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# PDMS Substrates with tunable stiffness for cardiac mechanobiology investigation: A nanoindentation study

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## Abstract

Micromechanical characterization by nanoindentation of PDMS substrates for cardiac mechanobiology studies.

## Introduction

Mechanical cues play a continuous, crucial regulatory role in growth, maintenance, and pathophysiology of cardiac tissue.<sup>1,2</sup> In particular, cardiac cells can translate changes in biophysical characteristics of the surrounding matrix, such as stiffness, into intracellular activation cascades with consequent evolution toward pro-inflammatory/pro-fibrotic phenotypes.<sup>3,4</sup> Thus, for studying *in vitro* cardiac cell mechanobiology, it is crucial to use substrates that mimic the mechanical properties of the native physiological or pathological environment. Here, specific Polydimethylsiloxane (PDMS) blends with tunable stiffness,<sup>5</sup> to be used as model substrates for cardiac mechanobiology investigations, were prepared. By allowing investigations at typical cell-length scales<sup>6,7</sup> and applying small forces suitable for soft materials, nanoindentation was adopted to mechanically characterize PDMS blends in physiological conditions (wet conditions, 37°C).

## Materials and Methods

PDMS substrates with tunable stiffness were prepared using pure Sylgard 184 and Sylgard 527 (Dow Corning, USA). Five dif-

ferent Sylgard mass ratios 184:527 (1:10, 1:20, 1:30, 1:50, 1:100) were blended. PDMS blends were poured into 50 mm diameter petri dishes (cured at 65°C) to create ~3 mm thick films for mechanical testing. The procedure was repeated in three different days producing three lots (seven specimens per lot) for checking the manufacturing reproducibility. Indentation of the specimens, immersed in demineralized water at room temperature (RT) or at 37°C, was performed in displacement-control (indentation depth = 2 µm, cantilever stiffness = 4.4 N/m for Sylgard 184, 0.5 N/m for the other specimens) using the PIUMA nanoindenter (Optics11, NL), which automatically provided the load-indentation curves and their respective elastic modulus (E) values (Hertz model fitting). Each PDMS specimen was indented in three different regions performing, in each region, indentation matrices of 25 points equally spaced of 50 µm. Three different indentation rates (v1 = 1 µm/s, v2 = 10 µm/s, v3 = 50 µm/s) were adopted. In parallel, preliminary indentation tests were performed on a sample of bovine myocardium. The specimen (~3x3x0.5 cm<sup>3</sup>), immersed in demineralized water at 37°C, was indented in a central region (5x5 points, step size = 100 µm) in displacement-control (indentation depth = 4.5 µm, cantilever stiffness = 0.5 N/m, indentation rate = 2 µm/s). Statistical analysis of variance was performed to assess the impact of temperature and, indentation rate on derived E values (p < 0.05).

## Results

No statistical differences emerged among PDMS lots in terms of elastic modulus, proving the reproducibility of the

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manufacturing. Considering specimens with decreasing mass ratio of Sylgard 184 with respect to Sylgard 527, the average of the measured E values (setting v1) ranged from 1.80±0.09 MPa to 10.25±2.76 kPa when tested at RT, and from 1.44±0.13 MPa to 10.87±1.47 kPa when tested at 37°C (Fig. 1). Interestingly, 1:10, 1:20 and, 1:30 blends matched the E values of fibrotic tissue (20-100 kPa), while 1:50 and 1:100 blends matched the E values of healthy cardiac tissue (10-15 kPa).<sup>8</sup> Pure Sylgard 527 showed

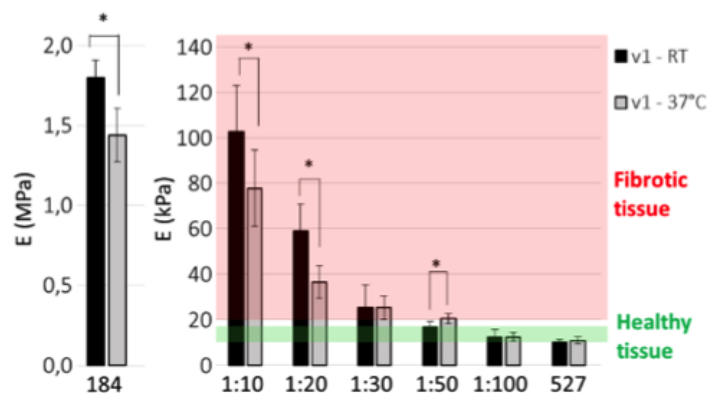


Figure 1. Average E values measured for all PDMS specimens at RT and at 37°C (v1 = 1 µm/s). The ranges of E values reported in literature for healthy (10-15 kPa, green band) and fibrotic (20-100 kPa, red band) cardiac tissue are shown (\*p<0.05).<sup>8</sup>

E values close to the lowest limit of the characteristic E values of healthy cardiac tissue. By increasing the indentation rate, the measured E values significantly increased, confirming the viscoelastic behavior of PDMS.

Furthermore, the statistical analysis indicated that the mechanical properties of the blends were less influenced by temperature increasing the quantity of Sylgard 527. As regards preliminary tests on bovine myocardium, the average of the measured E values ( $11.76 \pm 2.81$  kPa) was in agreement with literature data for normal heart muscle.

## Discussion and Conclusions

The findings support the use of specific PDMS blends as model substrates for mimicking *in vitro* the mechanical properties of physiological or pathological cardiac tissue. Interestingly, it emerged that the testing temperature has an effect on PDMS mechanical properties, in particular for blends stiffer than 30 kPa.

To our knowledge, this is the first sys-

tematic attempt to characterize at typical cell-length scale and in physiological conditions the mechanical properties of specific PDMS blends for mechanobiology applications, in view of future biological tests aimed at elucidating the role of substrate stiffness in cardiac mechanobiology mechanisms.

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