THE USE OF THE CHIARO NANOINDENTER IN MYOCARDIAL INFARCTION RESEARCH

Indenters have been widely used to study mechanical properties and mechanobiological processes in biomedical research, e.g. to characterize mechanical properties of different types of single cells, compare the stiffness of healthy and diseased tissues or to mimic the stiffness of native tissues when engineering new types of biomaterials (patches, vessels, organs). Here we want to underline the versatility of the CHIARO Nanoindenter in cardiovascular research and show the novel experiments that were enabled through the use of this instrument.

Introduction

Myocardial infarction (MI) causes the scarring of the tissue in the myocardium which has stiffer mechanical properties (1-2 orders of magnitude) and differential cellular composition compared to the healthy tissue. During MI, cardiomyocytes (CMs) are damaged and replaced by cardiac fibroblasts (CFs) which differentiate into cardiac myofibroblasts (CMFs). This process causes remodeling of the surroundings of newly formed CMFs into fibrotic tissue by overproduction of the collagenous extracellular matrix which constricts cardiac function. This study[1] aims to investigate the contractile mechanical signal propagation across CM-CMF interface as a model for MI boundary in healthy and heart-attack-mimicking matrix stiffness conditions.

Sample preparation

1. Three different PDMS substrates were prepared by using 1:100, 1:40 and 1: 20 ratios between base and curing agent.
2. MI boundary model was created on three PDMS substrates by seeding purified rat cardiomyocytes (~90% CMS and 10% CFs) cells on one half of Fibronectin (FN) coated PDMS and letting the CFS proliferate and differentiate into CMFs on the other half of PDMS which mimics the wound healing process after the injury to the heart. A control sample was made with only CMs culture on one half of PDMS and no cells on the other half to mimic the healthy tissue.

Figure 1. CHIARO Nanoindenter which can be mounted on an inverted microscope for single cell characterization. Not to scale.

3. Tissue cross sections were obtained from neonatal and adult rat hearts to measure the biomechanical properties of the healthy myocardium.

Mechanical tests performed with the CHIARO Nanoindenter

1. PDMS and native tissue characterization: cantilever-based probes with a spherical tip radius of 45µm and spring constants of 0.43N/m and 4.21N/m were used to perform indentations at three loading speeds: 50, 2 and 0.2µm/s.
2. CMFs characterization: single cells were measured following the same protocol but with probes equipped with 20.5µm tip radius and spring constant of 0.045N/m.
3. Contractile force measurements (dwelling): the probe with 2.7µm tip radius and 0.067N/m spring constant was used to indent the cell and keep in contact for 30s. The contractions of cell causes the change in the cell height and thus cantilever deflection in the transverse direction which is proportional to contractile force. Measurements were performed on CMs and CMFs by moving further away from the CM-CMFs interface (Fig. 2 A, B).

[1] Nguyen, Dung Trung, Neerajha Nagarajan, and Pinar Zorlutuna. "Effect of Substrate Stiffness on Mechanical Coupling and Force Propagation at the Infarct Boundary." *Biophysical Journal* 115, no. 10 (November 2018): 1966–1980. https://doi.org/10.1016/j.bpj.2018.08.050.

Results obtained by the CHIARO Nanoindenter:

1. PDMS 1:100, 1:40 and 1:20 had stiffness values of ~14, 83 and 484kPa at 0.2µm/s and showed an increase in stiffness with loading-velocity (Fig. 2 C).
2. Young's modulus of native tissue was ~11kPa at 0.2µm/s and also showed stiffening with the indentation velocity (Fig. 2 C).
3. CMFs cultured on PDMS increased their Young's modulus with the stiffness of the substrate and with the indentation velocity: 1.0-3.0, 2.1-5.0 and 1.6-5.5kPa, respectively (Fig. 2 D).
4. The beating force curves over time were measured on both sides of the boundary where CMs showed higher contractile force magnitude than CMFs (Fig. 2 E).
5. The contractile force magnitude of CMs was highest on the softest substrate which had similar stiffness as native tissue (Fig. 2 F).
6. The contractile force amplitude exponentially decreased from CM-CMFs boundary (Fig. 2 G). The mechanical signal was transmitted furthest on the softest substrate (Fig. 2 H) and the propagation distance was even greater on control samples lacking CMFs.

Note: The CHIARO Nanoindenter can also perform viscoelastic characterization, mapping of mechanical properties over large areas and lateral force measurements.

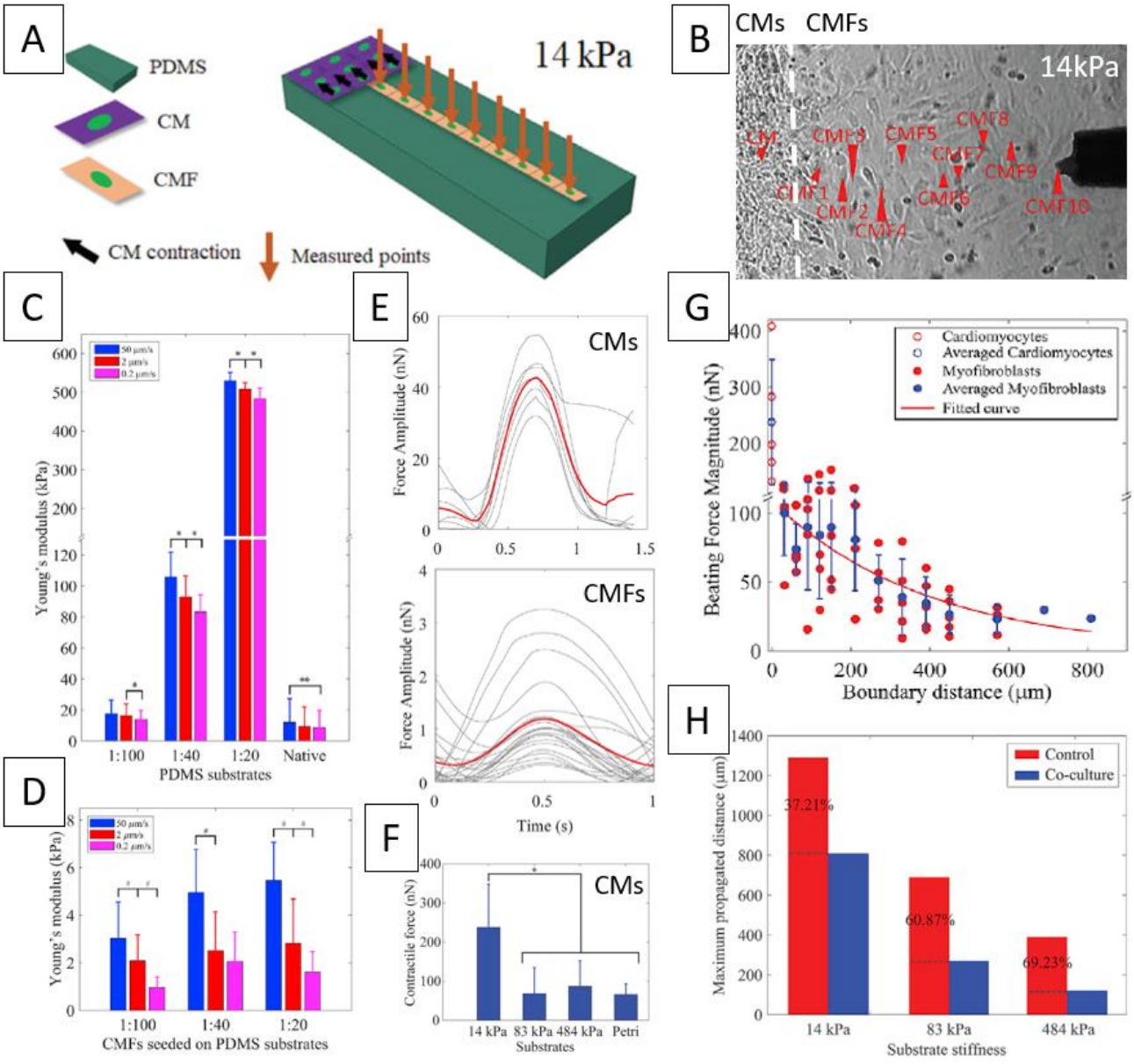

Figure 2. Reprinted from "Effect of Substrate Stiffness on Mechanical Coupling and Force Propagation at the Infarct Boundary" (p. 1966-1980) by Nguyen, Dung Trung, Neerajha Nagarajan, and Pinar Zorlutuna, 2018, *Biophysical Journal* 115, no. 10.

Written by Nelda Antonovaite
info@optics11.com www.optics11life.com