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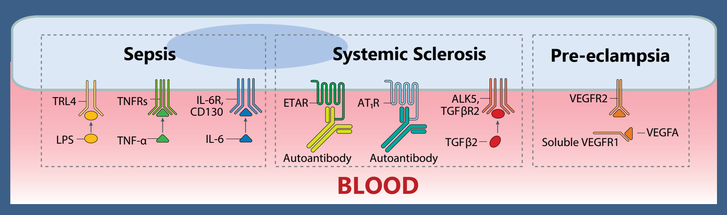
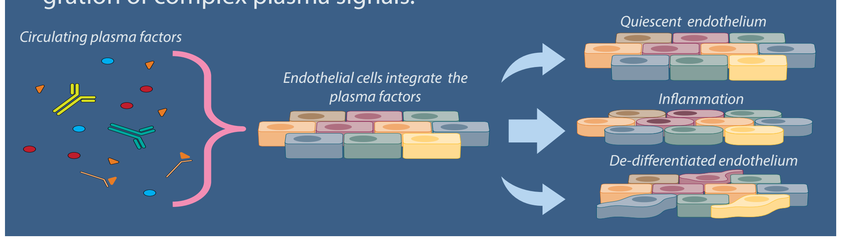
Novel morphological profiling assay to study the role of endothelial cells in the disease trajectory of liver cirrhosis *in vitro*

Introduction

- Studying the role of the endothelium in disease pathophysiology is important for a better understanding of many **SYSTEMIC DISEASES**.
- EC dysfunction in response to **CIRCULATING PLASMA FACTORS** is implicated in a variety of medical conditions, including, sepsis, cardiovascular diseases, diabetes, systemic sclerosis, chronic kidney disease.



- ECs will assume **DIFFERENT MORPHOLOGIES** depending on the integration of complex plasma signals.

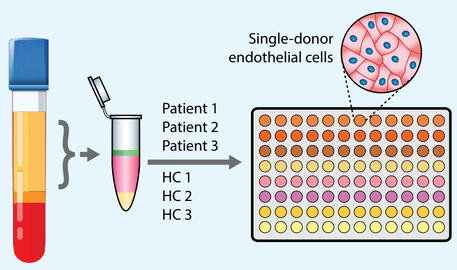


Aims

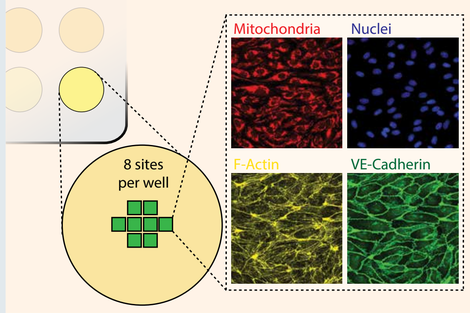
- **Develop an unbiased morphological profiling assay to assess the impact of patient-derived plasma on EC phenotype.**
- **Validate its performance using known stimulants.**
- **Stratify liver cirrhosis patients based on induced EC morphology.**

Methods

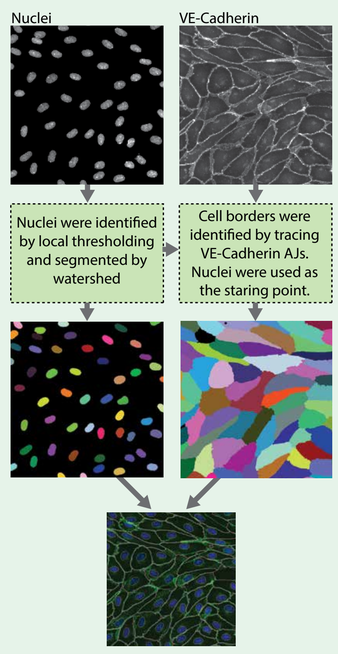
1: Exposure of endothelial cell monolayers to 25% stabilized and recalcified EDTA plasma



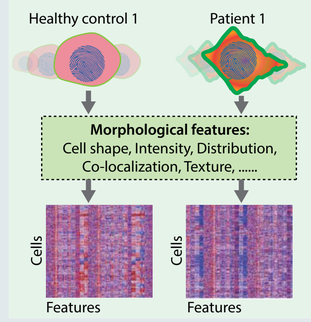
2: High-content imaging and quality control



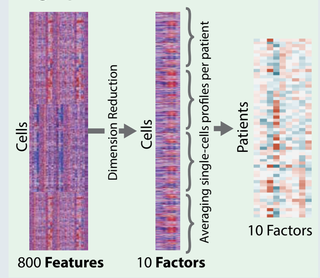
3: Single-cell segmentation



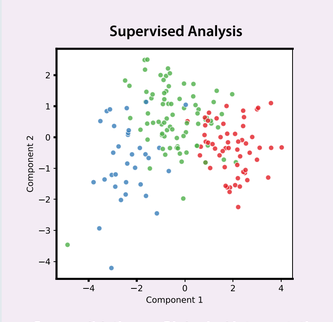
4: Extracting single-cell features



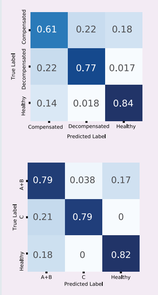
5: Computing morphological fingerprints



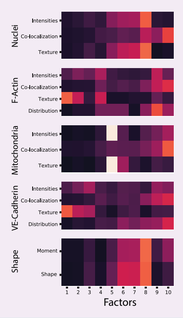
6: Data analysis



Cross-validation

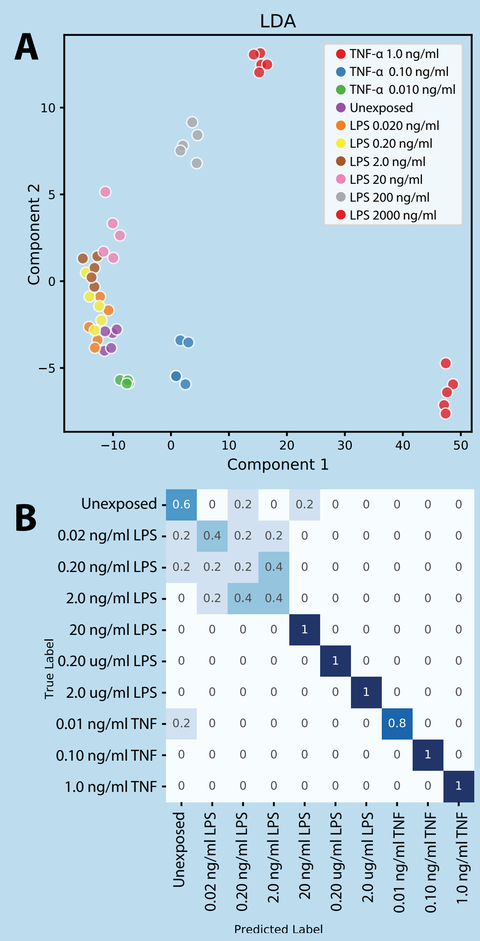


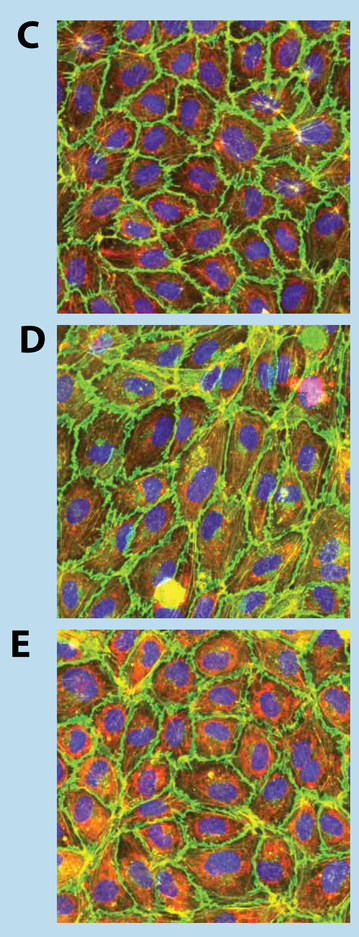
Biological interpretation



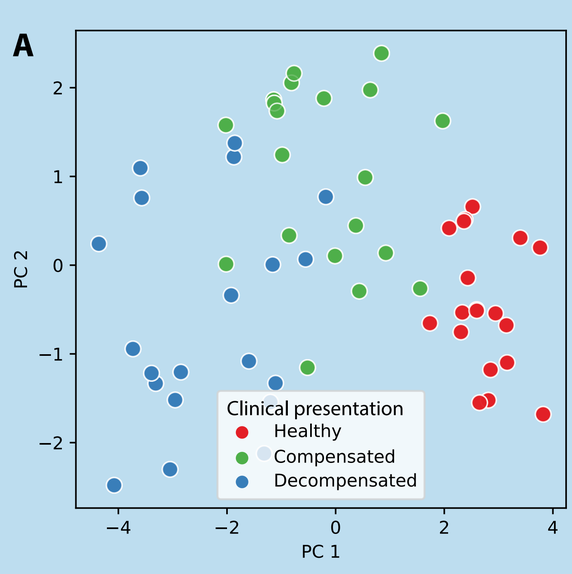
- Cultured endothelial cell (HUVEC) monolayers were exposed to 25% stabilized EDTA plasma from patients with **compensated cirrhosis**(n=20), **decompensated cirrhosis**(n=20) and **healthy controls**(n=20).
- Patient specific **PHENOTYPIC PROFILES** were generated and used for analysis:
  - Single-cell morphological profiles were extracted by automated image analysis following staining of multiple cellular components (mitochondria, nuclei, cytoskeleton, and adherence-junctions) and high-content imaging.
  - After dimension reduction by Factor Analysis, patient profiles were created by cell-to-patient data aggregation.
  - Multivariate data analysis was performed to **STRATIFY PATIENTS** and identify **discriminating morphological features**.

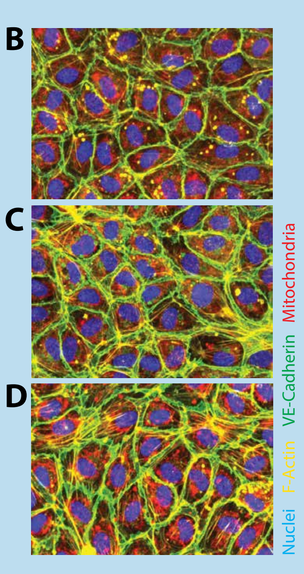
Results



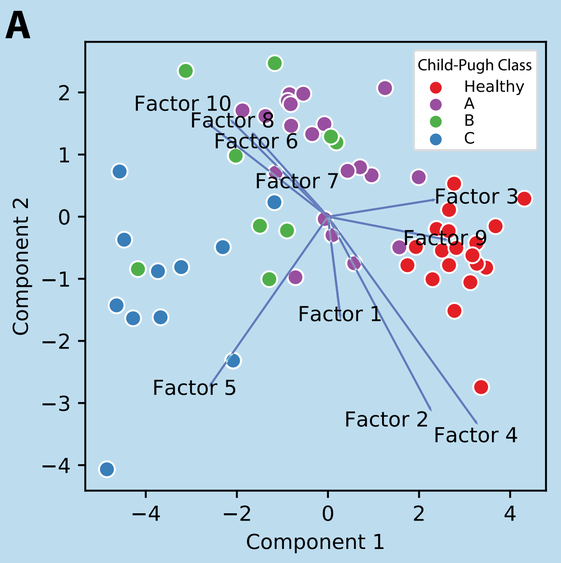


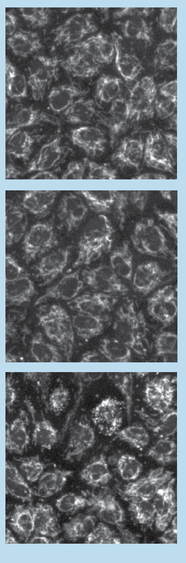
- Morphological profiles computed from HUVECs *(C)* stimulated by TNF-α *(D)*, or LPS *(E)* were compared using a supervised linear model.
- The score plot of this model *(A)* showed two different trajectories for each stimulant in a dose dependent manner.
- The excellent LOO cross-validation results *(B)* indicated that the morphological profiles indeed do contain generalizable discriminatory information.





- HUVECs were exposed to EDTA plasma from patients with **compensated cirrhosis** (C), **decompensated cirrhosis** (D) and **healthy controls** (B), resulting in visually identifiable morphological differences.
- Supervised analysis of the morphological profiles for clinical presentation (*Healthy / Compensated / Decompensated)* showed clear separation of the three groups, with overlap of the compensated group.
- The overlap between the compensated and decompensated group were further studied in the subsequent model by classifying the Child-Pugh severity score.





- Supervised analysis by CP-class (A) showed clear clustering of healthy controls.
- Importantly, CP-C patients showed separation from CP-B patients, determined by factor-5, representing mitochondrial morphology (B).
- Visual confirmation showed mitochondrial distress in CP-C (E), compared to CP-B (D) and CP-A (C)

Discussion

- Exposure of ECs to patient- or control plasma samples induced profound changes in morphological profiles, displaying a clear overall distinction between controls and patients with decompensated cirrhosis.
- Patients with compensated cirrhosis exhibited an intermediate phenotype, showing overlap with both controls and patients with decompensated cirrhosis. Using supervised analysis, we were able to correlate Child-Pugh(CP) class to EC morphology and activation.
- Importantly, patients with CP-C cirrhosis showed a different EC phenotype, in which changes in mitochondrial morphology were determined to be most discriminative.

Conclusion

We introduce a new assay that integrates and displays the impact of circulating factors on endothelial cells and show that it may constitute a valuable tool to study the role of endothelial dysfunction in the disease trajectory of cirrhosis. We demonstrate that EC activation corresponds with the disease severity. Moreover, our results suggest the presence of mitochondrial dysfunction in ECs of decompensated cirrhosis patients.

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