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Commentary

Revolutionizing Single-Cell Cytokine Profiling: The HL-Chip and the Future of Immune Function Analysis

Kin Jang*

Department of Clinical Medical, Nanchang University, Jiangxi, China

DESCRIPTION

Protein secretion is a fundamental biological process underpinning key physiological functions from immune signaling and inflammation to tissue repair and disease progression. Among these secreted biomolecules, cytokines play a central role in immune regulation, serving as messengers that direct cellular activity and orchestrate responses to infection, cancer and other insults. Despite their importance, the ability to measure secreted proteins, particularly cytokines, at the single-cell level has remained technically challenging. Conventional bulk measurement tools obscure cellular heterogeneity and many single-cell methods suffer from limitations in multiplexing, throughput or true secretion detection. A new method based on an advanced hierarchical loading Microwell Chip (HL-Chip) is poised to transform this space by offering efficient, high-resolution and highly multiplexed analysis of protein secretion at the single-cell level.

Limitations of traditional and single-cell protein profiling methods

Traditional tools such as ELISA, antibody arrays and mass spectrometry have provided a wealth of data on protein secretion in bodily fluids or mixed cell populations. However, these methods measure average responses, failing to resolve critical nuances such as cellular diversity or polyfunctionality attributes that are particularly important in fields like immunotherapy or infectious disease. Single-cell methods like ELISpot and Intracellular Cytokine Staining (ICS) introduced a new level of granularity but carry inherent compromises. ELISpot captures secreted proteins but is limited in multiplexing, while ICS provides higher multiplicity but only assesses intracellular cytokine presence, not true secretion. Additionally, ICS involves secretion inhibitors and fixation, making downstream functional analysis or cell recovery impossible.

To address these gaps, researchers have turned to microfluidic platforms, which offer a promising avenue for miniaturized, high-throughput and controllable assays. Microwells, microchambers and droplet systems each have shown utility in capturing the real-time secretory profiles of single immune cells like T cells, B cells, macrophages and even tumor cells. Yet, despite advances, each of these approaches still faces barriers such as Poisson-distributed loading, limited multiplexing, or difficulty in precise cell-bead pairing.

The HL-Chip technology introduces an elegant solution to many of these challenges. Unlike earlier microwell approaches that relied on stochastic cell loading, this platform employs a hierarchical microwell architecture to deterministically pair single cells with multiple antibody-coated microbeads. The innovation here lies not only in the deterministic loading which minimizes empty wells and reduces sample waste but also in the use of beads with spectral and spatial barcodes. These microbeads are engineered to detect multiple proteins simultaneously, significantly increasing multiplexing capability. Each microwell hosts a single cell and two differently sized beads, with each bead carrying three different capture antibodies, enabling six-plex cytokine profiling.

This enhanced HL-Chip was successfully applied to profile cytokine secretion in T cells and macrophages, providing a real-world demonstration of its potential. In the case of antigen-specific T cell receptor-engineered T cells, the platform uncovered a transient but intense cytokine burst early after stimulation a phenomenon that may have been obscured in bulk or lower-resolution assays. Moreover, the system revealed polyfunctional heterogeneity in T cell responses, shedding light on how individual cells within a seemingly homogeneous population can differ drastically in their functional output.

Such discoveries are not just academically intriguing they have tangible clinical relevance. In cancer immunotherapy, for instance, the effectiveness of T cell-based treatments often hinges on the functionality of individual cells. A technology that can identify highly polyfunctional cells (which tend to be more potent in fighting tumors) could refine cell therapy manufacturing, improve patient stratification and even aid in the monitoring of therapeutic efficacy over time.

Another strength of the HL-Chip system is its compatibility with live-cell retrieval. Unlike ICS or fixed-cell assays, the HL-Chip

Correspondence: Kin Jang, Department of Clinical Medical, Nanchang University, Jiangxi, China, E-mail: jang@gmail.com

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allows researchers to recover viable cells after secretion profiling, opening the door to downstream analysis such as single-cell RNA sequencing, epigenetic profiling, or functional assays. This integrative capacity is vital for building a holistic view of immune cell behavior linking cytokine secretion to gene expression, receptor signaling, or metabolic states.

Importantly, the platform's versatility suggests applications beyond immunology. Any cell type that secretes biologically active proteins whether neurons releasing neurotransmitters, stem cells producing growth factors, or cancer cells modulating their microenvironment can be studied using this approach. Furthermore, with future improvements in bead coding and antibody conjugation, the multiplexing capacity could easily be expanded beyond six analytes, making it feasible to track dozens of proteins from a single cell simultaneously.

Despite these advances, challenges remain. The current reliance on antibody-coated beads necessitates rigorous validation of antibody specificity and efficiency. Additionally, while the deterministic loading addresses some limitations of traditional microfluidics, further automation and integration with analytical pipelines will be essential for widespread adoption, particularly in clinical settings.

CONCLUSION

In conclusion, the HL-Chip-based single-cell secretion assay represents a significant leap forward in the field of functional immunology and cellular phenotyping. By overcoming long-standing trade-offs between sensitivity, multiplexing and live-cell compatibility, this technology not only enhances our ability to dissect immune responses at unprecedented resolution but also provides a platform for the next generation of diagnostic tools and therapeutic monitoring systems. As single-cell analysis continues to drive biological discovery and precision medicine, tools like the HL-Chip will be at the forefront of translating cellular complexity into actionable insights.