INTRODUCTION: The cytoskeleton in a non-muscle cell is dynamic and versatile, and drives many essential processes ranging from cell division to wound healing. Through our work, we hope to understand how and where cells such as fibroblasts build and regulate the cytoskeletal machinery needed to carry out well-defined motile tasks. In essence, we aim to get, for non-muscle cells, the type of information routinely available to the muscle physiologist - detailed mechanics in addition to cytoskeletal kinematics. As a basis for this project, we need quantitative information on the spatial pattern and magnitude of the forces (tractions) applied by a cell to its surrounding matrix and neighbours, and a means for matching those patterns to identifiable cytoskeletal structures. The pioneering work of Harris [1] in growth of cells on transparent elastic substrata provides a means for estimating complex tractions through microscopy and digital image processing [2]. We have extended this approach to cells in collagen matrices, and we have automated the mechanics analysis.

METHODS: In our experiments, mouse 3T3 fibroblasts expressing GFP-alpha-actinin or YFP-actin are grown in a three-dimensional (3D) model extracellular matrix (ECM) composed of type I collagen hydrogel with specific isometric constraints and free-boundaries. Collagen is the primary constituent of connective tissue ECM and thus approximates a native environment for this cell type. The entire specimen is made small enough (5mm square x 0.3mm thickness) so that a large fraction of its volume is accessible to view with high-NA optics. By use of a perusable, thermostatted environmental chamber, the specimen can be maintained on the microscope for periods up to seven days. Our Automated Interactive Microscope (AIM) supports time-lapse, repetitive serial-focus operation, and switching between transmitted-light (Nomarski DIC) and fluorescence imaging modes. In the fluorescence illuminator light path, we utilize (Nomarski DIC) and fluorescence imaging modes. In the fluorescence illuminator light path, we utilize (Nomarski DIC) and fluorescence imaging modes.

RESULTS AND DISCUSSION: Over a period of several days in culture, 3T3 fibroblasts migrate long distances, proliferate, and contract the collagen gel to form a dense matrix resembling connective tissue. Deformations caused by the action of relatively isolated cells can produce anisotropy in the collagen which may affect other cells through contact guidance over 100-1000um distances. Sparse cells appear to organize into clusters or chains that
produce large-scale (non-elastic) condensation of the collagen. Single cells extend and retract long processes (pseudopods and filopods) and small lamellipods over 1- to 15-minute periods. In serum-deprived cultures, this activity produces little change in the model ECM. However, in growth medium (10% serum), cellular action leads to prominent elastic deformation of the collagen coupled to cell shape changes and locomotion. In fluorescence optical sections, GFP-alpha-actinin shows a highly non-uniform distribution throughout the cell (Fig.1), with major concentration in pseudopods and filopods. Deformation is quantified in corresponding image maps of collagen density increment and of strain (Fig.2). Further data analysis will test the hypothesis that alpha-actinin is concentrated in the actin cytoskeleton at locations under the greatest tensile stress. In addition to the analysis of single-cell mechanics, it is possible to quantify large-scale deformation in tiled image sets. This opens the way to analysis of fibroblast behaviour in reshaping tissue in terms of single-cell effects, direct effects on neighbours, and collective action mediated by long-range effects on material properties.


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