Modeling Contact Guidance and Invasion by Cancer Cells

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Abstract
The first step in the spread of cancer is invasion by malignant cells of the normal tissue surrounding a tumor. There is considerable evidence both \textit{in vitro} and \textit{in vivo} that mechanical interactions with the tissue, in particular with the biopolymer network that makes up the extracellular matrix (ECM), are important factors in invasion. The interactions take two forms: (i) contractile cells on the surface of the tumor act on the nearby ECM and remodel it; in some cases, they align the fibers of the biopolymers; (ii) the aligned fibers can enhance invasion via contact guidance, the tendency of motile cells to follow alignment. Here, we give evidence, mainly for \textit{in vitro} systems, that both effects are important. We discuss how alignment occurs in biopolymers such as collagen-I (a major component of the ECM). We propose a modeling framework for computing alignment and propose phenomenologic models for contact guidance.

\textbf{See all articles in this Cancer Research section, "Physics in Cancer Research."}

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Introduction
Cancer is a serious disease mainly because it spreads. The first stage of spreading is local invasion; in this process, cells escape from a tumor into surrounding tissue and migrate in the extracellular matrix (ECM). This is often a precursor for metastasis (spread throughout the body via the blood and lymph systems). In some cancers, for example, glioblastoma multiforme or glioma, there is no metastatic spread; invasion in the systems. In some cancers, for example, glioblastoma multiforme or glioma, there is no metastatic spread; invasion in the tissue of the brain is the main mechanism for dispersal (1, 2).

When cells are ready to invade, they usually take on a rodlike shape (the epithelial–mesenchymal transition; refs. 3, 4). Mesenchymal cells, such as fibroblasts, are usually highly contractile (5, 6); invading cancer cells share this property (7, 8). Thus, it is to be expected that tumors will exert considerable stress on the surrounding tissue. This effect has been observed \textit{in vivo} (9, 10) and \textit{in vitro} in systems of glioma tumor spheroids in collagen (7, 11). Furthermore, as we will see below, large stresses can align the fibers of the ECM (12–14). Thus, the migrating cells are subject to contact guidance, i.e., the tendency of cells to move along oriented tissue (15–17).

The picture that emerges from this discussion is remarkable. Cells in tumors produce "highways" in the surrounding tissue and then cells detach and move along these highways. The mechanics of this interplay is the subject of this review. Of course, as many of the previously cited authors have noted, there are biochemical signals that affect cell motility and invasion. The micromechanics of these systems has received far less attention.

These effects may have clinical implications. For example, it has long been known that the mechanical microenvironment of a tumor affects invasiveness (3, 18–20). Here, we investigate specific mechanical mechanisms that could be important in producing the observed effects.

The Mechanics of the ECM
To understand cell mechanics during invasion, we start by describing the mechanical properties of a representative component of the ECM, collagen-I. Other biopolymers, such as fibrin, have similar properties (21).

Mechanics of collagen
Collagen-I is a very abundant animal protein and a major component of the ECM. Its mechanical properties have been studied in considerable detail (22, 23). We give here a review of the important features. The parameters we quote here are from ref. 23.

Collagen-I consists of fibers (bundles of polymer chains) that are quite large and stiff. Their diameter is around 30 nm. They can be considered to be elastic beams with a bending modulus of $K_f = 32pN \cdot \mu^2$. They have a stretching modulus, $K_s = E/A$, where $A$ is the area of the fiber and $E$ is the Young modulus, which we take to be 50 MPa. The fibers in the ECM are a cross-linked polymer gel with mesh size of order of 1 μm. For confocal images of the network and a method of extracting the positions and connectivity of the fibers see ref. 24.

The stiffness of the fibers is such that there is little or no thermal cooling between cross-links. The thermal persistence length, $K_f/k_B T$ (the length over which the stiffness of the fiber prevents it from coiling due to thermal fluctuations), is about 1 cm (23), far larger than the distance between cross-links. Here, $k_B$ is the Boltzmann constant and $T$ is the temperature. Thus, in contrast with more floppy materials like actin, we can think of the mechanics of collagen in purely mechanical terms.

The stress–strain relation of this material is quite strikingly nonlinear; see Fig. 1. There is an elastic regime at small strain, but above about 10% strain the curve steepens; collagen is a strain-stiffening material. The linear elastic modulus is quite

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regime to stretching-dominated high strain (25). Our model corresponds to the shift from a bending-dominated low-strain elasticity of collagen-I (23) based on the idea that the transition to high strain, there is considerable alignment of cells can easily drive collagen nonlinear. When collagen-I is small so that the critical stress for the onset of nonlinear behavior is very small, approximately 1 Pa. Thus, as we will see, cells can easily drive collagen nonlinear. When collagen-I is stretched into this regime, there is considerable alignment of the fibers, as the Fig. 1, bottom, shows.

We have given a finite-element model for the nonlinear elasticity of collagen-I (23) based on the idea that the transition corresponds to the shift from a bending-dominated low-strain regime to stretching-dominated high strain (25). Our model treats each fiber as an elastic beam with the bending and stretching moduli listed above. Each cross-link is treated as an angular spring with a torsional modulus that is the only adjustable parameter in the theory. The geometry of the gel is taken directly from experiment using the method we developed in ref. 24. This theory is successful. The line in Fig. 1 is our theory. For an unscaled version of the plot, in which the comparison between theory and experiment is clearer, see ref. 23.

There is a feature of this computation that is important for the subject at hand. The transition occurs because at low-strain, regions of the collagen rotate with respect to one another; this is called nonaffine deformation. We find that at low strain, 95% of the energy of deformation is in bending. For high strain, fibers align and the remaining deformation is mainly stretching the aligned fibers. In fact, we showed that if we completely neglect the cross-links and the bending modulus, we still fit the measurements at very high strain. This is because the fibers are essentially lined-up. Figure 1 (bottom) shows an experimental picture of the alignment.

It is interesting to see how the transition from nonaffine, bending dominated, to affine, stretching dominated, works out in a simple two-dimensional (2D) lattice model (26, 27). Such models illustrate in an unambiguous way that linear elements can give rise to nonlinear stress–strain relations. The model consists of a triangular lattice of bonds. Each bond has a stretching modulus, \( K_s \). In addition, each line of bonds is considered to be a continuous fiber with a bending modulus, \( \kappa \). Six fibers meet at each lattice site, and the link there is considered to be a freely rotating hinge. The model becomes interesting when it is diluted, that is, as in percolation models, bonds are removed with probability \( 1 - p \) so that the fibers have a finite length.

For small dilution, the model is stable with \( \kappa = 0 \), and the shear modulus is determined by \( K_s \). However, there is a point in dilution, the isostatic point beyond which the shear modulus is 0 if \( \kappa = 0 \); for the triangular lattice, this point is \( p_c = 2/3 \). For \( p < p_c \) the shear modulus for the small strain regime is proportional to \( \kappa \), and small deformations are nonaffine (28). This is the regime in which we expect the model to behave qualitatively like collagen. In Fig. 2, we show that this is the case and also present a quantitative measure of the alignment that we discuss next. We see that the nonlinear regime is associated with a rapid growth of alignment.

Alignment

We have discussed alignment qualitatively thus far. We will find it useful for modeling to give a quantitative measure. Roughly speaking, we want to define the average direction of fibers near some point. We might be tempted to specify this with a unit vector, say \( \vec{n} \); such a vector is called the director. However, \( -\vec{n} \) would be just as good, so \( \vec{n} \) is not an ordinary vector. Instead, we must take over the notion of a tensor order parameter, the nematic tensor, from liquid crystal theory (29) to have a representation that behaves correctly under changes in coordinate directions.

Suppose we have a set of fibers labeled by \( i \), which individually have directors

\[ \vec{n}(i) = [n_x(i), n_y(i), n_z(i)] \]
Figure 2. Top, stress–strain relation for the 2D model under shear for various values of dilution. For $P$ below the isostatic point, we have considerable nonlinearity. Bottom, nematic order parameter for the same model for $P = 0.6$. Alignment grows with applied stress. The line is the result of the Landau theory discussed below. Courtesy of Jingchen Feng.
Then, we form a traceless tensor, the nematic order tensor, out of the components of \( \mathbf{h}(i) \):

\[
Q = \begin{pmatrix}
(n_x^2) - 1/3 & (n_x n_y) & (n_x n_z) \\
(n_y n_x) & (n_y^2) - 1/3 & (n_y n_z) \\
(n_z n_x) & (n_z n_y) & (n_z^2) - 1/3
\end{pmatrix}
\] (1)

Here, \( \langle \rangle \) denotes the average over \( i \). This symmetric matrix can be diagonalized.

Thus, if the director is along the z-axis we expect:

\[
Q = q \begin{pmatrix}
-1/3 \\
-1/3 \\
2/3
\end{pmatrix}
\] (2)

We have assumed that the x, y directions are equivalent, on the average. The quantity, \( q \), is the strength of the alignment; \( q = 0 \) for an isotropic system. We recognize \( q \) as 3/2 times the
largest eigenvalue of $Q$, and the director is the eigenvector. This is the quantity plotted in Fig. 2, bottom. For results for real collagen, see ref. 14 in which the strength of alignment is measured with confocal microscopy.

**Cell Motility and Contact Guidance**

With this background in mind, we turn to evidence that fiber alignment is large near masses of cancer cells and that invading cells follow the alignment.

**In vitro evidence**

In this work, we will be exclusively concerned with cell motility in three dimensions. Motion on a surface, which is much more commonly studied, may be quite different and is, in any case, more distantly related to cell motion in tissue.

A popular *in vitro* model for invasion is the 3D tumor spheroid assay (7, 11, 30). In this system, a small well, around 1 cm on an edge, is filled with ungelled collagen or (sometimes) Matrigel. A lump of cultured tumor cells is dropped in, and gelation is forced so that the spheroid is suspended in the well. In the experiments that we will analyze, the tumor cells came from a U87 glioma cell line. The tumor spheroid is typically around 250 $\mu$m in radius. Cells are observed to exit the spheroid and can be observed with various forms of microscopy; see Fig. 3, top. For many cell lines, including U87, single cells detach from the spheroid and migrate away. This is thought to be a reasonable model for invasion by glioma in the brain.

We analyzed the results in several ways. By looking at a bright-field microscope picture of the pattern of cells exiting, we were able to show that the pattern, for one cell line, could only be understood by assuming that there was a drift velocity outward (31).

However, there is a more direct way to see how the cells migrate. We can track them individually with confocal microscopy and analyze the motion. An example of the type of data that we have is given in Fig. 3, bottom. It is clear from a glance at the picture that the cells are not moving randomly, but are flowing radially outward from the tumor spheroid. We made this quantitative in refs. 12 and 13. We showed that we can describe the motion of the cells as an average radial velocity outward of around 10 $\mu$m/h. The azimuthal velocity averages to 0.

So far, we have shown only that the cell motion is directed. It seemed likely, at first, that some biochemical cue was directing the cells via chemotaxis. However, there is certainly evidence that cells in the tumor spheroid deform the environment. In ref. 7, fluorescent beads were seeded in the matrix, and their motion gave a measure of the stresses in the system. By measuring the force near a single cell the strength of contraction for glioma was estimated as 10 to 100 nN.

A very interesting experiment by Vader and colleagues (13, 14) pointed directly to cell motion along aligned fibers as the likely cause of the directed motion. In this experiment, two tumor spheroids were put in the well (by accident). If we assume that cells on the surface pull on the matrix, it is clear that between two spheroids there will be a concentration of stress. The experiment took images of the fibers, by confocal reflectance, and also GFP-labeled glioma cells. In the resulting image the fact that cells follow aligned fibers is very clear (see Fig. 4). This is evidence for contact guidance of glioma cells in this experiment.

We can show that the cell tractions are enough to align the fibers by using the contractile force measured in ref. 7 and an estimate of the cell density on the surface of the spheroid to give a total force. Then, if we replace the two spheroids by planes, we can use the finite-element code of ref. 23 to see whether the fibers align. We find very strong alignment; see ref. 32. Our theoretical result also showed another effect: fibers are recruited to regions of high stress. This is evident in the experiment of Fig. 4 also.

![Figure 4. Two U87 tumor spheroids 3-mm apart. The fibers are imaged by reflectance, and the cells by transfected GFP. Note the fiber alignment and the concentration of cells along the fibers. Adapted from Stein et al. (12).](image-url)
A direct measurement of contact guidance for glioma was given in ref. 33. In this work, nanofibers were produced in two forms, random or aligned. The motility of glioma cells was measured on scaffolds made of this artificial fiber network. The result was a very strong increase in motility for the aligned case (see Fig. 5).

In vivo evidence

Provenzano and colleagues have shown that ideas very similar to what we have emphasized for glioma in vitro apply to breast cancer in vivo (9, 10). This group observed that invasive breast cancer is associated with alignment of collagen in the stroma of the tumor and that cells flow out along the aligned fibers. They even propose that alignment, detected by two-photon spectroscopy, can be used as a prognostic signature (34): Tumors with more aligned collagen are markers for more aggressive cancers. In Fig. 6, we show some of the results.

Prospects for Modeling

Mechanics of contact guidance

We now pose a simple question. Can we understand contact guidance in mechanical terms? It is not hard to suggest a qualitative picture. It is well known that cells “prefer” hard substrates to soft ones—they move more easily on hard materials (35) and even move toward hard materials if there is a stiffness gradient (36); this is called durotaxis.

For the kind of cell motility that is often important for invasion, cells move by attaching filopods to collagen fibers via integrin docking. Then the cell contracts, exerting considerable force (8). This force breaks the rear contacts, and the cell moves forward. For a detailed mechanical analysis of this type of motion for Dictyostelium, see ref. 37.

An implication of this analysis is that, on a hard substrate, all of the work done in contraction goes to breaking integrin bonds. On a soft substrate, some of the work is wasted in deforming the substrate. This means that the cell does less work in moving on a hard substrate; this is a plausible explanation for the observed higher motility. However, for a cell moving along an oriented fiber bundle, the substrate is quite stiff—the cell must stretch the bundle to deform it. However, for motion across the bundle the effective substrate is much softer—the cell can rather easily bend fibers. This may, in some measure, account for contact guidance.

We should point out another fact. A single contractile cell exerts enough force to take collagen into the nonlinear regime. Suppose we take the contractile force exerted by a cell to be \( f_c = 100 \text{ nN} \) (7, 32). A simple linear elasticity calculation can be done. Imagine that the force is equivalent to a negative pressure, \( p_c \), on a sphere of radius \( a = 10-\mu \text{m} \) imbedded in the collagen: \( p_c = -f_c/4\pi a^2 \). This pressure is around 80 Pa. It is a simple matter to find the stresses outside the cell (38):

\[
\sigma_{rr} = \frac{p_c a^3}{r^3}; \quad \sigma_{\theta\theta} = \sigma_{\phi\phi} = -\frac{p_c a^3}{2r^3},
\]

where \( \sigma_{rr} \), \( \sigma_{\theta\theta} \), and \( \sigma_{\phi\phi} \) are the components of the stress tensor, i.e., the force per unit area on elements of volume of the collagen. We use polar coordinates centered on the cell.

This means that at the cell boundary, the stress is 80 Pa, well into the nonlinear regime. In fact, the nonlinear regime starts around 40-\( \mu \text{m} \) outside of the cell. See ref. 32 for some implications of this fact; also see ref. 39. One thing seems evident. If cells are closer together than about 40-\( \mu \text{m} \), they will have strong mechanical effects on each other.

There is an alternative point of view about cell interactions with substrates (40). In this view, cells adjust to a target stress just outside that is computed using continuum elasticity theory. This leads to predictions about cells turning into the direction of principal stress and has implications for durotaxis.
The relationship between this point of view and the ideas expressed here remains to be clarified. One thing is clear, however, from the last paragraph: using linear elasticity for cells in collagen is quite risky. The work in ref. 39 is relevant to this point.

Modeling alignment

The considerations above have led us to try to construct a coarse-grained model for the interactions of collagen with cells. We are trying to give a tractable theory that can be predictive in specific cases. We have started with an effective continuum model for the nonlinear elasticity of collagen (41).

Our idea is to write down a Landau-type theory (29) for collagen. This is an expression of the coarse-grained free energy in terms of order parameters, the main variables that control the physics. We assume that there are two coupled order parameters: the usual strain tensor or rather its nonlinear counterpart, the left Cauchy–Green tensor, and also the nematic tensor, \( Q \). We couple the two according to symmetry requirements and minimize the free energy with respect to their values. We have tried to calibrate the model by comparing with the simple lattice models described above (26, 27). Our preliminary results are encouraging. Fig. 2 gives an example of the comparison of direct simulation to our theory.

We then need to insert cells. We will start as we did above: a cell can be thought of as a contractile inclusion in the medium or, as in ref. 40, as a force dipole.

Finally, we need an “equation of motion” for cells. Of course, we need to derive this from experiments. To our knowledge, quantitative measurements of contact guidance...
in stressed materials have not been done. We can, however, suggest a target for eventual experiments: of course, this discussion is purely speculative until experimental evidence is available.

A cell can be considered, in some cases, to move randomly, i.e., to diffuse. We can characterize this motion on large scales with a diffusion tensor, $\mathbf{D}$. In the presence of contact guidance, the tensor should become anisotropic. The simplest model that is consistent with the symmetry and that couples motility to alignment is this:

$$\mathbf{D} = D_0 (\mathbf{I} + \alpha \mathbf{Q}).$$  \hspace{1cm} (4)

The leading behavior is taken to be isotropic (proportional to the unit tensor, $\mathbf{I}$), and $D_0$ is the effective cell diffusion coefficient. The next term couples to the alignment so that there is faster diffusion along the director. We have defined the first contact guidance coupling constant, $\alpha$, which must be measured. It should be noted that if $\mathbf{Q}$ depends on position, there is an effective force in this equation. It corresponds to nearby cells attracting each other.

If there is a gradient of some growth factor that gives rise to chemotaxis, we may continue in the spirit of finding the simplest form consistent with symmetry by writing down an expression for the drift velocity of a cell:

$$\mathbf{v} = (\mathbf{I} + \beta \mathbf{Q}) \mathbf{v}_0.$$  \hspace{1cm} (5)

Here, $\mathbf{v}_0$ is the drift velocity that would occur for an isotropic material, and $\beta$ is the second coupling constant, which must be measured. In a simple hopping model it is clear that $\alpha = \beta$. Whether this is true for real cells needs to be settled by experiment. Note that, according to this equation, if the gradient is not along the local director, the cell will move along some angle between the two directions.

Summary

In this brief review, we have emphasized a mechanical point of view of cancer invasion. We have reviewed evidence for glioma in 3D assays and breast cancer in vivo that cells can align fibers of ECM, and then follow the alignment in the course of invasion. We have given a modeling framework that could guide further studies of this subject.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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