Novel soft robotic cell-stretching device alters PIEZO1 expression in cardiac strain profiles

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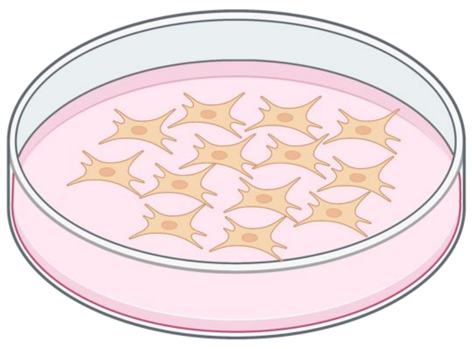
Introduction and methodology

Fig. 1



Traditional cell culture techniques lack physiologically relevant mechanical cues (Fig, 1).

Tissue culture plastic is in the GPa range, where the diseased heart experiences stiffness in the kPa range.

The mechanosensitive, Ca^{2+}-permeable ion channel PIEZO1 (Fig, 2) is present throughout the cardiovascular system and has distinct roles in physiology and disease.

Soft robotics present new opportunities to deliver physiologically relevant mechanical cues to cells, offering advantages over stiff tissue-culture to investigate mechanical insights into cardiovascular disease, including PIEZO1 regulation.

Fig. 2

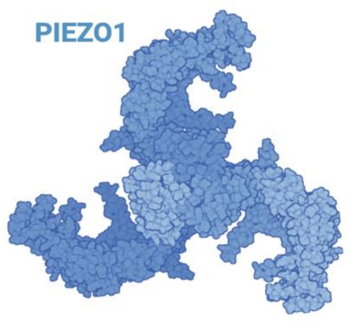


Fig. 3

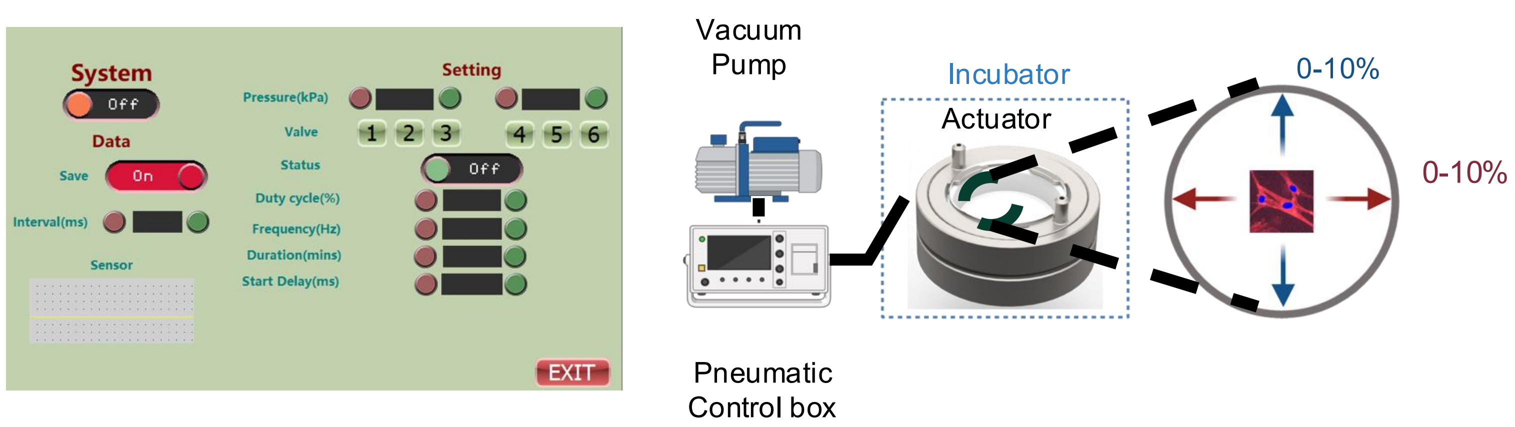


Fig. 3 CellQ-Twin System: A pneumatically controlled cell-stretching device that enables programmable strain profiles for cell culture.

PDMS substrates in medical-grade stainless steel housing, enabling cyclic strain profiles through six independent ports for patient-specific mechanical stimulation

Aim: Test a novel soft robotic cell-stretching device in primary human ventricular fibroblasts with cardiac-relevant strain profiles.

Hypothesis: The mRNA expression of PIEZO1 will be upregulated in line with the frequency of stretch applied.

Results

Fig. 4 Human ventricular fibroblasts plated on Collagen-1 coated PDMS actuators express fibroblast markers

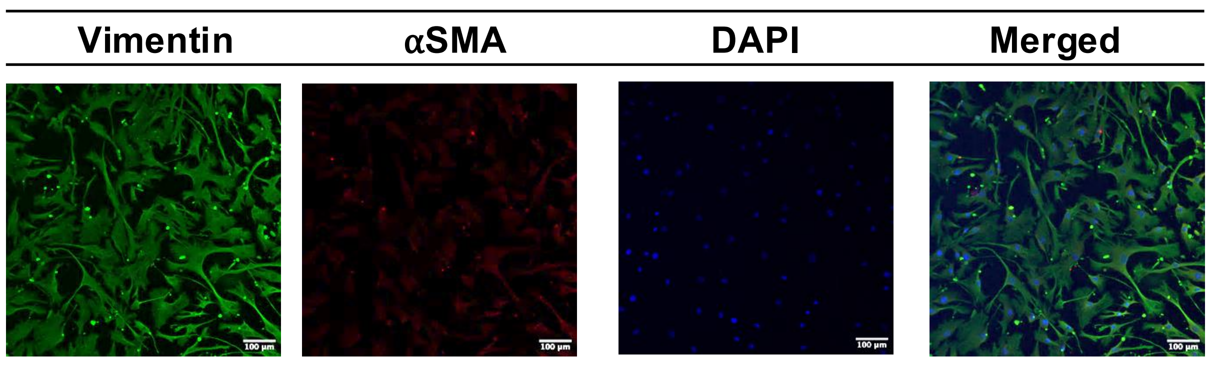


Fig. 4 Immunostaining analysis of PIEZO1 on ibidi slide preparations in patient-derived cardiac fibroblasts (N=1).
Example of staining of human ventricular fibroblasts plated on collagen 1 (rat tail) coated PDMS actuators. Staining for fibroblast markers, vimentin (green), α–SMA ((alpha smooth muscle actin), red), nuclei (DAPI, blue) and merged image. Cells exhibit an undifferentiated fibroblast-like phenotype. Scale bar 100 μm.

Fig. 5 Human cardiac fibroblast stretching by CellQ-Twin

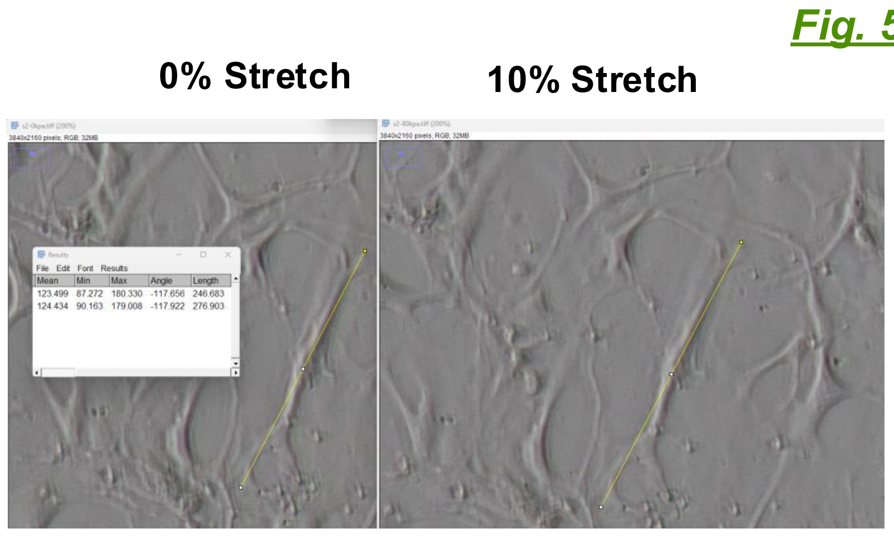


Fig. 5 Brightfield microscopy images of human cardiac fibroblasts in static conditions (left) and held at 10% stretch (right).
Human cardiac fibroblasts are physically stretched to 10% after the application of strain.
This confirms that the protocols defined in the control box are being transmitted into the cultured cells.

Fig. 6 Human cardiac fibroblast morphology post-stretching

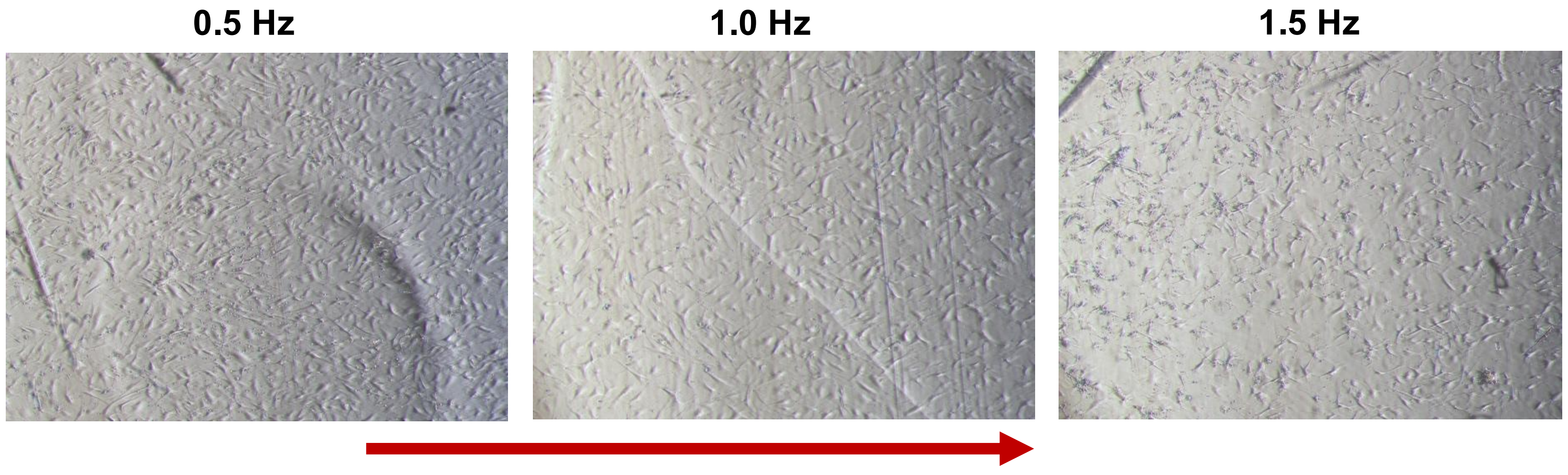


Fig. 6, Brightfield microscopy images of human cardiac fibroblasts after 4 hours of stretch at 10% strain, with differing frequencies (0.5, 1.0 and 1.5 Hz).
Cellular morphology changes with increasing frequency of stretch. There is more dead-space in between the cells, and they are elongated at 1.5Hz compared to 0.5Hz.

Fig. 7 Preliminary result – mechanical rhythm sweet point

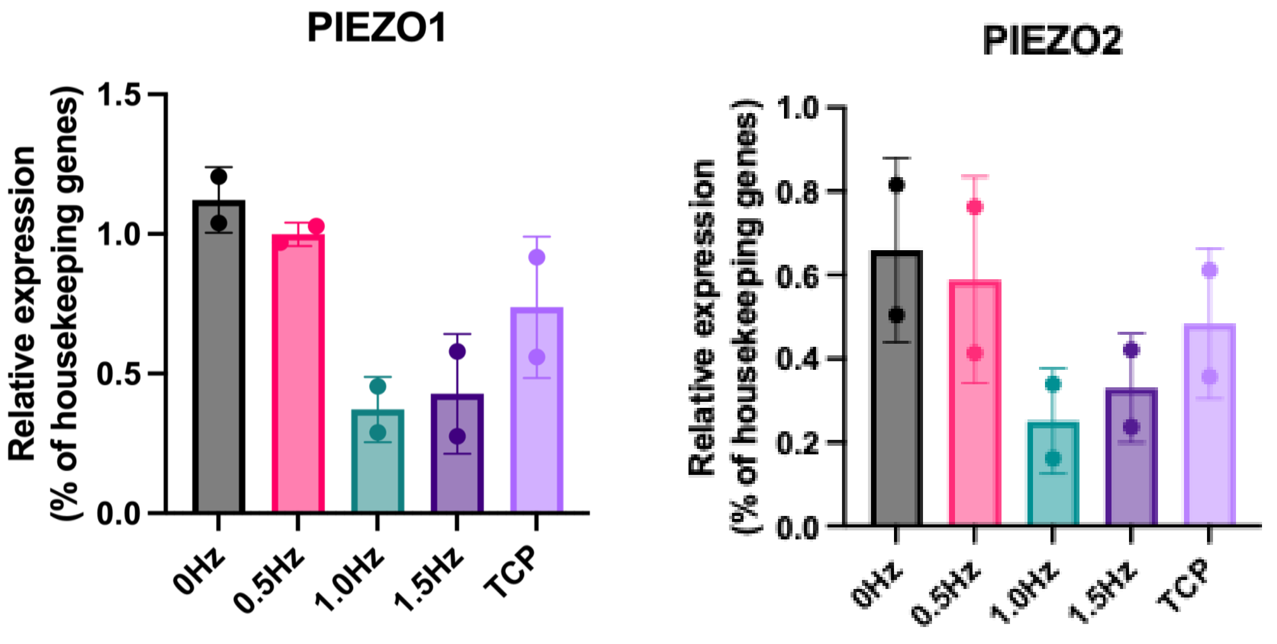


Fig. 7, RT-qPCR gene expression of PIEZO1 and PIEZO2 after 4 hours of mechanical stretch at 10% strain at either 0.5, 1.0 or 1.5 Hz (N=2, n=3).
PIEZO1 mRNA expression adapts to the mechanical frequency applied.
There is an indication of a sweet point of PIEZO1 mRNA expression at 1Hz (equivalent to 60bpm). The other conditions reflect greater mechanical load, in which PIEZO1 upregulates its mRNA expression.

Key Findings

- Isolated cells plated on the PDMS actuators have a fibroblast-like phenotype.
- Cellular morphology changes with increasing frequency of mechanical stretch from 0.5-1.5 Hz.
- PIEZO mRNA can sense mechanical frequency and adapt its mRNA output as a response. There may be a 'mechanical sweet point' of PIEZO mRNA expression which is 'just right' in the healthy myocardium.

Conclusion

The data highlights the potential for CellQ-Twin to deliver mechanically related insights into health and disease and supports the idea of PIEZO1's centrality in cardiac mechanical adaptation.

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