Noncooperative cell behavior

Under certain conditions cells interact with the biomaterial surface each individually
A brief review or relevant structures: cell membrane, transmembrane proteins, cell receptors (integrins), cytoplasm, matrix
Definition of unit cell process

Unit cell process confined conceptually in a control volume $dV$
A typified cell diagram showing cell-cell binding.

Diagram removed due to copyright restrictions.
Cell membrane sketch showing transmembrane proteins
Another model of a specific cell-matrix interaction

Diagram of fibronectin attaching cell to surface of collagen fiber removed due to copyright restrictions.
View of cytoplasm

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A biologically active ECM analog
Cells pull matrix

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Fibroblast contraction of a collagen–GAG matrix

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Modified cell force monitor used to study cell-matrix interactions quantitatively

Use to study unit cell processes quantitatively

Freyman et al., 2001
Fig. 2. Contractile force plotted against time, for several densities of attached fibroblasts at 22h (cell number in millions). Raw data is plotted for 2.3 and 4.4 million attached cells to show data scatter. Higher densities are shown by trend lines for clarity.
Fig. 4. Force plotted against attached cell number per sample at 22 h, showing a linear relationship at 2 h (solid line) and 10 hours (dashed line) post-seeding.

Table 1
Exponential curve fit parameters ($\tau$, $F_a$)

<table>
<thead>
<tr>
<th>Total no. of attached cells in matrix ($\times 10^6$)</th>
<th>Time constant, $\tau$ (h)</th>
<th>Asymptotic value, $F_a$ (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3 $\pm$ 0.31</td>
<td>5 $\pm$ 1.3</td>
<td>3.7 $\pm$ 0.6</td>
</tr>
<tr>
<td>4.4 $\pm$ 0.21</td>
<td>4 $\pm$ 0.5</td>
<td>5.4 $\pm$ 1.4</td>
</tr>
<tr>
<td>6.0 $\pm$ 0.13</td>
<td>5 $\pm$ 0.4</td>
<td>8.1 $\pm$ 0.5</td>
</tr>
<tr>
<td>7.2 $\pm$ 0.05</td>
<td>7 $\pm$ 1.5</td>
<td>10 $\pm$ 1.9</td>
</tr>
<tr>
<td>10 $\pm$ 0.23</td>
<td>4 $\pm$ 0.5</td>
<td>12 $\pm$ 0.7</td>
</tr>
</tbody>
</table>
Fig. 5. Light micrograph of hydrated matrix (scale bar = 100 μm).

Conclusions on Linearity vs. Cooperativity of Fibroblast Contraction of Matrix

• The contractile force increases linearly with cell density.
• The average contractile force is calculated at 1 nN per cell.
• The time constant for development of force is also independent of cell density.
• In this model cells must develop contractile forces individually, not cooperatively.
SECOND ARTICLE

Experimental Cell Research 269, 140–153 (2001)

Micromechanics of Fibroblast Contraction of a Collagen–GAG Matrix

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See Fig 2 (schematic of imaging setup), Fig. 4 and Fig. 5 (graphs of results). In Freyman et al. “Micromechanics of Fibroblast Contraction of a Collagen-GAG Matrix.” *Exp Cell Res* 269, no. 1 (2001): 140-153. 
http://dx.doi.org/10.1006/excr.2001.5302
Sequence showing a cell (arrow A) simultaneously elongating and deforming a matrix strut (arrow B). 

**FIG. 6.** Sequence of images depicting a cell (arrow A) simultaneously elongating and deforming a matrix strut (arrow B). As the ends of the strut were drawn closer (2-28 min), the cell extended toward the ends of the strut (arrows C); it did not contract along with the strut. The buckling of the strut coincided with the release of the cell from the cell matrix (arrow D). Which also seems to buckle to accommodate the cell.
Another sequence showing a cell (A) elongating and deforming matrix struts (B).

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Sequence shows cell (A) elongating on matrix strut (B). Later, adhesion sites near cell center are released (C); eventually one end of cell fails to attach and the cell retracts rapidly (D). Later, the cell elongates once more (E) and the process is repeated.
struts for a given displacement imposed by the cell. Note that following the onset of buckling, resistive force does not increase significantly for increase in deformation.
Conclusions on Micromechanics of Fibroblast Contraction

• The aspect ratio of cells increases with time and eventually saturates, just as the force does.
• Initiation of cell elongation occurs stochastically.
• The force plateau most simply results from buckling or bending of individual struts in the matrix by cells.
• Matrix deformation (contraction) occurs as a result of cell elongation, not cell contraction.
Fibroblast Contractile Force Is Independent of the Stiffness Which Resists the Contraction

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http://dx.doi.org/10.1006/excr.2001.5408
FIG. 5. Plot showing the effect of initial matrix stiffness on the average reduction in diameter of free-floating matrix disks over 2 weeks in culture. The attached cell number does not vary significantly with time or between initial stiffness groups.
FIG. 6. Light micrographs of H&E-stained GMA sections of free-floating matrix samples showing cell distribution and matrix microstructure changes with time. Less stiff matrix disks are shown in a, b, and c for time points 1, 6, and 15 days, respectively. Stiffer matrix disks are shown in d, e, and f for time points 1, 6, and 15 days, respectively. Scale bar, 200 μm.
FIG. 7. Schematic showing the centripetal motion of adhesion sites and the centrifugal motion of cytoplasm. This attempts to explain the phenomenon of simultaneous cell elongation and matrix contraction. (a) As the cell elongates, due to cytoplasm motion, new adhesion sites...
Conclusions on the Effect of Matrix Stiffness on Cell Contraction

• The contractile force generated by fibroblasts was independent of matrix stiffness in the range 0.7 – 10.7 N/m.

• Contractile forces generated by cells are force-limited, not displacement-limited.

• As cells elongate, cell-matrix adhesion sites hypothetically form at the cell periphery, increasing length of matrix strut under compressive load and decreasing load required to buckle the strut.