Multiscale Analysis of Macrophage Secretomes by Multiplex Nanobiochips

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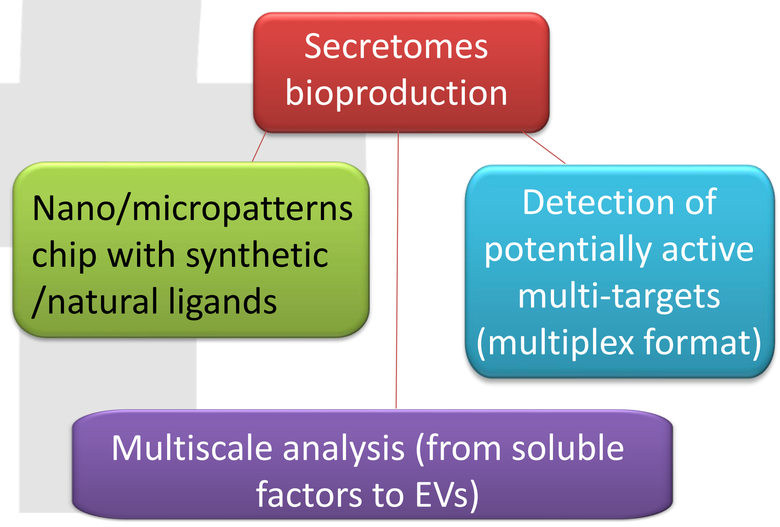
Context

Fibrosis results from chronic inflammation and dysregulated tissue repair. Macrophages and fibroblasts are key players that interact through their secretome — the set of secreted molecules including cytokines, growth factors, and extracellular vesicles (EVs). Depending on environmental cues, M1 macrophages drive inflammation, whereas M2 macrophages promote resolution and support fibroblast-mediated tissue remodeling. Deciphering the composition of M1 and M2 secretomes is essential to understand macrophage–fibroblast communication and identify mediators involved in fibrosis.

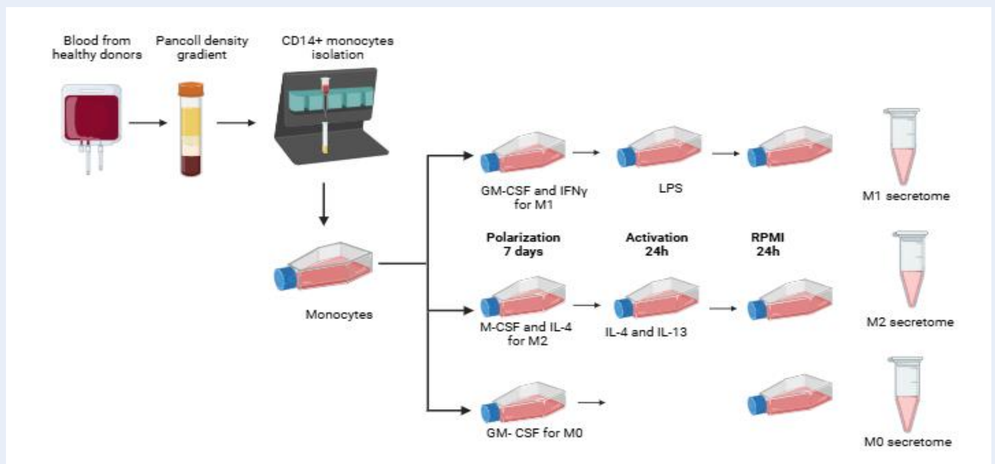
Goal

Develop a new generation of surface plasmon resonance (SPR) based biochips to characterize the generated macrophages secretomes.



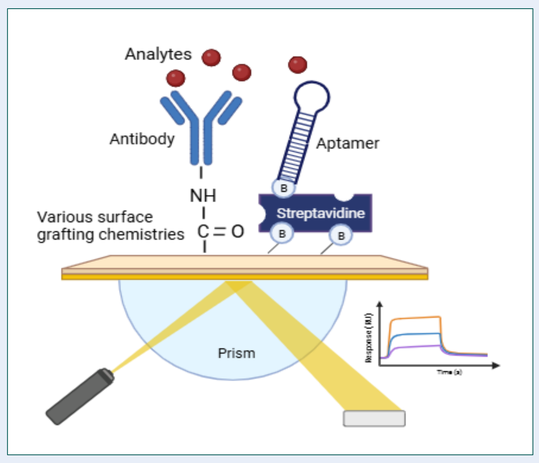
Methodology overview

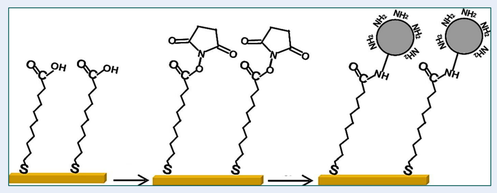
➢ Macrophages engineering and bioproduction of secretomes



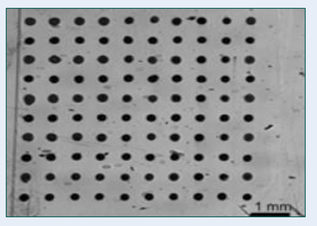
The secretome exhibits high molecular and vesicular complexity. As a proof of concept, we focus on the cytokine IL-6, which drive chronic inflammation, and on the characterization of EVs potentially involved in inflammatory signaling and tissue remodeling.

➢ Real-Time Detection of Molecular Interactions via SPR





EDC/ s-NHS chemistry

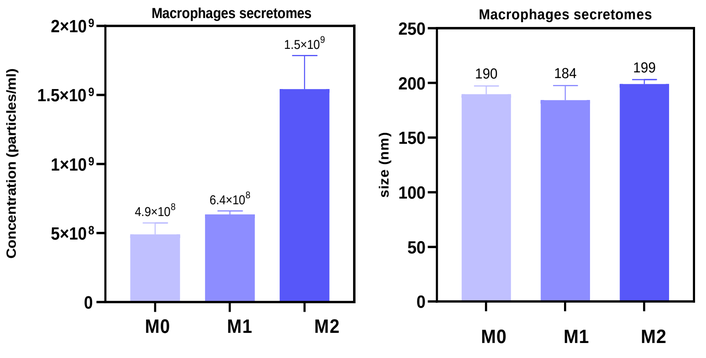


Multiplex biochip

Detection of synthetic IL-6 by SPR was used to validate the surface functionalization and ligands (antibodies and aptamers) immobilization. The multiplex format will be extended to include EVs detection.

Results

➢ Different EVs expression profiles among macrophages phenotypes

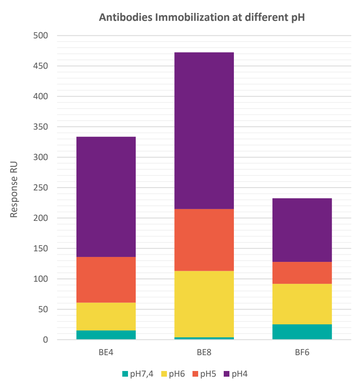


Videodrop analysis of macrophage secretomes. Measurements were performed on each of the 4 donors, then secretomes were pooled for comparative analysis.

M2 macrophages secreted a higher number of vesicles compared to M0 and M1.

Extracellular vesicles from M0, M1, and M2 showed similar size profiles.

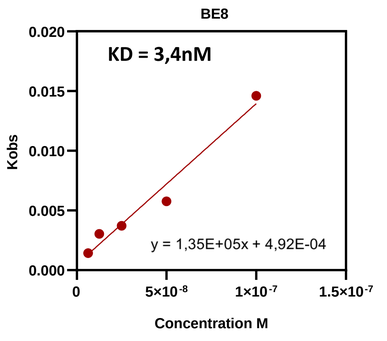
➢ Antibodies immobilization and kinetic validation by SPR

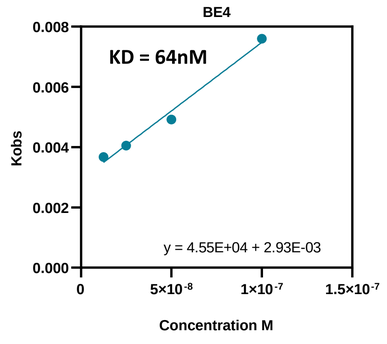


Antibody immobilization was achieved using EDC/s-NHS chemistry.

Optimal pH for antibody grafting was determined to be 4.

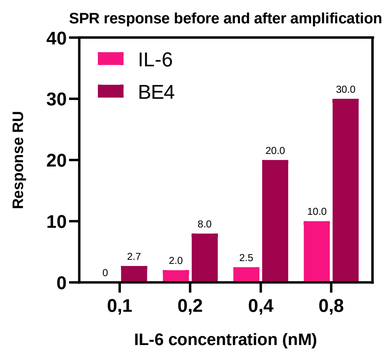
Validated anti-IL-6 were tested to confirm functionality and determine KD values.

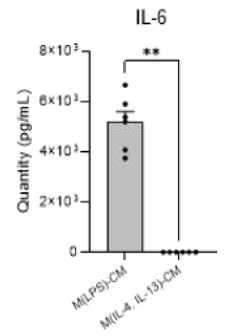




Plots of *kobs* vs concentration were used to extract kinetic parameters and calculate KD values

➢ Direct vs amplified IL-6 detection by SPR





IL-6 quantification in M1 and M2 secretomes by flow cytometry

Sandwich assay with BE4 secondary antibody enhances IL-6 detection limit to 0.2 nM.

The SPR detection limit closely matches IL-6 levels measured by flow cytometry in M1 secretomes (~0.24 nM), supporting the validity of the assay sensitivity.

Conclusion and perspectives

- Secretomes from macrophages contain extracellular vesicles, with M2 releasing higher numbers than M0/M1.
- Kinetic analyses (KD values) validate ligand immobilization and binding performance.
- SPR biochips allow direct detection of inflammatory cytokine IL-6 at physiological levels.

➢ **We aim next to extend multiplex SPR biochip for simultaneous cytokines and EVs detection in crude secretomes.**

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