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Potential use of Raman Spectroscopy in the Quality Control of blood products

CellTool
live sorting

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Introduction and Purpose

Legal basis for the relatively time-consuming and costly intensive quality control tests of blood products is the *Hämotherapierichtlinie der Bundesärztekammer, 2010* in combination with the *AMWHV*, the *EU directive 2005/62/EG*, the guidelines for laboratories of the German Medical Association (RiLiBäK, Bundesärztekammer, 2013) and the §25 of the German Social Code. Accordingly, the platelet content, e. g., must be >2*10^{11}/unit, less than 1 million leukocytes /unit and < 3 millions residual red blood cells (RBCs) per unit. The pH value should be between 6.4 and 7.8, and the product must be sterile till end of life. Platelet concentrates produced by the DRC North meanly content 3*10^{11}/unit, residual RBCs per unit are close to 0, the residual leukocytes in amount ca. 60000 in the mean, and the pH is approximately 6.9.

However, the quality control system used today is not able to proof one or more factors responsible for the biological function of the platelets. This gap could be closed by Raman Spectroscopy, which has been established as a photonic finger printing method of living cells during the last few years. Raman spectroscopy is an optical spectroscopy method that allows non-contact and label-free characterization and identification of cells and tissue under physiological, in vivo-like conditions. Only small amounts (about 10µl) are required for one-line monitoring.

In this context, with this preliminary study we wanted to check, whether Raman spectroscopy could be used for fast, non-invasive monitoring of platelet and RBC cultures derived from bags of their concentrates used in transfusion medicine.

Material and Methods

Material:
We measured three different donors of platelet concentrates (donor 647, 648 and 649) on the days 1, 2, 3, 7, 8, 10, 11, 17 and 21. Small amounts (about 10µl) of samples (1,200 E+09/l) were extracted directly from the blood bags using a fine needle syringe. Platelets were diluted in storage solution for platelets (SSP, SSP 1020 U, Macopharma, Langen Germany).

Raman measurements:
Raman spectra of 30-50 cells per time point were measured applying an accumulated measuring time of 3x10sec. (BioRam® System, 785nm laser, 80mW, 60x water immersion objective, Fig. 1).
Single platelets (circle) were kept within the focused laser beam utilizing the **laser trapping** effect. This effect is due to the strong electro-magnetic gradient that is formed when a laser beam is focused through a high numeric aperture objective. Charged samples such as single cells are dragged into the focal center and are held there as long as the laser is on. From each measured cell a bright field image is stored for later inspection. In the figure at the right, also aggregates of platelets can be seen.

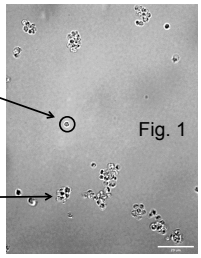
Fig. 1

Data Export:
The data were edited by cropping the spectra to the biological relevant area known between 700-3000cm^{-1}. Mean spectra of each sample were calculated for a first survey (Fig3).

Multivariate Data Analysis
Statistical analysis of the spectral data was performed applying Principal Component Analysis (PCA) using the statistical Camo Unscrambler X 10.3 software. This well established and accepted standardized statistical evaluation method allows to draw conclusions of the spectral peaks and wave numbers (i. e. 1/wavelength) responsible for the differences between the samples.
Prior to PCA spectral data were smoothed using a median filter (to abstract spikes) and normalized with the SNV (standard normal variate). The PCA was calculated using the NIPALS Algorithm with a cross validation of 10 segments.

Principle of Raman Spectroscopy

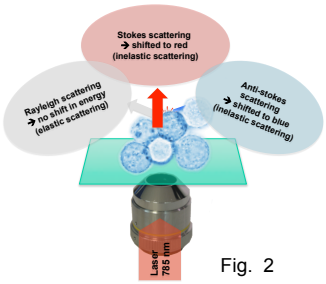

Fig. 2

Raman spectroscopy is molecular spectroscopic technique, based on the detection of light that has been **inelastically scattered** by a sample (“Raman effect”) . It was established in 1928 by a correspondent Indian physicist and displayed with the Nobel price for physics in 1930.
Raman spectroscopy is based on focused laser light shone into cells to excite molecular vibrations (Fig. 2). The shift in frequency of the emitted light is detected by a spectrograph.
The probability of Raman scattering is weak (about 1 per 10 million photons) but results in well-resolved peaks, uniquely associated with the biochemical properties of the samples (Fig. 4b).

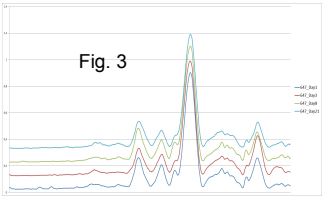
Fig. 3

The spectral contributions from **all molecules** within the cell yield a spectral sum - as characteristic as a **“fingerprint”** (Fig. 3). This information can be used to identify and characterize cell types, internal cellular states or the cellular response onto external influences such as drugs or environment.

Results

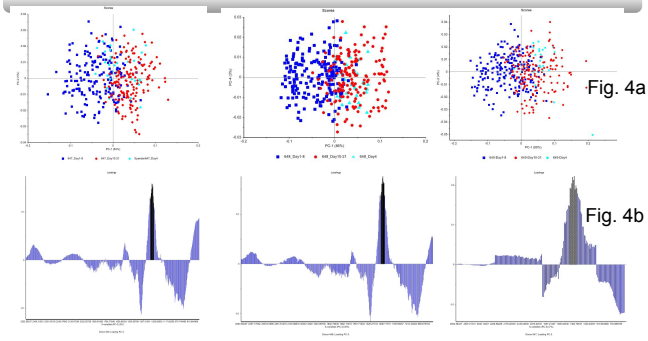
Fig. 4a

Fig. 4b

Setup for Data Comparison:
For all three donors we processed each measured cell respectively, comparing day one with day two, day one with day three and so on.
Spectra of cells from day one to day eight were identical, yielding a cluster (blue dots) clearly different from those of day ten to day 21 (red dots). Cells could be separated along PC1 (carbonic part) with a probability of up to 90 % (Fig. 4 a).
Loadings show which of the waver numbers are mainly responsible for this difference: They are in the range of 1296-1333cm$^{-1,}$ including changes in Guanine (several peaks in the range of 1313-1325cm^{-1}), Amide III of α-helix (1321cm^{-1}) and a region associated with DNA (1330cm^{-1}) (Ref. 1), highlighted with a black peak in figure 4 b.

Conclusion and further perspectives

By analyzing three different platelet concentrates by Raman Spectroscopy, we clearly showed that beginning with day 10 after blood donation respectively preparation the platelets start to differ in the wave number range of 1296-1333cm^{-1}. Differences shown in this range are well known to be associated with apoptotic cell death (e.g. Ref 2, 3). These data are in accordance with the life cycle of platelets in vivo and their therapeutic use within 7 days at maximum. For RBCs, comparable experiments are under investigation.
In conclusion, our data demonstrate that Raman spectroscopy could become a valuable tool to monitor the quality of blood products in a easy, quick and non-destructive way. Furthermore, Raman spectroscopy could help to identify additives or solutions that are optimal for their long storage and safe application. It even may be also used to study adhesion and aggregation of platelets. Moreover, Raman Spectroscopy could be used to identify and to assess protein and other modifications during the process of blood storage as well as of pathogen inactivation procedures or activation processes by signaling and transporter molecules of platelets and RBCs. In this context, Raman spectroscopy could support recent proteomic strategies in a better understanding of the very complex events during the therapy with blood products and could thus contribute to increased transfusion quality.

References:
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3) Verrier S., et al.; In Situ Monitoring of Cell Death Using Raman Microspectroscopy; Biopolymers,74: 157-162 (2004)

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