remains the same at each optical probing simplifies the analysis and facilitates consistency. The extent of dilution and/or solution used may depend on the particular application, priorities of interest and cartridge design. (Normalisation of pre-test Hb concentration aids with consistency in the effective magnitude of induced stress when performing a fragility test. Also, higher dilution allows assessment of RBC properties minimally influenced by RBC aggregation.)

When induced haemolysis is more of interest than pre-existing haemolysis (as in RBC MF testing), a spectrum of unstressed sample can be taken as a baseline, with haemolysis-related changes monitored. In that case, spectral contaminations from other agents (e.g. bilirubin or lipids introduced if using whole blood (WB)) can be assumed to remain unchanged when applying shear stress and, thus, their impact on analysis would be minimised as they would be included in the baseline. Spectral output of BluLoop LED light source used in this work does extend to 380 nm, thus not allowing Allen correction, as it is traditionally implemented for Hb. If a need for such would be found, a broader-range light source could be used.

This approach is likely to be sufficient for many applications; however, multi-wavelength analysis could optionally be used to de-convolute the spectrum and determine the relative concentrations of other interfering agents. While not currently implemented in the integrated system (which is discussed later), such analysis was tested here in post-processing of benchtop optical data, for accounting of different haemoglobin forms [18] with extinction coefficients of main Hb forms.[19] It was used for corrections in cases of significant MetHb and/or DeoxyHb presence in a sample (e.g. testing in hypoxic environment). Other absorbent proteins in a sample that may potentially interfere with haemoglobin measurements could be accounted for with multi-wavelength analysis as well. Alternatively, if desired, control ratios could be used to ascertain whether unacceptably high concentrations of interfering agents may be present; this may require adding LEDs with corresponding emission spectra to fit the relevant protein absorption profiles. With an average dilution factor of ~30-fold and a post-dilution test volume for the flexible cartridge chamber of 400–500 μL, a single test typically requires less than 20 μl of an original packed RBC unit (pRBC) or whole blood sample.

Direct optical techniques are regarded as highly reliable for measuring cell-free haemoglobin, with sensitivity reaching 1 mg dl^{-1} Hb.[13] Unlike added-chemical methods, where the outcome depends on the progress of a chemical reaction in the sample, direct optical methods show no time dependence. On the other hand, they may be more sensitive to the presence of interfering agents, both through contribution to absorbance and through changes in turbidity. Notably, such interference is minimal when plasma is not present, as in RBC solutions.[14] As such methods rely on OxyHb absorbance, they could provide erroneous results if other (e.g. MetHb) haemoglobin forms are present.

At present, the configuration has a limited sensitivity for detecting very low levels of haemolysis—thus being better suited currently for applications where haemolysis can be substantial (e.g. when it is deliberately induced *in vitro*—such as in fragility testing or in haemocompatibility testing of blood handling devices). At this time, free haemoglobin, measured without cell separation, can be reasonably detected down to ~25 mg dl^{-1} Hb, which corresponds to ~5% haemolysis, when using samples diluted to 0.5 g dl^{-1}. Accuracy and sensitivity of the method could be further improved, particularly if detection of low levels of haemolysis were prioritised instead of full profiling of fragility.

Flexible chambers are most suitable for systems combining haemolysis measurements with in-cuvette application of shear stress (as described below), but require additional means to determine the actual optical path length if absolute Hb concentrations (rather than percentage haemolysis results) are desired. Such arrangements could include pre-calibrated hard stops or use of additional calibrating wavelengths in the near-infra-red range, corresponding to the water absorption maximum. A fixed-thickness rigid chamber, on the other hand, would allow direct determination of total Hb concentration based on its absorption in the visible part of the spectrum (in addition to the fraction of cell-free Hb). While convenient for measuring haemolysis, this would often be less suitable for fragility testing, which involves also stressing the samples. Whether the chamber is flexible or rigid, the relative amount of extracellular haemoglobin—and thus percentage haemolysis—can be identified without centrifugation or other separation of the cells, as Hb absorbance in the visible range provides a

control for normalising measurements. (As knowing the total Hb is needed to provide percentage haemolysis, this can be obtained either theoretically (from Hb extinction coefficients and concentration before testing) or experimentally (from absorbance of fully-haemolysed sample). The latter approach is used in the present work.)

4. The stressing/lysing unit

A number of different methods had been used over time to induce mechanical stress upon RBC. Some examples of such approaches include concentric cylinders,[20] orifice and capillary,[21] rotating plates,[22] convex/concave,[23] bead agitation [24] and bead rocking [6]. However, not all means are equally feasible for the present application, as they differ, for example, in sufficiency of stress and amenability to precise control. Also, different approaches for the stressing can induce qualitatively different types of shear and/or other mechanical stress (or relative contributions thereof), the significance of which may vary according to test purpose or application. The extent and adequacy of correlations between physiological stresses and stresses that can be induced by mechanical means remains an area of ongoing inquiry.

After exploring test-beds for concentric-cylinder, pressurised capillary and sonication approaches, we decided to focus on bead oscillation (bead milling) using a single bead in a tube. An early cam-based prototype exhibited excessive noise and vibration, so electromagnetic (EM) approaches were pursued. One such approach is to utilise EM to move the overall cartridge, in that respect being analogous to cam motion; the other EM approach is to directly induce bead oscillation while the rest of the cartridge remains stationary. The latter approach naturally has the limitation that the bead must be magnetic or at least magnetically susceptible, but was favoured in part due to the appeal of minimising motion as well as vibration and noise. Significantly, moving the bead directly via EM affords more direct control of bead movement, compared to when it is a function of movement of the whole cartridge. Frequency and amplitude of oscillations may be worthwhile to separate, if stress intensity is to be truly distinct from stress duration. Actual duration is a function of run-time, frequency and the fraction of each oscillation period the bead is in actual motion—the latter itself being a function of both frequency and magnetic force. Intensity, essentially determined by bead velocity, is a function of oscillating magnetic force, properties of the media and cartridge/bead properties and dimensions.

Current implementation of the lysing system employs an electromagnetically driven bead mill incorporating a commercially available coil-based audio transducer. This commercial EM actuator, by default, comes with a magnetically suspended heavy piston that moves in response to amplified audio signal input. This piston can generate a high force transferrable to whatever the housing is attached to—which makes the component useful in its marketed uses of causing controlled vibration. As adapted here, the piston is removed and the cartridge is positioned inside the coil assembly such that the EM field directly acts upon the magnetic bead inside of the cartridge. Coil dimensions determine the optimal position range for cartridge placement.

Regarding the oscillating magnetic bead, exposure of its core to sample may cause undesirable chemical interactions, thus warranting a biocompatible coating. A common Cu-Ni coating, while biocompatible, was found to lack the durability to reliably withstand bead mill operation. Similarly lacking in durability were gold and parylene coatings. PTFE had excellent durability and biocompatibility; however, the thick material layer significantly reduced the size of the bead's magnetic core for a given bead size, negatively impacting efficiency of haemolysis. Of the coatings tested thus far, black epoxy seems to provide a suitable blend of durability and biocompatibility, combined with the option to use thin coating layers. A range of bead and tubing dimensions were explored and, for tubing with dimensions of 37 mm (inner length) $\times$ 4.75 mm (inner diameter), adequate lysing efficiency was obtained with beads ranging from 8–9 mm in length and 3.5–3.8 mm in diameter (including coating).

Chamber geometry, sample volume and chamber fill-fraction also can be significant factors affecting lysis (along with oscillation frequency and force). While higher lysis rates may be desirable for making the test faster, this may need to be balanced against having the lysis be spread out sufficiently over time so as to allow collecting a desired number of data points in a given profile/run. Also being explored are the relative contributions of the bead's design (e.g. size, shape), as well as various oscillation parameters, to the nature and/or extent of the stressing—and potential effects of such on the cells as reflected by fragility. It was found that non-conventional bead shapes (e.g. cylinders) can significantly increase lysis efficiency.

Figure 4 shows the lysing set-up, with a testing cartridge being positioned in the electromagnetic actuator; oscillation frequency and signal waveform are determined by a power supply, while duration is set via an incorporated timer (not shown). The fan positioned

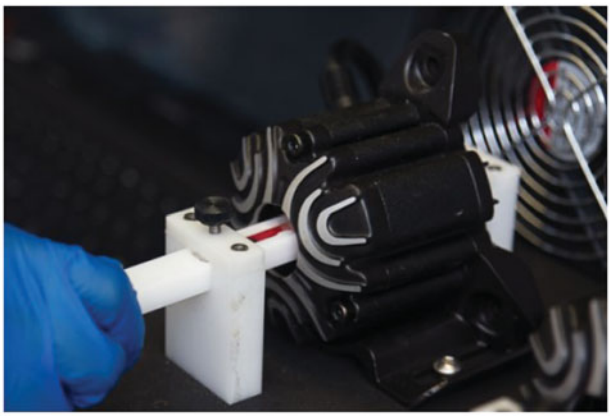

Figure 4. Electromagnetic lysis unit, with cartridge placed so as to have the sample-containing chamber inside.

behind the electromagnet provides cooling, supplemented when needed by additional airflow aimed directly at the cartridge. After running for a desired increment of oscillation, the cartridge is brought to the haemolysis measuring station described above. At this stage, machined cartridge exteriors are reused, with the interior tubing and beads being changed between runs.

5. Fragility profiling

Repeatedly stressing and measuring a given sample allows one to obtain a fragility profile—i.e. a set of data points corresponding to haemolysis levels associated with respective applied stress levels (e.g. durations). Such profiles enable various fragility-based indices to be interpolated or otherwise inferred. For example, the area under the profile curve can be used to reflect overall fragility. Alternatively, fragility can be characterised by particular haemolysis levels associated with given stress duration(s) or vice versa or indices can focus on particular regions of a profile (e.g. corresponding to relatively low or high stress durations). In any case, a raw profile can be processed for analysis using an appropriate best-fit function. Other possible indices could be slopes at particular profile points, derivatives or even qualitative categories of profile shapes.

Note that, for any index based on a profile, sensitivity to error from any given data point is dampened based on the number of data points in the profile. Also, aside from using different stress durations for the data points, a given sample could be sub-divided for separate runs at different intensities, to enable construction of a three-dimensional (3D) profile.

Figure 5 reflects the consistency of current fragility measurements using the combination of means

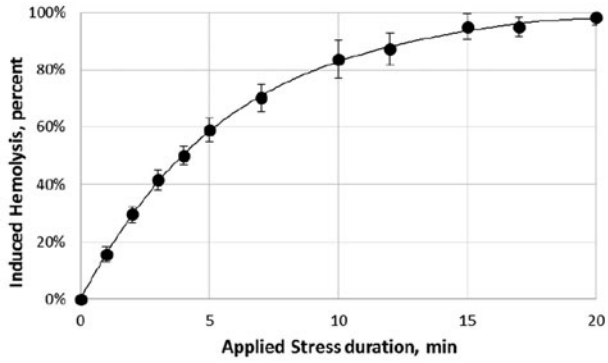

Figure 5. Sample RBC mechanical fragility profile obtained with use of both the bead milling and optical sub-systems. Presented are the results of five independent measurements. Profile was collected at oscillation of 5 Hz at 0.6 V, using N52 magnets with black epoxy coating (3.5 mm × 9 mm) and Tygon® ND-100-65 tubing (4.75 mm inner diameter and 37 mm inner length). Error bars are based on SD. Fit shown is a fourth-order polynomial and is for illustrative purposes only. RBC samples were as described in Figure 2.

discussed. Such testing has been suitable for preliminary proof of concept studies with select applications. Possible sources of current error include, to varying extents, as follows: non-uniformity of the flexible tubing (e.g. variability of tube segment cross-section, length or inner diameter), non-uniformity of the magnets/beads (e.g. variability of length or diameter) and non-uniformity of manually filling the tube. Ongoing efforts to standardise materials used and testing protocols aid in improving consistency, as will the eventual reduction of manual steps involved.

6. Integrated system

The integrated prototype system includes an overall base unit (Figures 6 and 7), which combines the haemolysis detection unit and the sample lysing unit, utilising a specially designed testing cartridge (Figure 8) that essentially serves the same functions as the cartridge described above—yet has some notable differences.

The sample is inserted into the cartridge by a gel-loading type pipette through a capillary connecting the sample entry port to the transparent flexible tubing constituting the sample chamber. Its current design and fill-line provide for loading to just under 100% of the cartridge's total fluid capacity (including chamber and capillary), which appears to facilitate consistency as well as avoid foaming during bead movement due to air presence. At the top (sample-loading end) is a screw-cap, which has an O-ring for sealing and a protruding insert to go in the capillary and down to the fill line when the cap is screwed on. At the other end

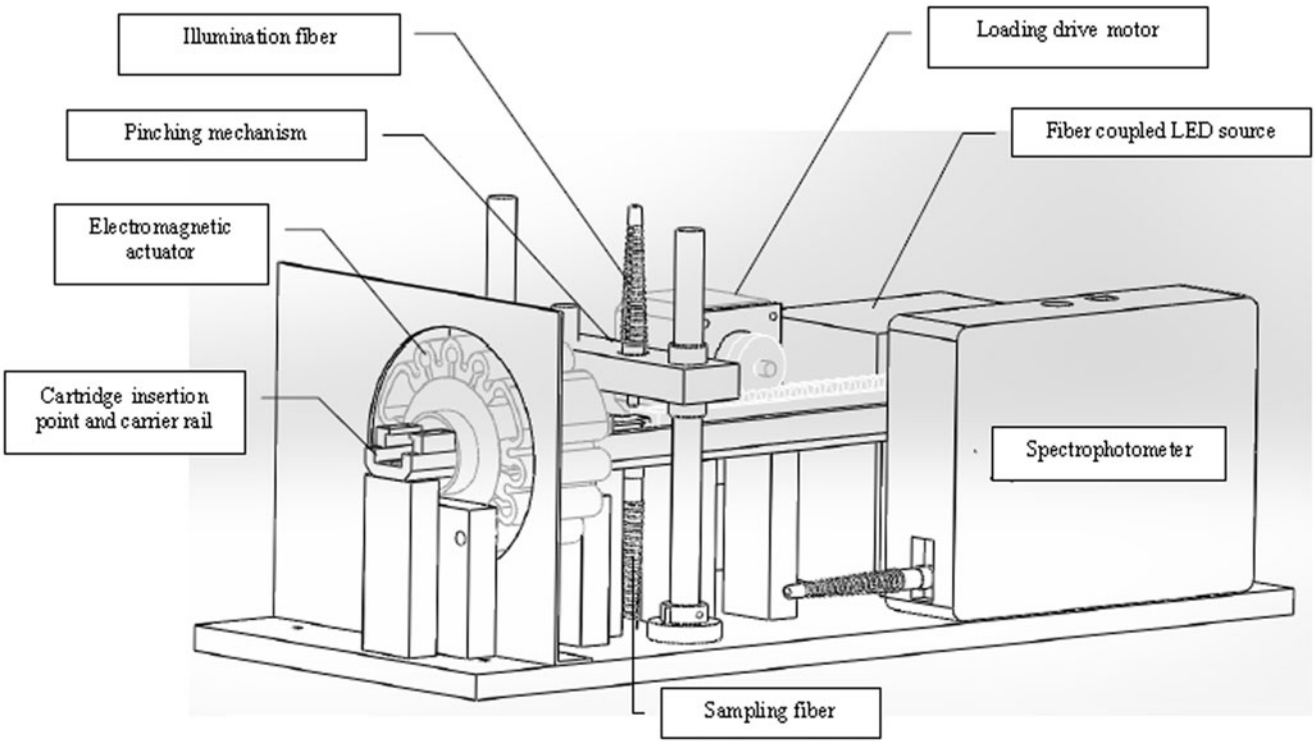

Figure 6. Drawing of integrated system base unit interior, showing key components.

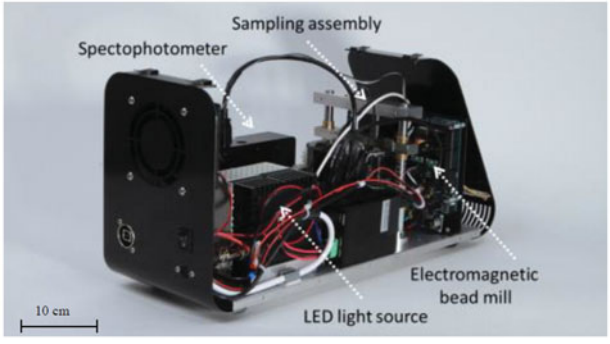

Figure 7. Photograph of integrated system base unit, without enclosure casing. Dimensions with casing are 45.7 cm long, 22.2 cm tall and 15.9 cm wide.

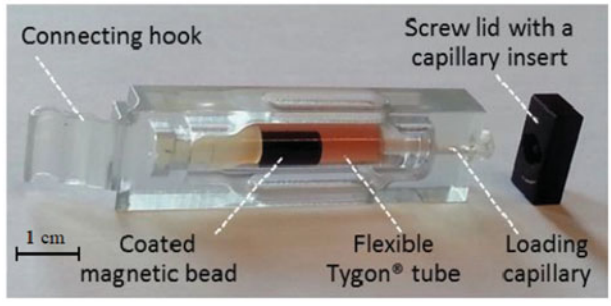

Figure 8. Photograph of cartridge for integrated system, with sample of RBC.

of the cartridge is a hook for attaching to the carrier when it is brought to the cartridge loading position on the rail. Inside the sample chamber is the magnetic bead that will be longitudinally oscillated between the two ends—each bounded by barbed plugs (one of which is partially hollowed, for the capillary path).

The cartridge slides along an automated rail between a loading position, two calibration positions and two operational positions. The test begins and ends with the cartridge in the loading position, protruding through an opening in the front of the casing. Upon initiation of testing, the cartridge moves first to the calibration positions, then proceeds to the "lysing position". After defined increments of stress application, the cartridge is moved to the "detection position" for optical probing; after the optical readings for each increment are complete, the cartridge returns to the lysing position.

The sampling assembly as integrated in the overall system includes a motor-driven "pincher" that drives a horizontal bar—with a mounted downward-protruding fibre-optic—to slide vertically along poles. A second, end-aligned fibre-optic is mounted in the rail that supports the cartridge carrier. The horizontal bar is designed to move downward to temporarily squeeze the transparent chamber of the cartridge and the sample therein between the fibre-optic ends. The bead within the cartridge needs to be moved out of the way during the optical probing; a permanent magnet fixed upon the rail does this as the cartridge approaches the detection position.

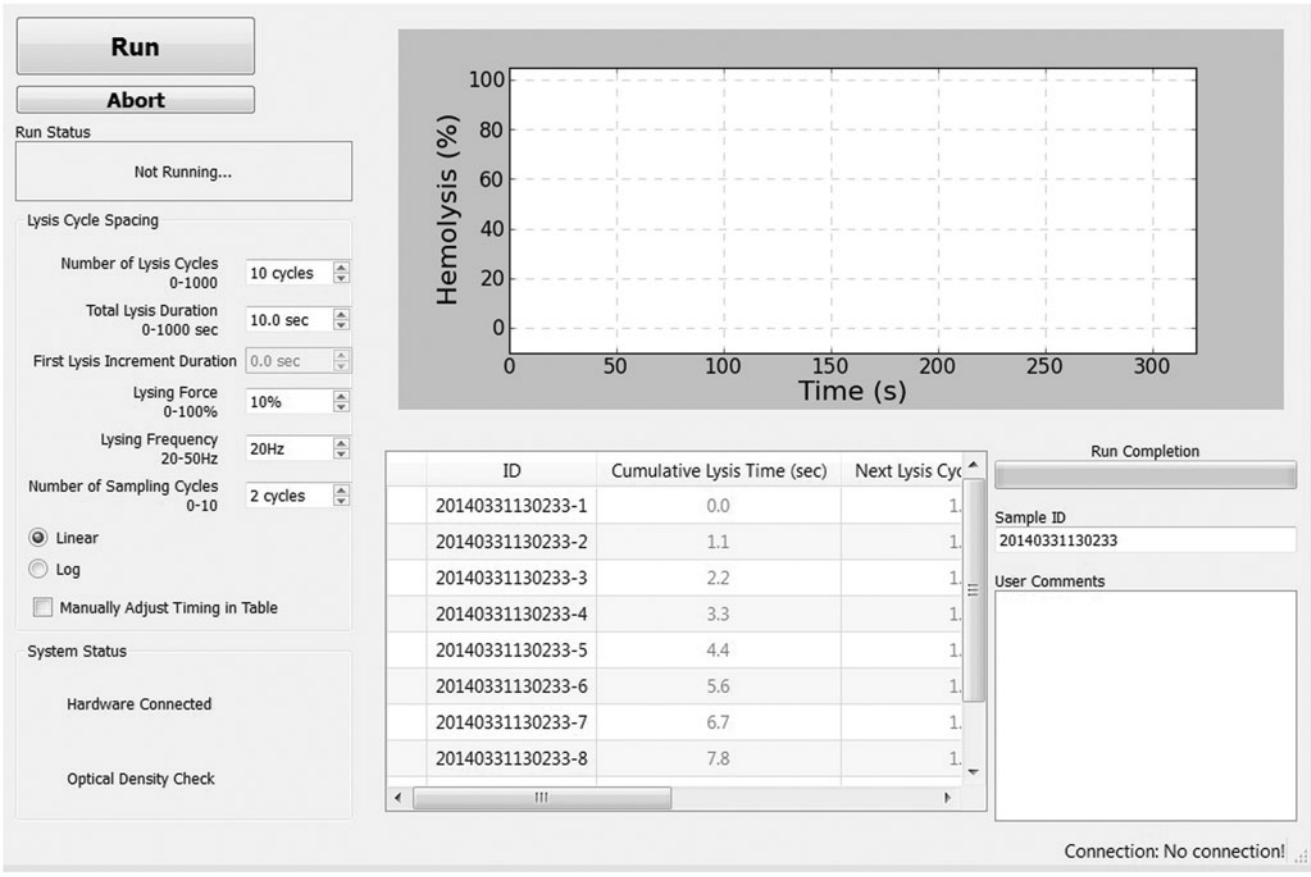

Figure 9. Screenshot with the general layout of the user interface for the integrated system, with system not in use; some example settings are entered.

Two positions on the transport rail are used for optical calibration at the start of each run: a "light" patch allows correction for absorption and reflection losses due to the chamber walls and is fashioned from the same plastic tubing as that from which the cartridge chamber is made; an opaque or "dark" position enables calibration for dark current of the spectrophotometer.

Multiple optical readings are taken during each individual pinch, and the "correct" one is ascertained retrospectively (by the software—discussed below) based on a pre-selected optical density at the 576 nm oxy-Hb maximum (currently using 0.12, so that absorbance at the Soret band maximum is ~1, but other combinations of wavelengths could be used). Multiple pinches can be performed at each increment of stress duration, to allow averaging of readings. Before each repeat pinch for each data point, the cartridge is carried back and forth once on the rail to gently mix the sample via cartridge movement relative to the bead which is kept stationary by the permanent magnet. Such mixing motion is slow, to avoid contributing to sample haemolysis (and likewise for the pinching motion).

As with the set-up discussed above, with the integrated system, fans are employed to maintain ambient room temperature for the sample. In addition, here, baffles are configured to help direct air flow within the casing.

After loading the sample and inserting the cartridge in the machine, a user specifies the run parameters and initiates the run. (These settings notably must be selected to be mutually compatible—e.g. to ensure the force is appropriate for the desired oscillation frequency.) Before commencing a run, the system performs a check to ensure that the sample Hb concentration is within the range suitable for testing. Also checked for potential flagging during the run is whether there has been excessive Hb conversion in the sample to other (non-oxyHb) forms, so that this could be accounted for in analysis.

The software to control the device and process the results (from a connected computer) uses a graphical user interface (GUI) shown in Figure 9. It allows the user to specify the desired stress intensity parameters (oscillation force and frequency) as well as the number and distribution of time intervals at which the stressing shall be paused to measure the incremental haemolysis. Such intervals can be distributed over the total stress duration linearly/evenly, logarithmically or

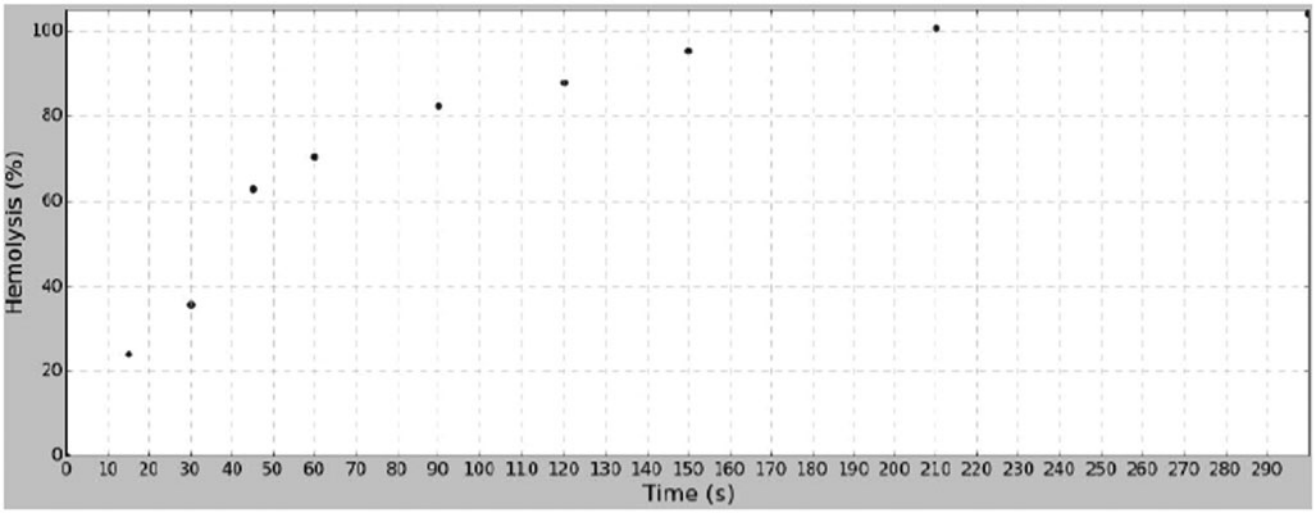

Figure 10. Actual RBC fragility profile (via direct screenshot) generated by the system in a fully-integrated/automated run. An option was selected that allows manual setting of the times for data point collection. "Time" here refers only to stressing time, not overall testing time (which also includes optical calibrations and the haemolysis measurements), which totalled ~15 min. Points at 0 s (set as 0% haemolysis) and 300 s (the final for this run) are obscured by grid corners. Run was at 30 Hz.

manually as desired (although they should ideally be set sufficient to minimise effects of bead mill ramp-up/down times).

During a run, the GUI shows a plot of the MF profile being acquired as data gets received from the base unit—with real time estimates of haemolysis calculated using the theoretical ratio between Hb extinction coefficients at 576 and 415 nm. The final results can if desired be adjusted based on measured cell-free haemoglobin at full lysis, if and when the fragility profile plateaus sufficiently to indicate that 100% haemolysis is achieved (mathematical criteria for defining such plateaus can be set in the code). Users can annotate, save and export results for subsequent data processing or analysis as desired.

The first profile successfully generated from a fully-integrated and fully-automated run of the system is shown in Figure 10.

7. Future work

Plans for both the sub-systems and the integrated system include refinements to improve accuracy and precision, quantitative system-level verification for the integrated system and comparative studies alongside other fragility testing approaches. The latter could aid standardisation of protocols to establish general MF profile-based parameters. Application-specific validation is planned for by prospective early adopters in particular fields of interest. Other work will look at the oscillating bead's acceleration profile, the nature and extent of stresses occurring, effects of optical interference from known agents and the effects of changing certain aspects of the test set-up or protocols. Once suitable specifications are determined for at least initial production and use, economical cartridge replication can be pursued to enable the entire cartridge to be single-use.

Future versions of the technology could include active temperature control, automated sample dilution and multiplex capability for facilitating higher throughput and/or concurrent 3D profiling (e.g. plotting haemolysis vs stress duration and intensity). Other possible design enhancements could include custom optics or a custom electromagnet (e.g. to eliminate cartridge transport between stressing and detection). Additional opportunities exist in expanding analytical capabilities to allow detection of other absorbing and/or fluorescent agents.

Unlike research-use versions for industrial and academic users, eventual clinical products based on the technology would involve regulatory clearance. Clinical models might offer features such as simplified user interfaces, integrated sample prep, shorter testing time, particularised output (e.g. specific MF index values deemed clinically relevant), a built-in computer terminal and CPU and networking capabilities.

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Disclosure statement

The presented work was funded entirely by Blaze Medical Devices, an early stage company working to develop and commercialise blood analysis technology and which fully owns the proprietary technology presented. Authors Alfano and Tarasev have equity and employment with Blaze. The other authors are with in2being, which is contracted by Blaze for development work. Aspects of the presented subject matter are described in patent material of Blaze.

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