Development of pathological Heart-on-Chip models to improve Extracellular Vesicle-based paracrine therapy against myocardial injury

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Introduction

Ischemic heart disease, often leading to cardiac fibrosis, remains the leading cause of mortality worldwide, driving the need for safe and effective cardiac regeneration strategies. Among the emerging approaches, non-viral direct reprogramming of cardiac fibroblasts (CFs) into induced cardiomyocytes (iCMs) via microRNA release has shown promise for in situ treatment and cardiac function recovery, though its efficiency remains a limitation [1].
In this context, advanced in vitro models, such as pathological Heart-on-Chip platforms, provide a promising avenue for developing and validating novel therapies [2].

Materials and Methods

In the context of RECOVERY project (pREcise Cardiac Organ-on-chip modeling to improve extracellular VEsicle-based paracrine therapy against myocaRdial injurY), in collaboration with Politecnico di Torino and Università degli Studi di Genova, we developed a microfluidic platform integrating bioactive hydrogels loaded with extracellular vesicles (EVs). This system aims to enhance the efficiency of microRNA-based reprogramming of CFs into iCMs while exerting cardioprotective and antifibrotic effects.
First, preliminary experiments to assess the cardioprotective effects of EV particles on CFs were conducted in 2D. In addition, we designed a 5-channel Heart-on-Chip model to evaluate different therapy strategies and we tested the injectability of bioactive hydrogels in the microfluidic device. Finally, we validated the 5-channel platform by assessing the efficacy of EV treatment on 3D CF constructs, evaluating fibrosis-related markers in presence or absence of EVs.
In parallel, we developed a non-invasive setup to monitor the fibrotic state of 3D CFs in real time using impedance measurements.Two electrodes configurations were tested to measure impedance, one in direct contact with the cellular constructs and another from the reservoirs.

Results

Geometrical and functional characterization of the designed 5-channel platform as well as injectability tests with bioactive hydrogels were successfully performed.
In parallel, we demontrated that our pathological Heart-on-Chip model effectively recapitulates fibrotic phenotype using both TGFβ1 treatment and mechanical stimulation. Using a preliminary platform we confirmed that daily real-time impedance measuring did not negatively impact CF constructs. Moreover, the two measuring configurations showed comparable results, detecting a slight increase in electrical impedance in fibrotic CFs. Immunofluorescence staining further confirmed the fibrotic state of 3D constructs on-chip.These results suggest that impedance measurements could serve as biomarker for realtime fibrosis assessment.
After determining the optimal hAFSC-EV dose with cardioprotective and antifibrotic effects in 2D cultures, we investigated the effectivenes of EV treatment in 3D. Ongoing experiments aim to establish the optimal EV dosage on-chip to maximize their protective effects.

Discussion

Our study demonstrates the potential of a pathological Heart-on-Chip model for evaluating extracellular vesicle (EV)-based therapies aimed at mitigating cardiac fibrosis. The successful recapitulation of fibrotic conditions, achieved through TGF-β1 treatment and mechanical stimulation, underscores the model's relevance in studying fibrosis progression and therapeutic responses. Additionally, impedance measurements proved to be a promising non-invasive biomarker for real-time fibrosis assessment, offering an alternative to conventional endpoint assays. By combining Organ-on-Chip approach with bioactive hydrogels and EVs we developed a promising tool for high-throughput drug screening and personalized medicine.

Conclusions

The development of this Heart-on-Chip model represents a significant step forward in cardiac disease modeling and therapeutic testing. The integration of EV-based treatments within a microfluidic environment provides a controlled platform to optimize regenerative strategies. Our findings support the use of impedance-based monitoring as a real-time functional readout for fibrosis progression and treatment efficacy. Ongoing investigations will focus on refining the optimal EV dosage and delivery strategy to maximize therapeutic benefits. Ultimately, this platform could serve as a valuable tool for accelerating the development of EV-based therapies for myocardial injury, bridging the gap between in vitro studies and clinical applications.

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