Raman spectroscopy as a Process Analytical Technology for cell therapy bioprocessing

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Background

Manufacturing cell therapies at scales required in phase III clinical trials and beyond will require step changes in industrial processes and controls. As more of these therapies are gaining market authorisation, attention is turning to the bioprocesses used for their manufacture, in particular the challenge of gaining higher levels of process control to deliver product of a consistent quality. These processes are diverse, often spanning days to weeks. Thus, monitoring the quality of the cells during manufacture is highly desirable, yet very difficult to implement currently. In this study, we investigated the use of Raman spectroscopy as an in-line optical sensor for bioprocess monitoring

Method

T-cells enrichment from leukapheresis material (half collection) was performed using the CliniMACS®plus (Milytenyi Biotech GmbH) device by positive selection of CD4+/CD8+ cells. T-cell activation and expansion was performed in a DASbox Parallel Mini Bioreactor System equipped with Eppendorf BioBLU 300mL single-use vessels using a fed-batch process. Each vessel was initially filled with 110mL TexMACS media (Miltenyi Biotech GMbH) supplemented with 5% human serum (SeraLab), then fitted with dissolved oxygen (DO), pH, temperature and Raman probes (**Fig.1**). Cells were maintained in the bioreactors with addition of TexMACS media, 5% human serum and IL-2 (120 IU/mL) on days 2 (90mL) and 5 (50mL). T-cells banked from 4 different donors were cultured in parallel in 4 Eppendorf BioBLU single use vessels for 12 days. A total of 3 experiments with the same 4 T-cell banks were performed. The bioreactors were sampled daily to measure cell density and viability using the Vi-Cell XR (Beckman Coulter) cell counter. Culture supernatants were used to obtain offline reference data for glucose, lactate, glutamine, glutamate, and ammonia concentrations using the CuBiAn HT270 automated biochemistry analyzer (OptoCell). Raman spectra were acquired hourly (**Fig.2A**). For chemometric modelling (**Fig.2C**), the target analytes were calibrated by multivariate regression with the Projection to Latent Structures approach from the Raman spectra. Baseline-corrected Raman intensities were normalised and correlation analysis against metabolite concentrations was used (**Fig.3**) to identified surrogate univariate Raman markers whose patterns over time best matched those of off-line measurements (**Fig.4**).

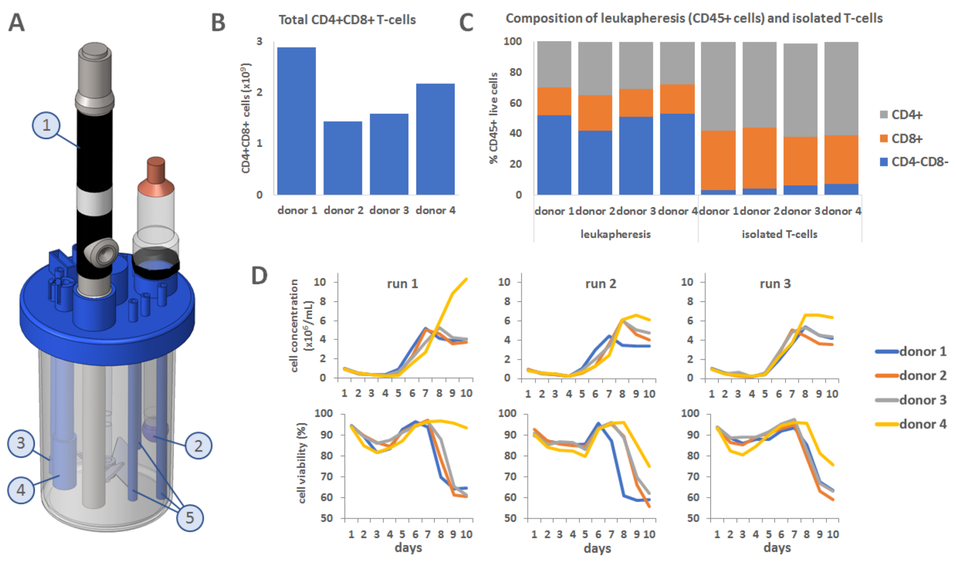

Figure 1. A – Computer-assisted design showing the setup of the Eppendorf BioBLU 300 mL single-use stirred tank bioreactor with Raman spectroscopy probe (1), pH probe (2), temperature probe (3), dissolved oxygen probe (4) and fluid addition and sampling lines (5). **B –** Donor-to-donor variability, expressed in total number of CD4+CD8+ cells in the leukapheresis material. **C –** CD4/CD8 composition of the CD45+ cells from leukapheresis samples before T-cell isolation (first four bars on the left) and after T-cell selection (last 4 bars on the right). **D –** Top row: cell concentration in the bioreactors over time, for all four donors and across all three process runs. Bottom row: corresponding cell viability curves.

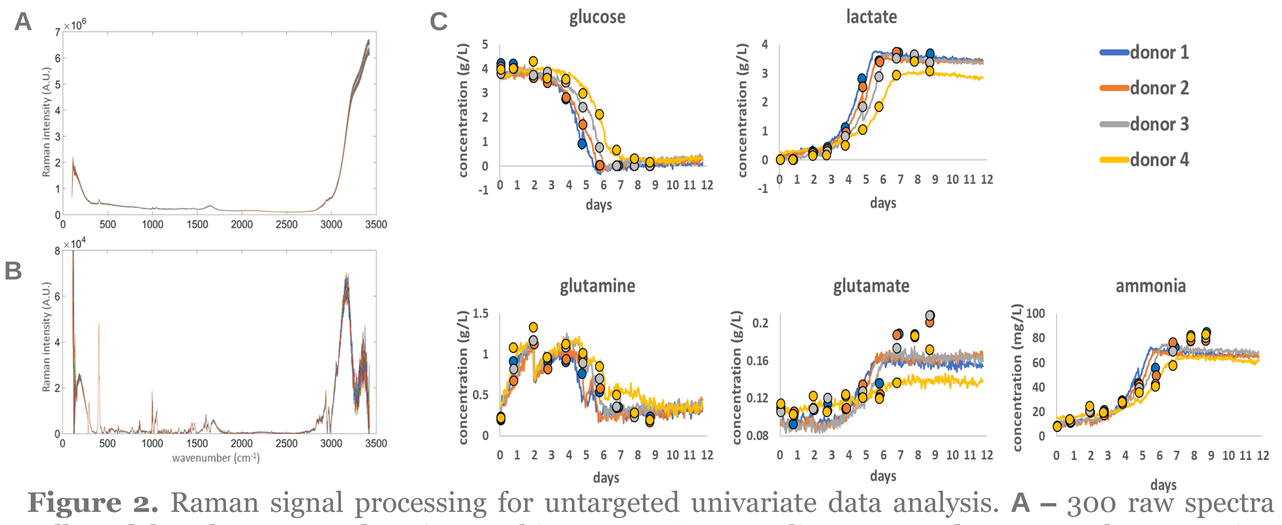

Figure 2. Raman signal processing for untargeted univariate data analysis. **A –** 300 raw spectra collected hourly over 12 days in one bioreactor. **B –** Baseline-corrected spectra. The correction highlights the potential peaks of interest. **C –** Chemometric modelling of glucose, lactate, glutamine, glutamate and ammonia applied to monitoring the T-cell cultures from 4 donors during one process run. Chemometric models were developed using CuBiAn bioanalyser data collected daily from the start of the culture process and over 9 days (dots). Raman spectra were collected hourly over 12 days. The continuous curves are the metabolite concentrations predicted by the models.

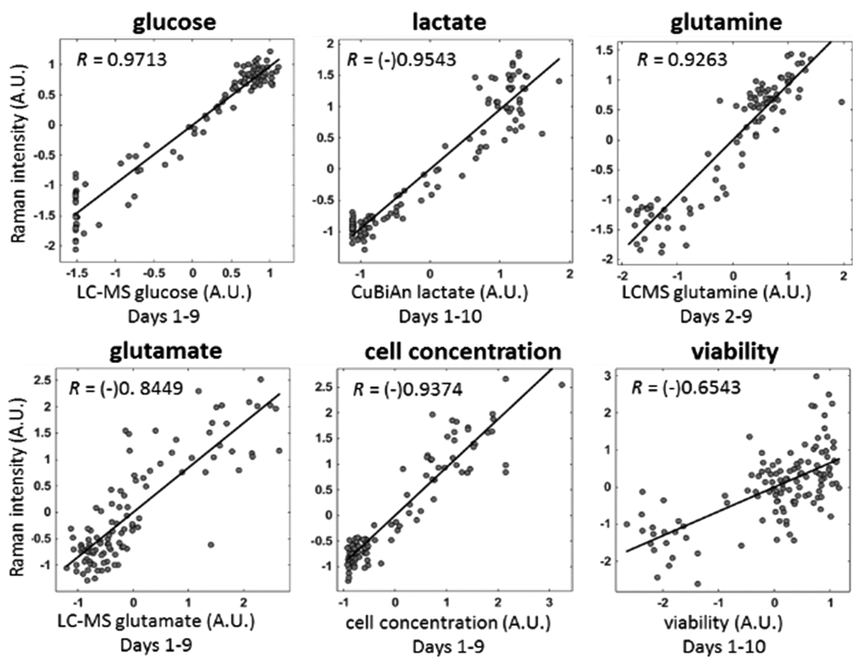

Figure 3. Best correlations observed between univariate Raman markers and off-line measurements for each nutrient, metabolites, cell concentration and viability. All off-line measurements from all donors and all runs where used in these plots, with an average of 108 points used per comparison. The pairwise datasets were auto-scaled using standardization and expressed in arbitrary units (A.U.) to make the scales comparable.

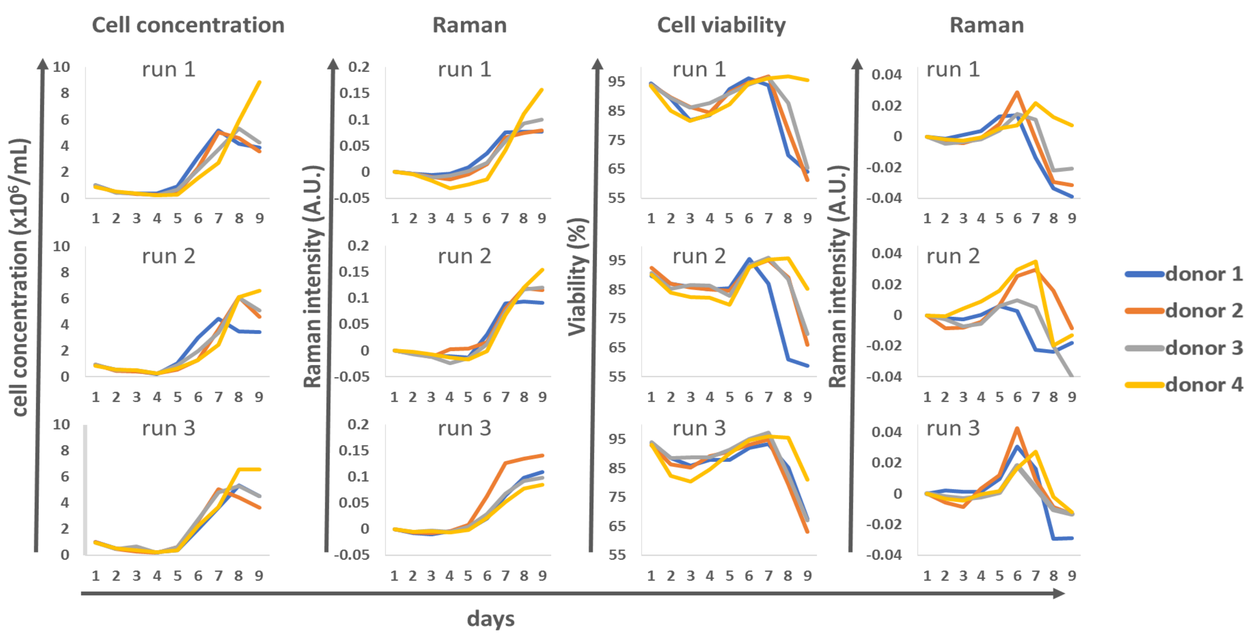

Figure 4. Discrete time course data for process critical parameters such as cell concentration and viability, for all donors and all runs, compared to matching univariate Raman data.

Conclusion

- Despite similar starting compositions, the 4 donor cells exhibited variable proliferation profiles, demonstrating the need for accurate real-time monitoring.
- Chemometric modelling using Raman spectroscopy provided models that can be used to monitor nutrients and metabolites in real-time without the need for sampling the cultures.
- Univariate Raman data was also shown to potentially allow for the real-time monitoring of cell proliferation.
- On these foundations, inferential in-line monitoring could be integrated for rapid automated process controls.

Further reading

Application of Raman Spectroscopy and Univariate Modelling As a Process Analytical Technology for Cell Therapy Bioprocessing. Baradez MO, Biziato D, Hassan E, Marshall D. Front Med (Lausanne). 2018 Mar 5;5:47. doi: 10.3389/fmed.2018.00047. eCollection 2018.

We work with Innovate UK

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