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A miniaturized EHT platform for contractile tissue measurements

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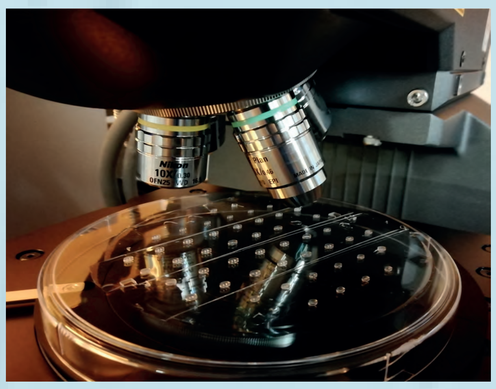
[2]Leiden University Medical Center, Leiden, the Netherlands

Motivation

* **Organ-on-chip (OoC)** [1] is an emerging technology that promises a valid alternative to current time-consuming and costly drug trials [2], whose high attrition rate is due to use of insufficiently representative models of human physiology [3].

* **Engineered heart tissues (EHTs)** are OoC devices consisting of a bundle of cells self-assembled around two anchoring pillars. By building a complex 3D model of a human tissue, EHTs allow in-depth study of contractile tissue properties.

* **We present a miniaturized EHT platform fabricated at wafer-level using silicon-based micromachining and polymer moulding.** Our EHT platform is an anisometrically downscaled version of HeartDyno [4]. It was mechanically characterised by nanoindentation, and is **the smallest and best characterised** to date.



Wafer-scale batch fabrication and inspection of PDMS-based EHTs

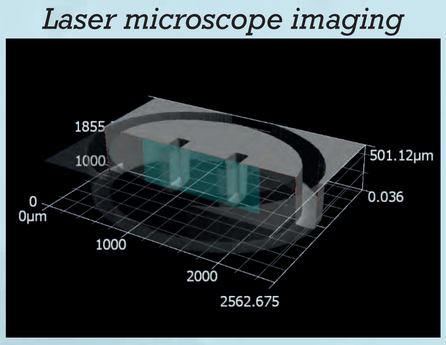


PDMS chips transferred to a 96-well plate for cell culturing experiments

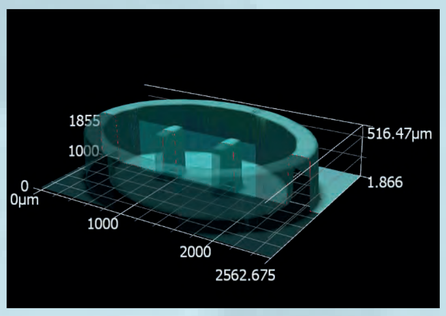
Microfabrication of the EHT platform

A 4-inch deep reactive ion-etched Si wafer was used as mould for the polymer structures. A perfluorinated silane-based anti-adhesion self-assembled monolayer (SAM) was deposited on the Si wafer to make the surface hydrophobic prior to spin-coating of polydimethylsiloxane (PDMS). After demoulding, PDMS chips of three different sizes were diced and transferred to a 96-well plate.

Laser microscope imaging

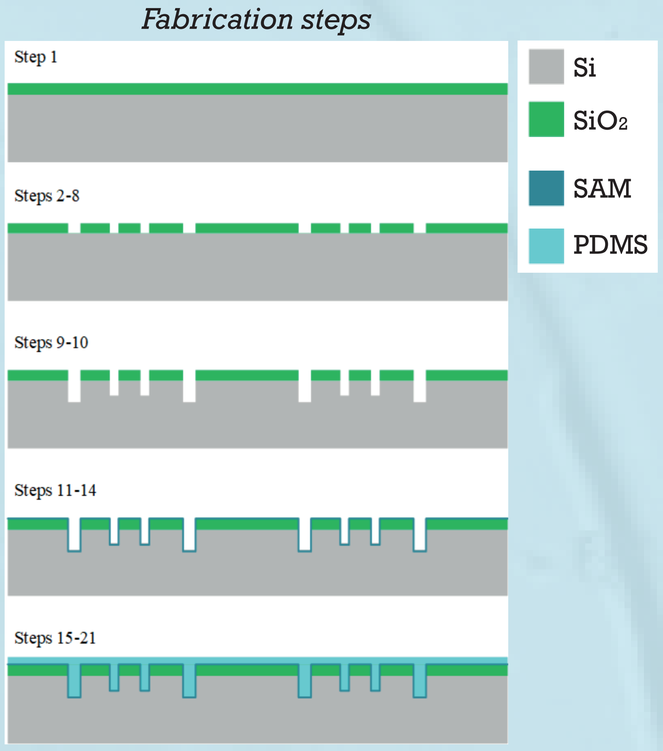


Micromachined Si mould



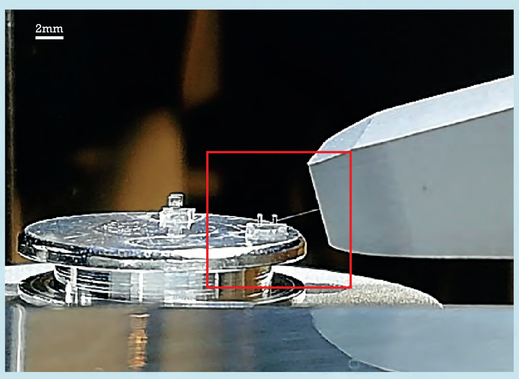
Final PDMS structure

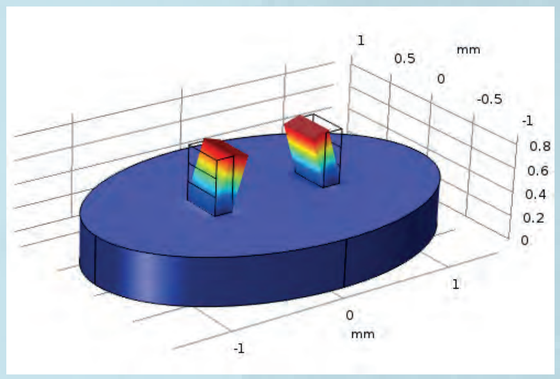
Fabrication steps

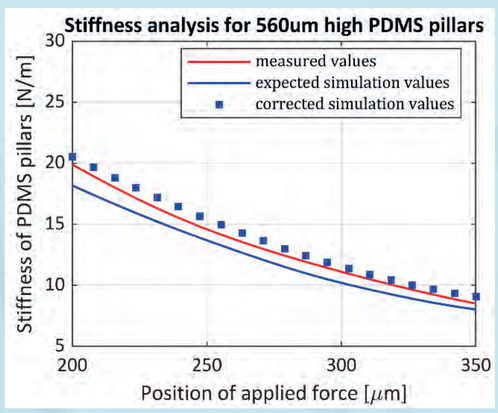


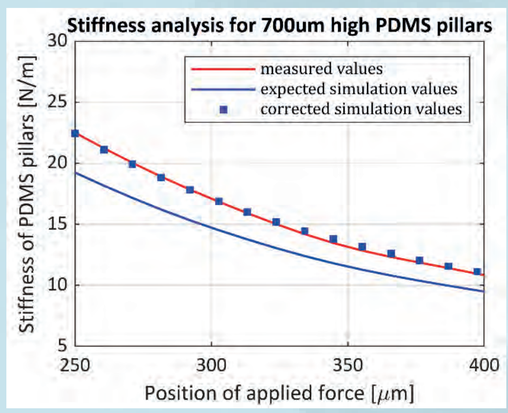
Mechanical characterization and modeling

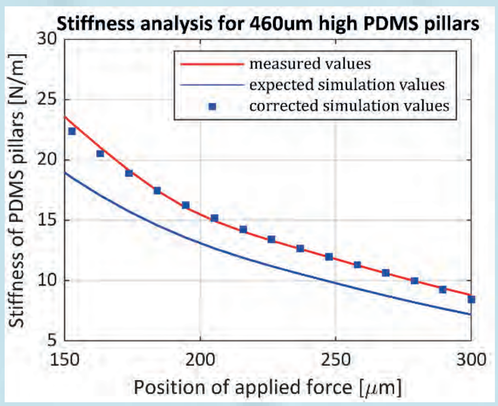
Stiffness of the pillars was measured using a nanoindentation tool and simulated.
* A specific force in the µN range was applied at different heights of the pillars by a silicon tip and the displacement of pillars was measured with a piezosensor.
* In parallel, finite-element method was used to simulate the mechanical behaviour of the pillars in Comsol Multiphysics.











Data from simulations were compared and fitted to experimental data to obtain an accurate estimation of the Young's modulus of PDMS (1.7 MPa) and of the stiffness of the three types of pillars.

Experiments with cells

Chips were seeded with 80% cardiomyiocytes and 20% fibroblasts. Tissue compaction started after an hour, and **the tissues formed succesfully in all different chip sizes.** Experiments were conducted for 18 days and the tissues were functional for the whole time.

3 µl (47000 cells)

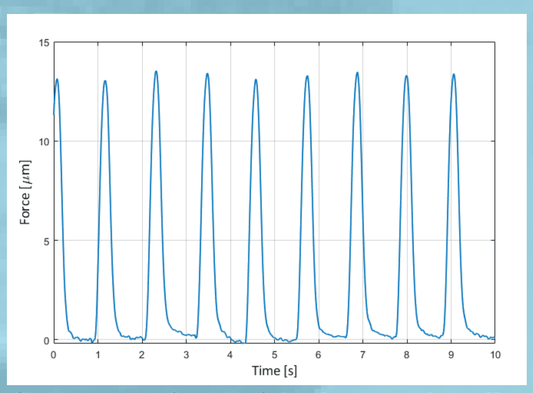


2 µl (31000 cells)



1 µl (16000 cells)



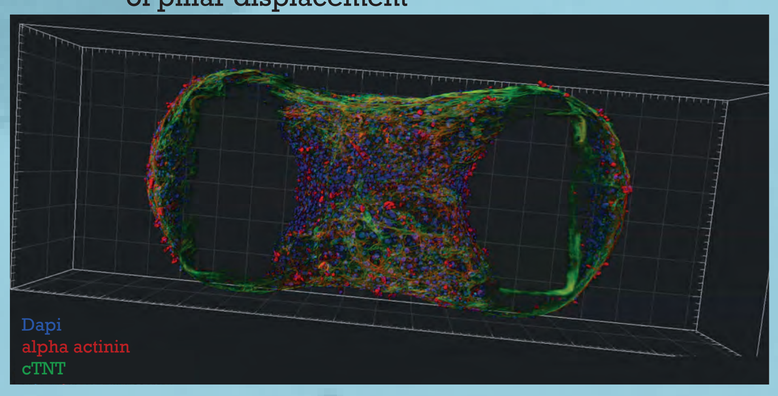


Contraction force of the beating bundle estimated by optical tracking of pillar displacement

Scan QR codes to watch the videos

Bundle contraction

Staining of the tissue



EHTs were stained for the cardiac markers alpha-actin (red) and cardiac troponin T (green), while cell nuclei were stained with Dapi (blue)

References

[1] B. Zhang *et al.*, *Nature Reviews Materials* 3, 257-278 (2018)
[2] M. Mastrangeli *et al.*, *ALTEX - Alternatives to Animal Experimentation* 36 (4), 650-668 (2019)
[3] U. Marx *et al.*, *ALTEX - Alternatives to Animal Experimentation* 33 (3), 272-321 (2016)
[4] R. Mills *et al.*, *Proceedings of the National Academy of Sciences* 114 (40), E8372-E8381 (2017)

Conclusion and outlook

We presented the smallest and best characterised EHT devices to date. The devices were fabricated by wafer-scale silicon and polymer processing, characterised by nanoindentation and finite-element simulations, and transferred to 96-well plates for cell seeding and optical tracking of bundle contraction. Cell bundles remained functional for at least 18 days. Pacing electrodes and strain sensors will be added for improved bundle control.