APPLICATION NOTE

Bruker Proteomic Product Suite for Cellular and Regenerative Medicine

Bruker's functional proteomics reveals unique secretomic signatures and insights into cellular and regenerative medicine

In this Application Note we outline:

- Overcoming challenges in cellular and regenerative medicine
- How understanding cellular immune function is critical for predicting protective immunity
- How detecting multiplexed serum protein is critical in predicting response in cellular & regenerative medicine
- Resolving heterogeneity in cytokine production profiles among hematopoietic stem and progenitor cells (HSPCs)
- Revealing aberrant cytokine signatures of progenitor myeloid cells in disease progression
- Informing targeted combination therapy to overcome resistance with single-cell intracellular proteomics

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High Level Challenges and Applications

Application 1: Accelerating Regenerative Medicine with Fibroblasts

Application 2: Resolving Heterogeneity in Cytokine Production Profile Among HSPCs

Application 3: Revealing Aberrant Cytokine Signatures of Progenitor Myeloid Cells in Disease Progression

Application 4: Informing Targeted Combination Therapy to Overcome Resistance with Single-Cell Intracellular Proteomics

Bruker Product Types that Address These Challenges:

- CodePlex Secretome
- Single-Cell Secretome (Human)
- Single-Cell Secretome (Mouse)
- Single-Cell Intracellular Proteome

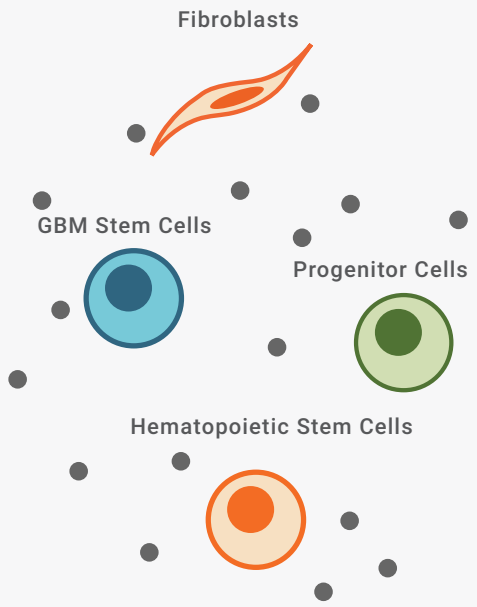
Overcoming Challenges in Cellular and Regenerative Medicine

Stem cells have been employed in the treatment of many different types of diseases due to their ability to differentiate into various cell types. Hematopoietic stem cells (HSCs), can differentiate into precursor progenitor cells, which can then differentiate into blood cells. Stem cells in regenerative medicine provide the opportunity to encourage the production of deficient cells and help restore normal function.

Heterogeneity in cytokine production and aberrant cytokine signatures can create challenges in cellular and regenerative medicine. Bruker's platform is helping leaders accelerate their workflows by functionally defining each cell type to inform more effective therapies.

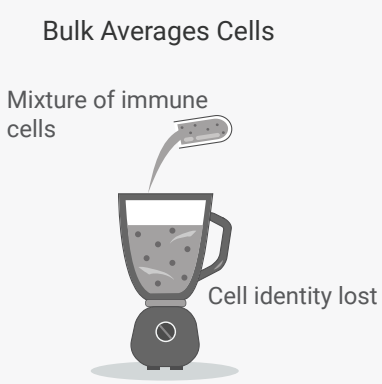
- **Challenge 1: Requires Highly Multiplexed CodePlex Secretome Solution**
- **Challenge 2: Requires Single-Cell Functional Proteomics and CodePlex Secretome Solution**
- **Challenges 3 & 4: Require Single-Cell Functional Proteomics Solution**

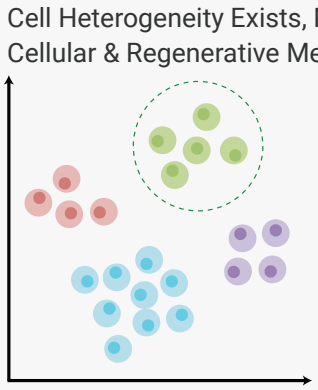
Cell Types and Cytokines Implicated



Functionally defining each cell type involved in the immune response for cellular and regenerative medicine.

Why Cell Subsets for Multiplexing Cytokines Matter in Cellular & Regenerative Medicine





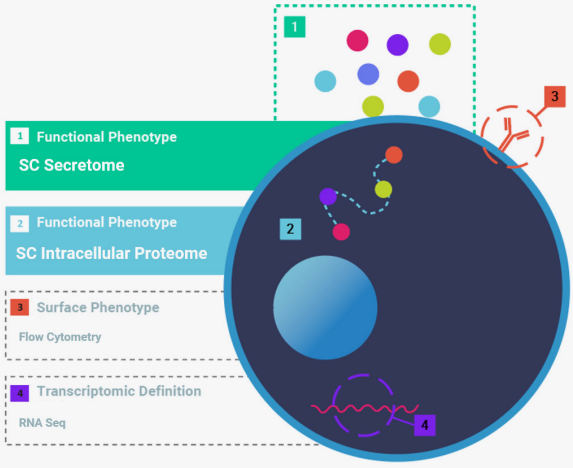
Traditional technologies average serum protein information from all cells. Bruker's cellullar functional phenotyping uncovers cellular differences to identify functional mechanisms in cellular and regenerative medicine.

Understanding Cellular Immune Function is Critical for Understanding Cell Differentiation and Regenerative Capabilities

Traditional technologies average serum protein information from all cells. In a variety of trials†, stratification of responders from non-responders is not possible with status quo technologies. Data shows that what specific cytokines are produced by each heterogenous immune cell matters, and Bruker's cellullar functional phenotyping uncovers these cellular differences.

Through analysis of cellular RNA or surface phenotypes alone, you may be missing essential functional extracellular phenotypic differences that reveal the biological drivers of patient response. Bruker's single-cell functional proteomics fills the existing gap in complete cellular characterization.

Multiplexed Proteomic Characterization: Filling the Existing Gap in Full Cellular Characterization from Single-Cells



Through analysis of cellular RNA or surface phenotypes alone, functional extracellular and intracellular phenotypic differences that reveal the biological drivers of patient response may be missed.

Detecting Multiplexed Serum Protein from Ultra Low Sample Volume is Critical in Predicting Response in Cellular & Regenerative Medicine

The IsoLight is the only system that enables researchers to obtain highly multiplexed cytokine data without advanced training and without interaction with the samples. Furthermore, the IsoLight is also the only system to:

(1) Perform multiplexed proteomic detection of 20-40 cytokine markers simultaneously, to provide early predictive metrics of functional and inflammatory cytokines

(2) Provide an automated, all-in-one system, for increased work-away time

(3) Handle a smaller amount of sample volume if large blood draws are not possible, making it capable of handling a wider range of clinical sample sizes

By functionally defining each cell type involved in the immune response, researchers can better understand the functional mechanisms for the development of patient biomarkers and novel therapies in cellular and regenerative medicine.

CodePlex Secretome Panels

Panel Menu

Granzyme B, IFN-y, MIP-1a, Perforin, TNF-a, TNF-B, GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21, CCL11, IP-10, MIP-1B, RANTES, IL-4, IL-10, IL-13, IL-22, sCD137, sCD40L, IL-1B, IL-6, IL-17a, IL17F, MCP1, MCP-4, IL-18, TGF-a, BCA-1, IL-12-p40, MIF, EGF, PDGF-BB

Stem Cell Signaling

IL-17A, MIP-1a, MIP-1b, IL-6, IL-8, IFN-y, GM-CSF, IL-4, IL-10, TNF-a, MCP-1, IL-2, IL-15, Rantes (MPN), IL1a, IL1b, IL12, CCL2, CXCL5 * (MPN)

Human Adaptive Immune

IL-17A, MIP-1a, IL-9, MIP-1b, IL-6, IL-7, IL-8, IFN-y, IP-10, GM-CSF, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-13, IL-2, Perforin, sCD40L, sCD137, TNF-b, Granzyme B, IL-15

Human Cytokine Storm Panel

IL-1b, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, MCP-1, GM-CSF, MIP-1a, MIP-1b, TNF-a, IFN-y

Human Innate Immune

IL-17A, MIP-1a, MIP-1b, IL-6, IL-7, IL-8, IFN-y, IP-10, GM-CSF, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-2, Perforin, sCD40L, sCD137, TNF-b, Granzyme B, IL-15, PDGF-BB

Cancer Signaling

IL-6, IL-7, IFN-y, IL-4, IL-5, IL-10, TNF-a, MCP-1, IL-13, IL-2, EGF, PDGF-BB, Rantes (MPN), MIF, FGF, HGF, IL1a, IL1b, IL12

Status Quo Multiplexed Bulk Analysis	
X	Up to 100-200 uL per sample (for replicates)
X	6-10 hours of hands-on sample prep time
X	Workflow requires multiple steps and user interaction points
X	Fill 96 samples before run
X	Multiple systems required to generate and analyze data
X	Limit of Detection: 5-5000 pg/ml
X	Data analysis and visualizations require much user input and are not automated

CodePlex Secretome	
√	11 uL per samples (for replicates)
√	5 minutes of hands-on time
√	Completely automated workflow
√	Modular, load 8-64 samples per run
√	One system: The IsoLight
√	Limit of Detection: 5-5000 pg/ml
√	State-of-the-art data analysis software with advanced visualizations

The CodePlex Secretome Solution measures 30+ cytokines in bulk, automated on the IsoLight system, and can selectively run eight conditions a chip in “MacroChambers” across eight chips on a single run. Easily run replicates with a small sample volume: 5.5 uL per microwell (11 uL per sample replicate).

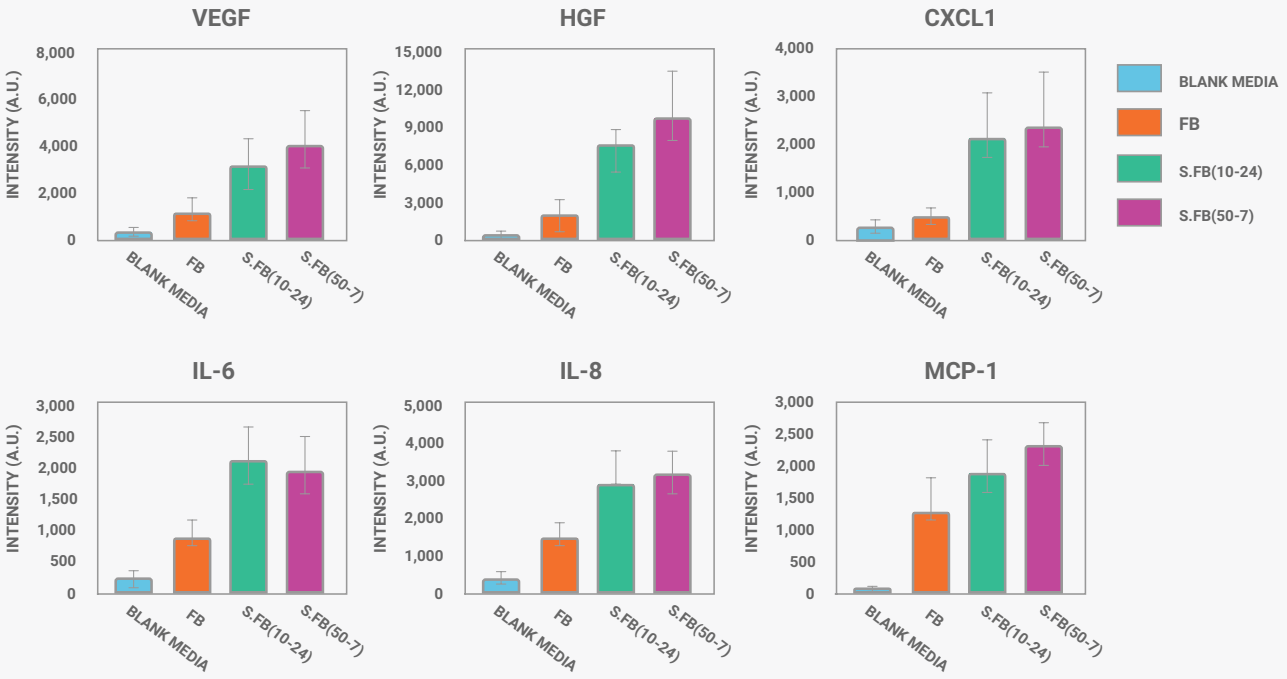
Application 1 – Accelerating Regenerative Medicine with Fibroblasts

CodePlex Proteomics Reveals Insight into Fibroblast Driven Cellular Senescence

Products Used

CodePlex Secretome

Uncovering the Secretome of Senescent Fibroblasts with CodePlex Secretome



Secreted factors from senescent fibroblasts that contribute to cellular regeneration [1].

Applying CodePlex Secretome to Cellular Senescence

- Factors from senescent fibroblasts contribute to inflammation and promotion of cancer development.
- Functional proteomics reveals insight into various challenges associated with delivering growth factors and cytokines for various therapeutic applications.
- Growth factor production from senescent fibroblasts was significantly increased compared to the presenescent fibroblasts, which demonstrates potential for promoting microvasculature formation *in vitro* and *in vivo*.

Reference: [1]

Application 2 – Resolving Heterogeneity in Cytokine Production Profile Among HSPCs

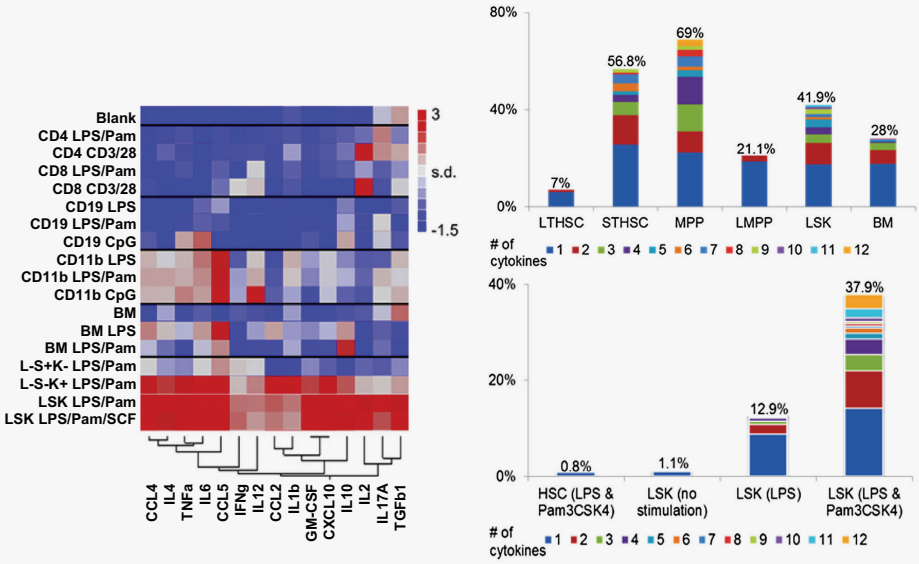
Bruker's Functional Proteomics Reveals Secretomic Signatures in Stem and Progenitor Cells

Products Used

CodePlex Secretome

Single-Cell Secretome

Single Cell and Highly-Multiplexed Bulk Analysis of Stem and Progenitor Cell Types in Response to Infectious Pathogens



Left: Highly-multiplexed proteomic quantification of cytokines in bulk cell-culture medium in order to compare cytokine production of different cell subsets. Right: Single-cell secretomic analysis of different cell subsets in response to infectious pathogens [2].

Highlights of Bruker's Functional Proteomics and Functional Significance of HSPC-Produced Cytokines

- Bruker's single-cell technology helped show that ST-HSCs and MPPs can translate danger signals arising from an infection into cytokine signals that can directly regulate stress-induced hematopoiesis.
- CodePlex technology showed that in response to stimulation, T, B, and myeloid cells were still significantly less potent cytokine producers than LSK cells.
- This study has uncovered an important property of HSPCs that enables them to convert danger signals into versatile cytokine signals for the regulation of stress hematopoiesis.

Reference: [2]

Application 3 – Revealing Aberrant Cytokine Signatures of Progenitor Myeloid Cells in Disease Progression in Mice

Bruker Single-Cell Secretome Technology Reveals Distinct Cytokine Profiles Associated with Pathogenesis

Products Used

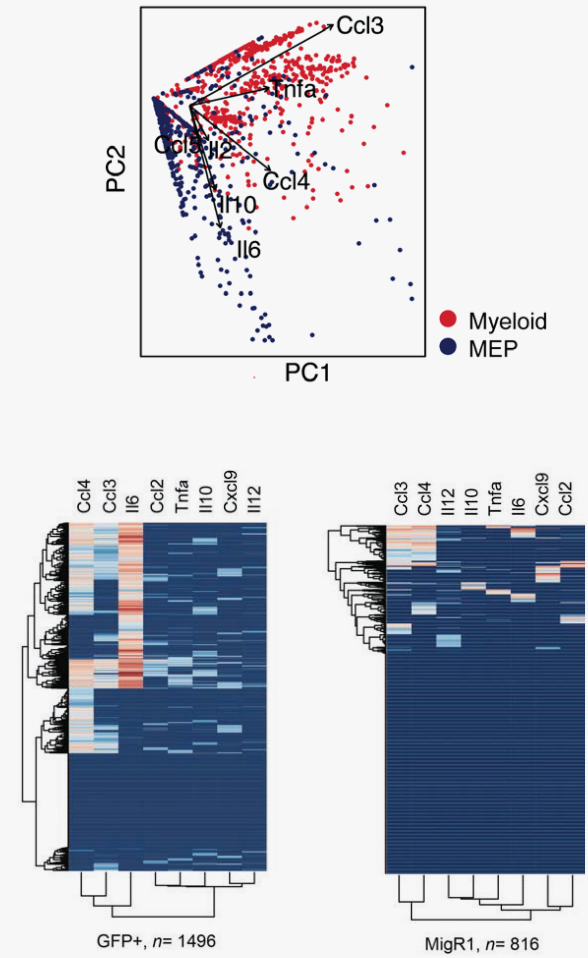
Single-Cell Secretome (Mouse)

Highlights of Identifying Aberrant Cytokine Signatures in Mature and Progenitor Myeloid Cells

- Data generated with the single-cell secretome solution demonstrated that JAK1/2 inhibition leads to a rapid, potent reduction in serum cytokine levels, consistent with the rapid clinical benefits seen with JAK inhibitor therapy and demonstrating this is a direct effect of JAK kinase inhibition on cytokine production.
- Hematopoietic cells in Myelofibrosis (MF) show significant up-regulation of a spectrum of pro-inflammatory cytokines, elevation of cellular heterogeneity in cytokine secretion, and increased multifunctional cytokine production, which are not observed in normal hematopoietic cells.
- Both mature and progenitor myeloid cells contribute to increased cytokine production, and more interestingly they show distinct cytokine profiles suggesting their different roles in MF pathogenesis.

Reference: [3]

Pathogenic Secretion of Multiple Cytokines by MF Cells



Top: PCA analysis of single cell cytokine secretion data from MEP and myeloid cells identified two principal components, largely defined by production of Il6 and Il10 (PC1, MEP) Ccl3, and Tnfa (PC2, myeloid). Bottom: Single-cell secretomic analysis of Bone Marrow (BM) cells revealed a striking increase in the cytokine production levels and heterogeneity [3.]

Application 4 – Informing Targeted Combination Therapy to Overcome Resistance with Single-Cell Intracellular Proteomics

Single-Cell Phosphoproteomics Identifies Adaptive Mechanism of Resistance Independent of GBM Cancer Stem Cell Phenotype

Products Used

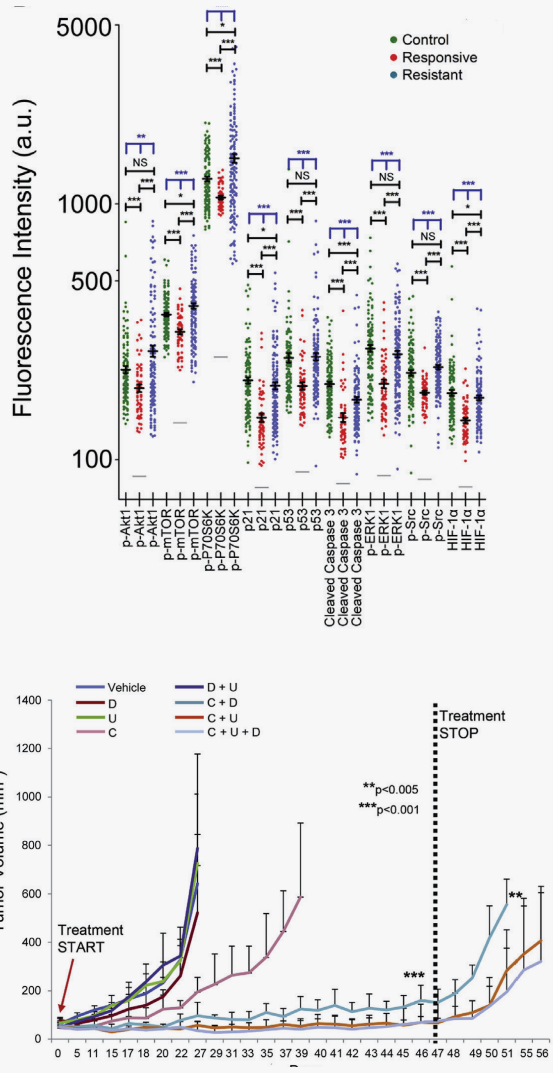
Single-Cell Intracellullar Proteome

Highlights of Informing Targeted Combination Therapies with Single-Cell Intracellular Proteomics

- Single-cell intracellular proteomics uncovers rewiring of signaling pathways, revealing dominant mechanism of resistance.
- Single-cell intracellular proteomics identify changes in signaling nodes missed by genomic analysis.
- Targeting these signaling nodes before treatment blocks resistance, demonstrating the importance of single-cell intracellular proteomics and network re-wiring for predicting cancer treatment responses.

Reference: [4]

Informing Better Combination Therapies to Overcome Resistance



Top: Single-Cell Intracellular Proteome Analysis of Glioblastoma. Bottom: Results for the seven monotherapy or combination therapies based upon the predictions from the Single Cell Intracellular Proteomics. All seven predictions proved correct [4].

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Challenges & Applications

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Application 4: Informing Targeted Combination Therapy to Overcome Resistance with Single-Cell Intracellular Proteomics

Solutions

- Functional proteomics reveals insight into various challenges associated with delivering growth factors and cytokines for various therapeutic applications
- Single-cell and CodePlex analysis uncovered an important property of HSPCs that enables them to convert danger signals into versatile regulatory signals
- Bruker Single-Cell Secretome technology reveals distinct cytokine profiles associated with pathogenesis
- Single-cell intracellular proteomics uncovers rewiring of signaling pathways, revealing dominant mechanism of resistance

References

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2. Zhao JL, et al. Conversion of Danger Signals into Cytokine Signals by Hematopoietic Stem and Progenitor Cells for Regulation of Stress-Induced Hematopoiesis. Cell Stem Cell 14: 445-459, 2014.
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4. Wei W, et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. Cancer Cell 29, 563–573, 2016.