OPEN ACCESS Freely available online

ISSN: 2153-2435
Pharmaceutica Analytica Acta

Mini Review

Evolving Ghost Cytometry may take away Labels for Manufacturing of Cell-Based Therapy Products

Keisuke Wagatsuma[1], Hiroko Nomaru[1], Hirofumi Nakayama[1], Sadao Ota[1,2*]

[1]*Thinkcyte, K.K., 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan*

[2]*Research Center for Advanced Science and Technology, The University of Tokyo, Meguroku, Tokyo, Japan*

ABSTRACT

Cell-based therapies, including stem cell therapies and regenerative medicine, offer transformative solutions for previously untreatable diseases. However, their manufacturing processes differ significantly from traditional drug production, requiring stringent Quality Control (QC) measures for the cells to ensure safety, efficacy and reproducibility. Fluorescence-based Flow Cytometry (FCM) is one of current QC methods, but rely on molecular markers that have potential impact on cell states and functionality, while also increasing costs. Moreover, there are cases that appropriate markers for accurately predicting cell function or differentiation are not available. Label-Free Ghost Cytometry (LF-GC) addresses these challenges by leveraging high-resolution and high-content morphological data and machine learning to classify and sort cells without fluorescent labels. LF-GC enables non-invasive, real-time analysis, preserving cell functionality and reducing manufacturing costs. Its applications extend beyond basic QC to include cell differentiation assessment and enrichment of potentially therapeutic cell populations. Recent studies have demonstrated its utility in analyzing blood and immune cells, induced pluripotent stem cells and retinal progenitor cells, highlighting its potential for improving cell manufacturing processes. Looking ahead, integrating LF-GC with unsupervised learning and other molecular techniques such as single cell sequencing will further expand the utility of LF-GC. As a scalable platform that can be automated, LF-GC has the potential to improve cell manufacturing by making advanced cell therapies safer, more accessible and cost-effective.

Keywords: Cell-based therapy; Machine learning; Label-free cell analysis; Ghost cytometry

INTRODUCTION

Cell-based gene therapies, stem cell therapies and regenerative medicine are rapidly advancing to provide solutions for previously untreatable diseases [1]. These therapies, however, differ fundamentally from traditional small-molecule drugs in their manufacturing requirements. They demand detailed control of biological variability and strict adherence to Good Manufacturing Practices (GMP) to ensure efficacy, reproducibility and safety. Meeting these requirements has significantly increased production costs, often making these life-saving therapies inaccessible to many patients [2,3].

To address these challenges, automation and process optimization have been pursued [3]. However, QC remains one of bottlenecks, with fluorescence-based FCM as the current gold standard [4,5]. While FCM provides high-throughput and reproducible cell assessments with minimal human intervention, it also involves staining with antibodies or dyes. These reagents can introduce cytotoxicity or alter cell functionality potentially compromising its downstream research and therapeutic applications [6]. Furthermore, the reliance on trained operator's increases production costs and potentially limits scalability [4,7,8]. Lastly, appropriate markers to define the desired cell function or differentiation are not always available.

Correspondence to: Sadao Ota, Research Center for Advanced Science and Technology, The University of Tokyo, Meguroku, Tokyo, Japan, E-mail: sadaota@g.ecc.u-tokyo.ac.jp

Received: 20-Jan-2025, Manuscript No. PAA-25-28312; **Editor assigned:** 22-Jan-2025, PreQc No. PAA-25-28312 (PQ); **Reviewed:** 05-Feb-2025, QC No. PAA-25-28312; **Revised:** 12-Feb-2025, Manuscript No. PAA-25-28312 (R); **Published:** 19-Feb-2025, DOI: 10.35248/2153-2435.25.16.802

Citation: Wagatsuma K, Nomaru H, Nakayama H, Ota S (2025). Evolving Ghost Cytometry may take away Labels for Manufacturing of Cell-Based Therapy Products. Pharm Anal Acta. 16:802.

To mitigate the challenges associated with labeling, several label-free cell characterization techniques, such as digital holographic microscopy, Raman spectroscopy and infrared spectroscopy, have been developed [7, 9-12]. These approaches monitor key metrics such as cell viability, metabolites and T-cell subset ratios. However, these methods are limited by slow image acquisition, intensive data processing and an inability to sort cells. These shortcomings drive the need for high throughput, label-free technology for cell assessment and sorting.

LF-GC can address these limitations by leveraging high-content cell morphology measures and machine learning methods to evaluate a wide range of functional cell phenotypes [13-17]. By eliminating the need for fluorescent labels, LF-GC tackles the safety, cost and scalability challenges associated with conventional QC methods, making it a potentially transformative tool for cell manufacturing. Importantly, LF-GC-based cell sorters can be used to enrich for morphologically expressed phenotypes and then validate them using molecular expression and cellular function in downstream assays. This review explores recent advancements in LF-GC and its applications in cell manufacturing, while also discussing its limitations and future directions for expanding its use in this evolving field.

LITERATURE REVIEW

Label-free ghost cytometry and its unique value in cell manufacturing

Ghost Cytometry (GC), introduced in 2018, is an image reconstruction-free image analysis approach that enabled the first high-throughput, fluorescence "Imaging" cell sorter [18]. Unlike conventional imaging methods, GC relies on the direct analysis of compressively measured temporal Ghost Motion Imaging (GMI) signals that are obtained with a single pixel detector when cells pass through a structured light pattern. These signals are processed using machine learning algorithms to classify and sort cells in real-time at high throughput.

LF-GC expands the original GC methodology by incorporating multiple label-free optical modalities, including forward scattering (fsGMI), backscattering (bsGMI), diffractive (dGMI) and bright-field Ghost Motion Imaging (bfGMI) waveform signals. This integration enhances the ability of LF-GC to capture intricate morphological data, allowing for precise classification of cell types, functions and states.

Thus far, the demonstrated advantages of LF-GC in cell manufacturing lie in its ability to eliminate the need for fluorescent dyes or antibodies and, by preserving the natural state of cells, LF-GC ensures functionality for downstream applications. It also holds potential to distinguish the cell function or differentiations by morphological analysis even if there are no appropriate markers. Furthermore, the process is non-invasive, cost-effective and compatible with automation. These attributes make LF-GC especially well-suited for cell assessment in the manufacturing process, where consistency, scalability and prompt and quantitative decision-making are critical.

Applications of LF-GC in cell manufacturing

Over the last few years, LF-GC has demonstrated its utility in various applications that are important to manufacturing of cell-based therapies. One of its core functions is QC, where it excels in tasks such as cell counting, viability assessment and impurity detection [17]. For example, LF-GC can accurately quantify cell count and distinguish live, apoptotic and dead cells in heterogeneous Peripheral Blood Mononuclear Cells (PBMCs) [17]. and induced Pluripotent Stem Cells (iPSCs) [16]. This function allows the proportion of viable cells in a therapeutic product to be efficiently evaluated. Additionally, its high-resolution analysis enables the detection of non-cellular particulates with sizes similar to cells, such as polystyrene microbeads or cellulose particles [17], which are often challenging to identify using traditional flow cytometers that can analyze the average information for each cell. While being underestimated, the contamination of cellular products by microplastic or other particles can interfere with the therapeutic effects of products [19-22], so we expect to see increasing attention to this issue and a need for their rapid detection.

Beyond the basic QC, LF-GC has proven effective in classifying cell differentiations without the use of molecular markers [13-15]. The first applications assessed the differentiation of iPSCs into Neuro Ectodermal Cells (NECs) and Hepatic Endodermal Cells (HECs) [13]. In this study, surface markers were used to create ground truth labels for training machine learning models that classify cells based on their multimodal label-free morphological information. Once trained, the model could successfully predict the surface marker-based labels from the label-free waveforms without observing the surface markers. A more recent study showed a new strategy of applying LF-GC to assess the differentiation states of iPSC-derived Retinal Progenitor Cells (RPCs) and sort them from dissociated retinal organoids, even in the absence of ground truth surface markers [14]. Here, a machine-based classifier was pre-trained with an RPC fluorescent protein reporter ES cell line and then applied to RPCs differentiated from a non-reporter iPSC line, successfully allowing for the label-free enrichment of specific populations at desired differentiation stages. Enrichment of RPCs differentiated from iPSC line was validated through gene expression analysis and immuno- fluorescence. The maturations of photoreceptors were also confirmed with transplantation of retinal spheroids in the retinal degeneration rats. In addition, LF-GC was also employed to classify the macrophage polarization from M0 to M1 using THP-1 cell in high-content CRISPR screening [15]. These collective findings highlight the potential of LF-GC not only as a tool for QC analysis but also as an integral component of cell manufacturing processes. By enabling the selective sorting of untouched, differentiated cells, LF-GC offers a path to safer, versatile and efficient cell production, ultimately leading to improving the quality of therapeutic cell products.

Furthermore, LF-GC also shows accuracy and versatility in identifying other diverse cell types. It has been applied to classify immune cell subtypes, including T cells and non-T cells within white blood cell populations [17]. Also, LF-GC can evaluate differences in cell states, such as distinguishing activated from

resting T cells [17], or even detecting subtle distinctions, such as exhaustion from non-exhausted immune cells [15]. Emerging data, though not yet published, suggests that LF-GC can also predict cellular metabolic or proliferation activities based on morphological information, indicating its potential to assess the functional properties and estimate the efficacy of therapeutic cells. These capabilities highlight LF-GC's analytic power, enabling it to provide critical insights into the therapeutic functionality of cell products, surpassing current quality control standards and enhancing their clinical and therapeutic value.

DISCUSSION

Future developments

Thus far, the demonstrated strategies of training the morphological classifier in LF-GC have mostly relied on ground truth labels based on molecular markers. However, there are cases that suitable molecular markers or training cell samples may not be available for training the morphologic classifier. For example, when protein expression markers lack sufficient specificity to identify the desired cell function, even if cell morphologies contain predictive information, non-target cells may be included in the labeled population, compromising the classifier's performance [23]. To address these challenges, integrating unsupervised learning methods and other molecular analysis techniques such as single-cell RNA sequencing methods could be a promising alternative and further expand the utility of LF-GC.

From the perspective of future personalized cell therapy, LF-GC can present exciting potential by leveraging its diagnostic capabilities to analyze cell morphology in patient's samples [16, 24]. This approach may bridge the gap between precision diagnostics and therapeutic innovations, ultimately envisioning more tailored and effective treatments. Future development of generalized models that integrate the analysis of diverse therapeutic and diagnostic cell types may further improve the robustness, efficacy and clinical relevance of LF-GC.

In addition, the implementation of LF-GC in in-line analyzers and cell sorters will enable continuous cell monitoring and sorting based on detailed optical characteristics. These would simplify workflows and minimize contamination risks and reduce manual intervention, which can significantly improve manufacturing efficiency and reliability.

CONCLUSION

In summary, label-free ghost cytometry is set to contribute to quality control and improvement in therapeutic cell manufacturing. By eliminating the need for fluorescent labeling, it reduces costs, increases safety and preserves the functionality of therapeutic cells. Its ability to perform real-time high-content morphological analysis positions it as a potentially key tool for ensuring consistency and quality in cell-based products. With further innovation, LF-GC can help to make advanced cell therapies more accessible, affordable and effective.

ACKNOWLEDGEMENT

Sadao Ota is the founder and shareholder of ThinkCyte K.K, a company engaged in the development of an ultrafast machine-vision-based flow cytometers. Keisuke Wagatsuma, Hiroko Nomaru and Hirofumi Nakayama are employees of ThinkCyte K.K. and have shares of stock options from ThinkCyte K.K.. Sadao Ota is a board member of ThinkCyte K.K., and Hirofumi Nakayama and Sadao Ota filed patents and applications related to the LF-GC method.

REFERENCES

1. Bashor CJ, Hilton IB, Bandukwala H, Smith DM, Veiseh O. Engineering the next generation of cell-based therapeutics. Nat Rev Drug Discov. 2022;21(9):655-675.
2. Johanna I, Daudeij A, Devina F, Nijenhuis C, Nuijen B, Romberg B, et al. Basics of advanced therapy medicinal product development in academic pharma and the role of a GMP simulation unit. Immunooncol Technol. 2023;20:100411.
3. Kaiser AD, Assenmacher M, Schröder B, Meyer M, Orentas R, Bethke U, et al. Towards a commercial process for the manufacture of genetically modified T cells for therapy. Cancer Gene Ther. 2015;22(2):72-78.
4. Campbell JD, Fraser AR. Flow cytometric assays for identity, safety and potency of cellular therapies. Cytometry B Clin Cytom. 2018;94(5):725-735.
5. Sarikonda G, Mathieu M, Natalia M, Pahuja A, Xue Q, Pierog PL, et al. Best practices for the development, analytical validation and clinical implementation of flow cytometric methods for chimeric antigen receptor T cell analyses. Cytometry B Clin Cytom. 2021;100(1):79-91.
6. Andrzejewska A, Jablonska A, Seta M, Dabrowska S, Walczak P, Janowski M, et al. Labeling of human mesenchymal stem cells with different classes of vital stains: Robustness and toxicity. Stem Cell Res Ther. 2019;10:1-6.
7. Cossarizza A, Chang HD, Radbruch A, Acs A, Adam D, Adam-Klages S, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies. Eur J Immunol. 2019;49(10): 1457-1973.
8. Mues M, Winkels M, Lange K, Stiem L, Janz E, Biedermann S, et al. Flow cytometric assays for CAR T cell manufacturing and patient immunomonitoring, comprising specific CAR detection reagents, stabilized pre-mixed cocktails and automated data acquisition and analysis. Cytotherapy. 2020;22(5):S200.
9. Pais DA, Galrão PR, Kryzhanska A, Barbau J, Isidro IA, Alves PM. Holographic imaging of insect cell cultures: Online non-invasive monitoring of adeno-associated virus production and cell concentration. Processes. 2020;8(4):487.
10. Tulsyan A, Wang T, Schorner G, Khodabandehlou H, Coufal M, Undey C. Automatic real-time calibration, assessment and maintenance of generic Raman models for online monitoring of cell culture processes. Biotechnol Bioeng. 2020;117(2):406-416.
11. Zavala-Ortiz DA, Ebel B, Li MY, Barradas-Dermitz DM, Hayward-Jones PM, Aguilar-Uscanga MG, et al. Interest of locally weighted regression to overcome nonlinear effects during *in situ* NIR monitoring of CHO cell culture parameters and antibody glycosylation. Biotechnol Prog. 2020;36(1):e2924.
12. Chen M, McReynolds N, Campbell EC, Mazilu M, Barbosa J, Dholakia K, et al. The use of wavelength modulated Raman spectroscopy in label-free identification of T lymphocyte subsets, natural killer cells and dendritic cells. PLoS One. 2015;10(5):e0125158.

13. Masashi U, Yoko K, Keisuke T, Kazuki T, Hikari M, Hiroaki A, et al. *In silico*-labeled ghost cytometry. Elife. 2021;10.
14. Iwama Y, Nomaru H, Masuda T, Kawamura Y, Matsumura M, Murata Y, et al. Label-free enrichment of human pluripotent stem cell-derived early retinal progenitor cells for cell-based regenerative therapies. Stem Cell Reports. 2024;19(2):254-269.
15. Tsubouchi A, An Y, Kawamura Y, Yanagihashi Y, Nakayama H, Murata Y, et al. Pooled CRISPR screening of high-content cellular phenotypes using ghost cytometry. Cell Rep Methods. 2024;4(3).
16. Kawamura Y, Nakanishi K, Murata Y, Teranishi K, Miyazaki R, Toda K, et al. Label-free cell detection of acute leukemia using ghost cytometry. Cytometry A. 2024;105(3):196-202.
17. Teranishi K, Wagatsuma K, Toda K, Nomaru H, Yanagihashi Y, Ochiai H, et al. Label-free ghost cytometry for manufacturing of cell therapy products. Sci Rep. 2024;14(1):21848.
18. Ota S, Horisaki R, Kawamura Y, Ugawa M, Sato I, Hashimoto K, et al. Ghost cytometry. Science 2018;360:1246–1251.
19. Molina SA, Davies SJ, Sethi D, Oh S, Durand N, Scott M, et al. Particulates are everywhere, but are they harmful in cell and gene therapies?. Cytotherapy. 2022;24(12):1195-1200.
20. Jack T, Brent BE, Boehne M, Müller M, Sewald K, Braun A, et al. Analysis of particulate contaminations of infusion solutions in a pediatric intensive care unit. Intensive Care Med. 2010;36:707-11.
21. Gustafson HH, Holt-Casper D, Grainger DW, Ghandehari H. Nanoparticle uptake: The phagocyte problem. Nano Today. 2015;10(4):487-510.
22. Perez M, Maiguy-Foinard A, Barthélémy C, Décaudin B, Odou P. Particulate matter in injectable drugs: Evaluation of risks to patients. Pharm Technol Int. 2016;1(2):91-103.
23. Scala S, Aiuti A. *In vivo* dynamics of human hematopoietic stem cells: Novel concepts and future directions. Blood Adv. 2019;3(12): 1916-1924.
24. Suzuki K, Watanabe N, Torii S, Arakawa S, Ochi K, Tsuchiya S, et al. BCR: ABL1-induced mitochondrial morphological alterations as a potential clinical biomarker in chronic myeloid leukemia. Cancer Sci. 2024.