CHARACTERIZATION OF PANCREATIC DUCTAL ADENOCARCINOMA CELLS MIGRATION AND TRACTION FORCE ON STIFFNESS-TUNABLE SUBSTRATES

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal malignancies [1]. In PDAC, stellate cell activation results in the excessive production of extracellular matrix (ECM), causing a significant increase of tissue stiffness (Young's modulus E~1-4 kPa in healthy pancreas, E~4-43 kPa in neoplastic tissue [2]), which affects tissue vascularization and limits chemotherapy effectiveness [3]. Solid stress and cancer-related stiffness are also associated with increased invasive potential. To identify potential candidates for PDAC targeting, it is crucial to understand how PDAC cells respond to tissue stiffness and to detect the key players in the mechanotransduction processes. In this study, we developed stiffness-tunable hydrogels and micropillar arrays and used them to investigate *in vitro* the influence of substrate stiffness on PDAC collective and single cell behavior.

Methods

To mimic the stiffness of healthy pancreas and PDAC, two polyacrylamide (PAM) substrates (PAM low and PAM high, respectively) were fabricated as thin films bound to coverslips, following a published protocol [4]. The effective modulus E^* of the PAM substrates, without and with a collagen coating, was characterized by nanoindentation tests (PIUMA, Optics11) performed in wet conditions (PBS) at 37°C. Human pancreatic cancer cell line (PANC-1) cells were then seeded on the collagen-coated substrates (n=3 for each type), cultured at 37 °C and 5% CO_2, and imaged every 10 min for 6 h for random migration assays. The migration rate v (μm/min) between two consecutive time points was calculated using MtrackJ plugin of ImageJ (NIH). Finally, for characterizing the traction forces exerted by the PANC-1 cells, two polydimethylsiloxane (PDMS) micropillar arrays with different bending stiffness (k_L = 72.3 nN/μm and k_H = 217.2 nN/μm) were designed (Solidworks) and fabricated by soft litography. PANC-1 cells were then seeded on the fibronectin-coated micropillars, stained with rhodamine phalloidin after 24 h, and after additional 24 h fluorescence images were acquired and analysed (ImageJ, Matlab), measuring pillar deflections, and evaluating traction forces (F) as:

$$F = k \cdot x \qquad (1)$$

where k is the pillar bending stiffness (nN/μm) and x is the measured pillar deflection (μm).

Results

The PAM low and PAM high substrates, without and with a collagen coating, exhibited effective modulus values in the range of pancreatic healthy and tumor tissue, respectively (PAM low: E^*=0.56±0.36 kPa and E^*=1.05±0.76 kPa w/ collagen; PAM high: E^*=18.79±5.29 and E^*=15.98±5.08 w/ collagen, Fig. 1A). PANC-1 cells seeded on PAM high substrates showed a higher migration rate (v=0.34±0.004 μm/min for PAM high; v=0.18±0.003 μm/min for PAM low, Fig. 1B). When seeded on micropillar arrays, PANC-1 cells exerted significantly higher mean traction forces on stiffer micropillars (F=14.2±3.9 nN for k_L, F=32.5±9.7 nN for k_H, Fig. 1C).

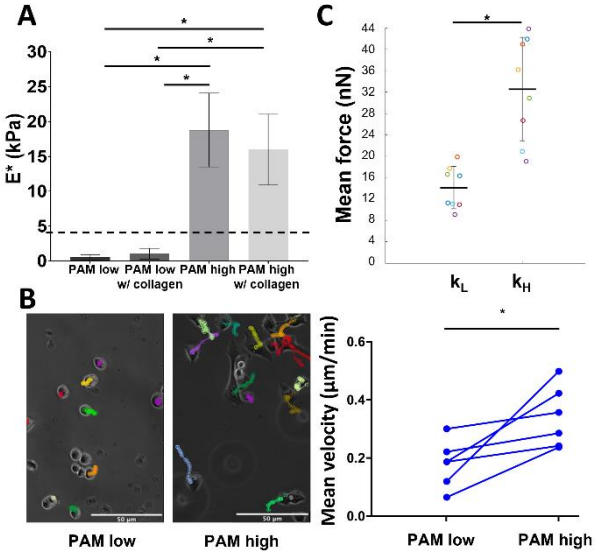

*Figure 1: A) Effective modulus of PAM substrates; B) Random migration assay: cell trajectories and mean cell velocity; C) Mean cell traction forces. (*p<0.01).*

Discussion

Nanoindentation tests confirmed the suitability of PAM substrates in mimicking the stiffness of pancreatic healthy and tumor tissue. Biological tests showed that PANC-1 cell migration is faster on PAM high substrates and higher mean traction forces are generated on stiffer micropillars, indicating that the physical environment affects cell behavior. Thus, the proposed approach could provide further insights into PDAC mechano-transduction processes. Tests on co-cultures of PANC-1 and fibroblasts on PAM hydrogels are ongoing, along with further optimization of micropillar arrays.

References

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