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DEVELOPMENT OF A CELL POPULATION MIGRATION ASSAY

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INTRODUCTION: Cellular migration is central to many physiological and pathological processes, from wound healing and the immune response to cancer cell invasion. Many *in-vitro* assays have been developed to study these processes and can be classified by the nature of the assay, for example migration of single cells or entire populations, as well as migration on a planar surface or through a 3D structure.
This work concerns the development of an assay to study the migration of whole populations of cells across a deformable planar substratum, which may be coated in adsorbed adhesion molecules. The technique detailed below was developed from the Teflon fence assay.[1]

METHODS: QSil216 silicone elastomer (ACC Silicones Ltd, UK) was cast into a mould to yield optically clear membranes, each containing a 0.5cm^2 square hole. The deformable substrates were made of polyurethane (PU) (grades Z1A1 and Z3A1, Biomer Technology Ltd., Runcorn, Cheshire, UK), cast from solution (10% in DMF), from which discs with a diameter of 15mm were punched. The latter were secured in the bases of 24-well tissue culture plates (Sigma). Solutions of adhesion molecules, such as fibronectin, were applied to the discs as required for the adsorption of the protein to the PU surface and then subsequently washed. Square wells were created by placing the silicone membrane onto the PU discs. Into these square wells 150µl suspensions of human aortic smooth muscle cells, at a concentration of 5x10^5 ml^{-1} in medium 231 supplemented with SMGS (Invitrogen, UK), were placed. Within 24-48 hours the cells had attached to the PU surface and migrated and proliferated to completely cover the base of the square wells. Upon removal of the silicone membrane the cells started to migrate and populate the cell-free area.
Phase-contrast images of each well were captured at regular intervals using an AxioImager Z1 microscope (Zeiss) to record the motion of the cells as they migrated into the unpopulated regions. The "Mark and Find" function of the microscope software was used to track cell motion at specific locations in each sample whereas the image analysis software ImageJ (NIH, USA) was used to determine average rates of migration. The latter were determined by measuring the mean displacement of all cells at each time point relative to the initial boundary.

RESULTS: The Figure below shows a sequence of graphically-enhanced phase-contrast images of a representative sample area of cells migrating into the adjacent cell-free area over a period of 72 hours in culture. The rate of migration over this time period was relatively constant and the leading edge of cells uniform along the perimeter of the initial cell confinement area.

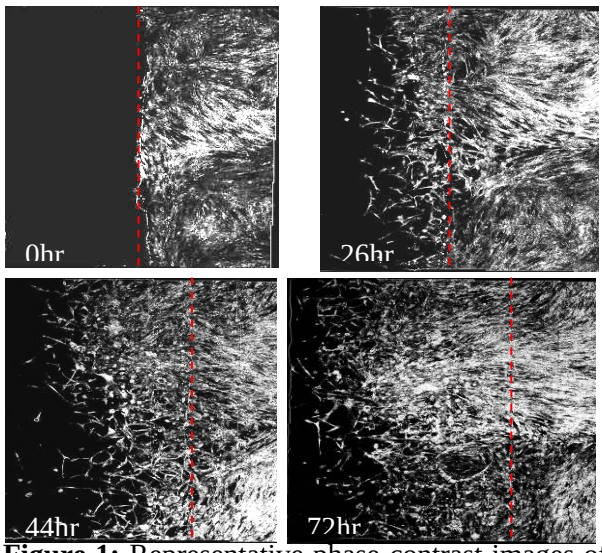

Figure 1: Representative phase-contrast images of the migration of cell population after the removal of the constraint at t=0 hours.

DISCUSSION & CONCLUSIONS: The assay developed here builds on the original Teflon fence assay for the purpose of providing a robust and reproducible method of measuring cell population migration rates on compliant substrates coated in adsorbed proteins. The circular fence of the original assay was replaced by a square one to aid in the processing of data while the Teflon construction material was replaced with a silicone elastomer. This allowed for a better contact with the PU, preventing cells "escaping" under the barrier before the start of the experiment in more samples. The optical clarity of the silicone allowed the determination of such a breach of the barrier and the sample to be discarded.

REFERENCES: [1] Pratt, B. M. et al. A. *Am. J. Pathol*. 1984, 117 (3), 349-354.