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"ASSAYING THE RIGIDITY GUIDED MIGRATION OF HUMAN TUMOUR STROMAL MYOFIBROBLASTS (TSM) ON POLYACRYLAMIDE SUBSTRATES MIMICKING HEALTHY AND FIBROTIC TISSUE TRANSITION BOUNDARY"

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While acute fibrosis is associated to tissue repair, chronic fibrosis associated with solid carcinomas results in perpetual deregulation of the normal wound healing process with upregulation/secretion of extracellular matrix (ECM) proteins and cytokines. In the later, human Tumour Stromal Myofibroblasts (TSMs) get activated and adopt a myofibroblast-like phenotype with a high contractile, proliferative phenotype, and increased ECM/cytokine production/secretion profile. This creates a new and dynamic environment characterized by remarkable matrix stiffness, which contributes to maintain TSM' activated phenotype and increased tissue tension. It is known that TSMs can migrate towards the tumour area in response to chemokine activation and cross talk with cancer cells to promote tumour growth. However, whether or not TSM can migrate to the tumour guided by mechanical taxis has not been studied yet. Elucidating the ability of TSM to display durotactic behaviour and studying this in detail will provide crucial information for the development of therapies for solid carcinomas and fibrosis.

In this project Tumour Stromal Myofibroblasts were plated on PAA gels with double rigidity gradient – 4 kPa, low rigidity healthy tissue stiffness and 25 kPa, fibrotic tissue rigidity, to assess cell ability to migrate towards less compliant substrate mimicking movement towards fibrotic sites. Fluorescent nanobeads labelling the 25 kPa gel side indicated the transition region between both rigidities. Acrylamide/bisacrylamide 4 kPa and 25 kPa working solutions were prepared from reagents according to the table 1. 25 kPa solution was labelled by adding 2.5 µl FluoSpheres carboxylate 0.2 um, yellow-green (Molecular Probes, cat. F8811). To create a gradient rigidity for the durotaxis assay two droplets, each containing 4 µl of the 4 kPa and 25 kPa gel, were placed adjacent to each other on a hydrophobic microscope slide. A 13 mm activated coverslip was placed on the droplets avoiding the liquids to mix. Regions of different rigidities were distinguished by fluorescent beads embedded in the stiffer part of the substrate. After 45 minutes of polymerization gel-coated coverslips were gently removed and crosslinked with fibronectin by photoactivated Sulfo-SANPAH. Cells were seeded following the one hour gel incubation with the culture medium. Thirty minutes after seeding the migration of cells was recorded by video time lapse microscopy with phase

contrast for 5 h 45 min. Observation was made in boundary, soft and stiff region with ~70 cells per region. The rigidity gradient from soft to stiff is marked / imaged by increasing density of fluorescent beads which contained the 25 kPa gel (Fig. 1). Cell movement distance in each stiffness and boundary region was calculated by subtraction of initial (at 0 h) and final (at 5 h 45 min) x, in gradient axis, and y, perpendicular to gradient axis, cell position coordinates.

The results demonstrated that the highly effective durotactic response is induced and directed by the rigidity gradient. It has been shown that fibroblasts are able to migrate towards higher rigidity[1, 2], but in this experiment durotaxis was shown for the first time in the context of modelling the migration of TSM into fibrotic sites. Our results demonstrated that TSM subjected to relatively lower gradients, mimicking diseased tissue, are also able to sense the rigidity difference and undergo the durotactic response. This outcome adds another important factor that has to be taken into account in designing anti-fibrotic treatment. The gradient model as proposed in our research, resembles changes of a soft to stiff microenvironment, which can be caused by TSM ECM production residing at wound/fibrotic sites. We demonstrated that increased migration appears only in the gradient region and neighter the compliant nor the rigid substrate trigerred this effect. It was further demonstrated that directed movement occurs only in the axis corresponding to the gradient.

This initial research, involving experiments mimicking physiological conditions in which Tumour Stromal Myofibroblasts in a fibrotic tissue are exposed to various microenvironment stiffness, revealed that cells are able to migrate from healthy to fibrotic stiffness substrate and can contribute to the progression of the disease.

1. Kidoaki, S. & Matsuda, T. Microelastic gradient gelatinous gels to induce cellular mechanotaxis. *J Biotechnol* **133**, 225-230 (2008).
2. Lo, C.-M., Wang, H.-B., Dembo, M. & Wang, Y.-l. Cell Movement Is Guided by the Rigidity of the Substrate. *Biophysical Journal* **79**, 144-152 (2000).

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Table 1 Reagents proportion to obtain polyacrylamide gel with desired rigidity

stiffness [kPa]	acrylamide concentration %	PBS volume [µl]	APS [µl]	TEMED [µl]	acrylamide/bisacrylamide (29:1) 40% vol [µl]
1.3	2.7	461.6	2.5	1	34.9
4	4.6	437.1	2.5	1	59.4
12	6.1	417.7	2.5	1	78.8
14.8	7.6	398.3	2.5	1	98.2
25	9.7	371.2	2.5	1	125.3

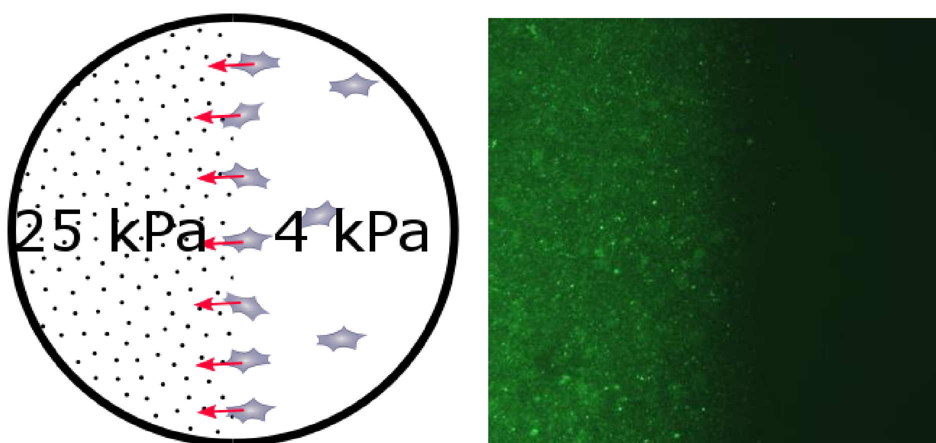

Figure 1 Schematic of double rigidity polyacrylamide gel to study cell durotactic response