THROMBOLUX – THE FIRST ROUTINE PLATELET TRANSFUSION QUALITY TEST

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INTRODUCTION

Platelets are blood cells that prevent and stop bleeding. In North America every year 4.2 million platelet transfusions are a life-saving part of cancer therapy and the treatment of bleeding patients.[1] However, 1 in 6 or 17% of all platelet transfusions are ineffective and do not have the desired clinical outcome.[2] Currently no in vitro quality test is used routinely to identify ineffective platelet transfusions and distinguish them from effective ones.[3] Instead, platelets are transfused without a pre-transfusion quality test and the outcome in the patient is the only indicator of platelet effectiveness. This practice is time consuming, costly and a significant risk to patients. It was our objective to solve this problem by developing ThromboLUX, a quick, easy and inexpensive optical platelet quality test intended for routine use prior to administering a platelet transfusion.

BACKGROUND

The ThromboLUX device uses the principle of dynamic light scattering (DLS) to determine what kind of particles are in the platelet concentrate, how many of the particles exist, and how they respond to temperature stress by measuring their relative size change when activated.[4] ThromboLUX uses the entire spectrum of particles suspended in plasma and the functional response of platelets to temperature changes and calculates an easy to use ThromboLUX score, which is a measure of the platelet quality. The ThromboLUX score ranges from 0-40 with a lower score indicating poorer quality and a higher score indicating better quality of platelets.[2]

The ThromboLUX system consists of a bench-top instrument with integrated analysis software and a disposable sterile sample transfer set used to obtain platelets from the cell storage bag.

In a previous study we have shown that the ThromboLUX score correlates with the clinical outcome of platelet transfusions,[2] which provides incentive to use this exciting new technology. By eliminating the use of ineffective platelets, improved clinical efficiency and significant cost reduction could be gained.

METHODS

Dynamic Light Scattering – an Absolute Sizing Technique

Dynamic light scattering (DLS) uses the principles of Brownian motion and Doppler shift to determine the size and number of particles in a suspension.

The particles in the suspension undergo continuous random movement called Brownian motion. To characterize this motion, laser light is focused into the suspension and a single photon counting detector is positioned at an angle with respect to the laser. As the particles move through the suspension, they pass through the laser beam, which causes some of the light to scatter in all directions. Because the particles are moving, the scattered light undergoes a Doppler shift, which causes a small frequency change of the scattered light compared to the unscattered light. Larger particles move slowly, and exhibit a small Doppler shift, whereas small particles move quickly and exhibit a large Doppler shift.

The photon counter is able to detect the light scattered in its direction, and measures the fluctuations in light intensity that result from the Doppler shift. The intensity fluctuations are measured for a period of time and the data is processed into a correlation function — a mathematical function that identifies the patterns in the measured intensity data. The exponential decay of the correlation function is characterized by the translational diffusion constant D_T. The Stokes-Einstein equation indirectly relates the diffusion constant to the hydro-dynamic radius R_H of the particles (Boltzmann constant k_B, temperature T, viscosity η), making dynamic light scattering an absolute sizing technique.

$$D_T = k_B T / 6\pi\eta R_H \tag{1}$$

If a sample such as a platelet concentrate consists of a mixture of particles of different sizes, a polydispersity analysis is performed on the correlation function. The polydispersity analysis identifies the size distributions of particles in the suspension and the relative number of particles of each size.

Platelet and microparticle sizing by microscopy

Differential Interference Contrast (DIC) microscopy was used to verify the presence of microparticles and platelets on a Leica DMRA2 microscope (Leica, Richmond Hill, ON, Canada) equipped with a 100x oil-immersion objective (NA = 1.4) and a digital camera (Retiga EXi Fast Cooled Mono 12-bit; QImaging, Surrey, BC, Canada). We followed the protocol as described by Devine et al.[5]

Detection of platelet activation by flow cytometry

Flow cytometry was performed on a FACS Canto II flow cytometer (BD Biosciences) using the FACSDiva software. Platelet activation was detected with a phycoerythrin (PE) - labeled antibody against CD62 as described by Leytin et. al.[6]

RESULTS

ThromboLUX

A user friendly device was designed to allow quick and easy testing of the quality of platelet concentrates.[7] ThromboLUX has a tray to hold the platelet bag during testing, draws the sample into a special sterile disposable sample transfer set, performs the dynamic light scattering measurement and automatically calculates and displays the ThromboLUX score on the touch screen.

Figure 1. Rendering of the ThromboLUX device for automated platelet quality testing.

The device was developed to allow an operator to use the instrument with minimal training, and does not necessitate the use of reagents, special techniques or any other laboratory equipment. Tests are quick and results are displayed within 20 minutes.

Platelet Quality

Platelets respond to temperature variation and "activate" when cooled. Platelet activation manifests as shape change, whereby platelets contract and form pseudopods: protrusions extending from the cell surface. Viable platelets activate readily when cooled and revert to a generally discoid shape upon re-warming,[8] whereas non-viable platelets will not respond to temperature stress.

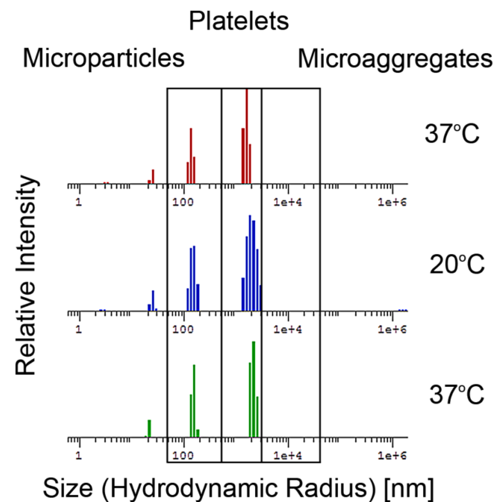

Figure 2. ThromboLUX result showing microparticle and platelet distributions at the three temperatures used for platelet quality testing.

The smallest size distribution of interest represents microparticles, which increase in number during storage of platelet concentrates as a consequence of platelet activation and aging.[9] Over time, small pieces of the platelet pseudopods fracture from the cells to become microparticles. The presence of microparticles is therefore an indicator of diminished platelet quality. Higher quality platelet concentrates contain fewer microparticles, indicating that the platelets are intact and have not deteriorated.

The large particle size distribution stems from platelets; the width of the distribution indicates the degree of heterogeneity of the platelet population. Exposure to low temperature activates platelets, which gives rise to pseudopod formation and reduced Brownian motion; the change in size and speed results

in a broadening and right-shift of the platelet size distribution.

The overall quality of the platelet concentrate can therefore be determined by considering the platelet count, the temperature response of the platelets, and the ratio of microparticles to platelets.

The ThromboLUX score was developed to take into account the ratio of microparticles to platelets, the degree of platelet heterogeneity, and the temperature response of the platelets. This score was then used to compare with the results of microscopy and flow cytometry.

Verification of ThromboLUX results by microscopy

Platelets can be viewed by microscopy with a 100x oil-immersion objective. In a fresh platelet concentrate, platelets are mostly discoid. After storage of concentrates for 5 days or more, platelets often have undergone shape change and appear as spiny spheres. The determination of platelet quality by microscopy is time consuming, subjective and only a limited number of cells can be evaluated.[10] Platelet viability and the number of microparticles is very challenging to assess by microscopy. Microparticles are extremely difficult to capture due to their small size of about 300 nm in diameter, and their quick movement in and out of focus.

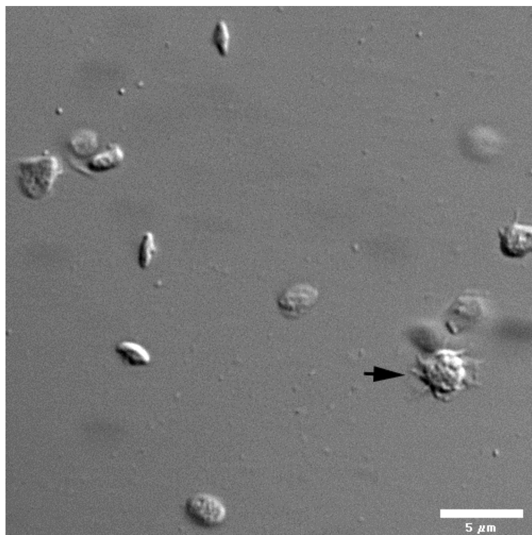

Figure 3. Differential Interference contrast microscopy of platelets and microparticles in a sample from a platelet concentrate. The scale bar represents 5 microns. The arrow points to an activated “spiny sphere” platelet.

Platelet concentrates were assessed for quality and compared to the ThromboLUX score. See Table 1 for results.

Verification of ThromboLUX results with flow cytometry

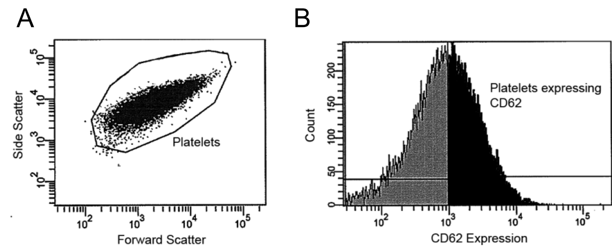

Figure 4. Flow cytometric detection of platelets by (A) static light scattering (forward scatter vs. side scatter) and (B) determination of activation status by CD62 expression.

Flow cytometry uses static light scattering to differentiate particles according to their size. The forward vs. side scatter dot plot shows the empirically determined platelet population. In contrast to dynamic light scattering, flow cytometry is not an absolute sizing technique and particle sizes can only be determined relative to particles of known size such as latex calibration beads.

In addition, the platelet surface can be probed with fluorescently labeled antibodies raised against surface markers for platelet activation. The most commonly used marker is CD62, which is expressed when alpha granules fuse with the plasma membrane during the activation dependent release reaction. The histogram in Figure 4B shows expression of CD62 on approximately half of the platelets due to activation. The expression of CD62 is always determined in relation to the binding of a fluorescently labeled non-specific antibody as negative control and the expression following full platelet activation stimulated by thrombin as positive control.

Platelet concentrates were assessed for degree of activation by microscopy (morphology score) and flow cytometry (CD62 expression) compared to the ThromboLUX score. The results are shown in Table 1.

Table 1.

	ThromboLUX Score	**Morphology Score**	**CD62 Expression**
Low Quality	0 -12	250 ± 30	29 ± 12%
High Quality	13 - 40	330 ± 45	21 ± 9%

Platelets with high quality show a higher ThromboLUX score, more discoid platelets corresponding to a higher morphology score and less activation indicated by lower CD62 expression.

DISCUSSION

The global blood industry represents a diverse assortment of companies, groups and organizations that have evolved over time to provide safe collection, testing, delivery and storage of human blood, synthetic blood and blood products for transfusion.[1] These blood transfusions are of critical importance to those with both acute and chronic medical conditions in which blood is depleted or non-functioning. US statistics indicate that 4.5 million Americans would die each year without blood transfusions.[1] The National Blood Services of England and Wales state that in 2005 blood donors saved or improved approximately one million lives.[11]

Historically, the quality of platelet concentrates has been considered “good” if the platelet count was within an acceptable range for a given volume. Samples would be taken from the platelet concentrate and analyzed using automated cell counters to determine the platelet count.[12] Counting platelets, however, does not test the functionality of the cells and does not account for microparticles. The two important indicators of platelet quality determined by the ThromboLUX that relate to platelet viability are the temperature response of the platelets and the presence of microparticles. In addition to the platelet count the temperature response of platelets and the number of microparticles significantly contribute to the ThromboLUX score.

Prior to the ThromboLUX, no single in vitro test existed that could be used individually to evaluate the quality and effectiveness of platelet concentrates for transfusion.[10] As hospitals currently cannot predict effective from ineffective transfusions prior to seeing patient responses, they transfuse all platelet products they acquire.

The ThromboLUX score correlates well with the platelet morphology observed by microscopy and the expression of CD62 determined by flow cytometry. Generally, a higher ThromboLUX score parallels a higher number of discoid platelets, a lower number of microparticles and a more pronounced temperature response of platelets. In contrast to the temperature response, the measurement of CD62 expression by flow cytometry requires significant sample preparation and data handling by a highly trained user. Thus ThromboLUX has successfully combined several important indicators of platelet quality and viability in a quick and easy-to-perform test that produces a simple output in the form of a numerical score.

ThromboLUX will reduce the risk of ineffective transfusions that are given primarily to cancer and bleeding patients. Consequently, the complications associated with ineffective transfusions will also be reduced significantly, which in turn will dramatically lower the cost of platelet transfusion therapy to hospitals and the health care systems.

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