Systems Medicine Lecture Notes

Uri Alon (Spring 2019)

https://youtu.be/5GJlYh8j-xQ

Lecture 8

Inflammation and fibrosis as a bistable system

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Introduction:

Fibrosis, or excess scarring, is a medical problem that unites many diseases. The scar replaces functional tissues and the organ loses function. Fibrosis occurs in organs such as the liver, lung, kidney and heart, and is a major contributor to age related diseases. There is currently no cure for fibrosis.

In this lecture we will understand inflammation and fibrosis in more depth, and explore how tissues in the body become scarred and lose function. Our basic question is how a single biological process, tissue repair, can lead to two very different results: full healing or fibrosis. We will use this understanding to consider potential avenues for therapy to prevent and reverse fibrosis.

Injury leads to inflammation, which goes to either healing or fibrosis

As we all know from our childhood injuries, the injury gets red, swollen, hot and painful- this is **inflammation** (flames). The wound develops a scar in a few days. The scar usually vanishes after a couple of weeks and the tissue is perfectly healed. But sometimes we get permanent scars that last a lifetime. These scars are examples of **fibrosis**.

There is a universal sequence across tissues:

$$injury \rightarrow inflammation \begin{matrix} \nearrow fibrosis \\ \searrow healing \end{matrix}$$

Thus, tissue repair can lead to two different outcomes, depending on the duration and intensity of the injury. Generally, injury which is prolonged, repetitive or extensive leads to fibrosis. Transient or small injury leads to healing.

Fibrosis has a physiological function; if there is a pathogen or a foreign object that cannot be removed, the body tries to encapsulate it in fibrous tissue rich with collagen- the scar. For example, the virus hepatis C in the liver causes liver fibrosis (cirrhosis). Likewise, a large wound need to be filled in, especially in tissues with low regenerative ability, and fibrosis can do the job.

Fibrosis has a dark side in aging. In the last lecture we saw the example of IPF which is a lethal loss of lung function. Similarly, kidney dysfunction is due to massive scarring of the kidney, cardiac failure is accompanied by massive scarring of the heart. Fibrosis also occurs in non-age-related diseases. Alcoholism leads to liver fibrosis, and non-alcoholic fatty liver disease which goes with obesity afflicts ~30% of world population. Of these a

fraction progress to chronic inflammation and to fibrosis and loss of liver function. There is no effective treatment for fibrosis.
Therefore, in many medical situations doctors try to stop inflammation quickly to prevent fibrosis. For example, after surgery, stroke and heart-attack. There is usually a **limited time window** of about two days in which stopping inflammation can avoid fibrosis. If the window is exceeded, fibrosis is inevitable, even if inflammation is stopped. Why this time-window? In this lecture we will try to find the origin.
Another intriguing question is the slow timescale of healing and scar formation. Despite the short time window, we just discussed, of days, it takes *months to a year for the scar to mature*- that is, to reach its final steady-state composition. Likewise, it can take two weeks for healing to be completed. Where does this long timescale come from? This is another mystery we will try to explain.
Inflammation and fibrosis is a busy research field in biology and medicine, and is currently focused on a large number of molecular facts. Many signaling molecules activate and inhibit immune cells and fibroblasts, and these participating cells have many possible states. We will take a big-picture view, putting the main facts in a mathematical model that captures the essential core features. This model has a basic property called **multi-stability**- the ability for the same equations to show two or more very different stable steady-states. Multi-stability can shed light on inflammation and how it can lead either to healing, if the injury is brief, or to fibrosis, if the injury is prolonged. This understanding can also point to potential strategies to overcome and even reverse fibrosis.

Inflammation includes a massive influx of immune cells and activation of myofibroblasts

Injury to a tissue, such as the skin, causes the cells of the tissue to release factors that cause **inflammation**. These factors include IL6, Il1 and TNFa. The purpose of inflammation is to fight pathogens, and to start the repair process. Inflammation has four main features, which are easy to remember by the Latin rhyme rubor (redness) calor (heat), tumor (swelling) and dolor (pain).
Redness, swelling and heat are caused by the dilation of nearby blood vessels and their opening up to let immune cells flow into the tissue, together with blood proteins that fight pathogens (Fig 8.1). The immune cells are white blood cells that specialize in fighting bacteria, called neutrophils, and blood monocytes that turn into **macrophages** ('big eaters'), cells that can engulf pathogens and foreign bodies. Macrophages play a big role in fibrosis and healing, which we will describe soon.

In parallel to letting in macrophages, injury sets off a process to lay down fibrous material, mainly collagen, to seal up the injury. To do so, damaged cells as well as the incoming macrophages secrete factors (including $TGF\beta$) that activate a cell-type found in every tissue called **fibroblasts** (fiber-forming cells). This signal causes fibroblasts to change their shape and become super-fiber-forming **myofibroblasts** ('muscle-bound' fiber-former), Fig 8.1.

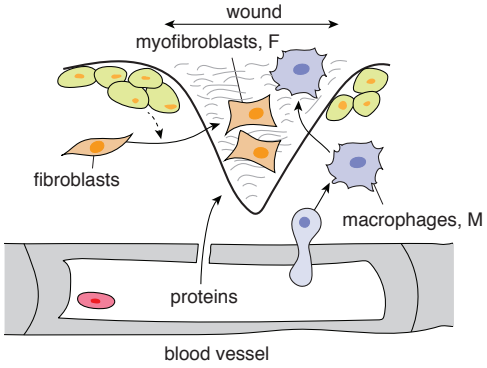

Figure 8.1

The two main cell types in our story are thus the incoming macrophages, which we will denote M, and the myofibroblasts, F. These two cell types activate each other's proliferation. They do so by secreting **growth factors** for each other – small proteins that diffuse in the tissue, and are sensed by receptors on the cell surface. The binding of the growth factor to the receptors makes the cells divide rather than die. The cells take in the receptor bound to the growth factor and degrades the growth factor – a process called **endocytosis** (Fig 8.2). Interestingly, F cells also secrete the growth factor for themselves, in an example of an **autocrine loop**. (Fig 8.3)

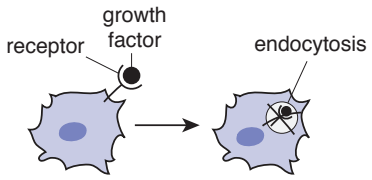

Figure 8.2

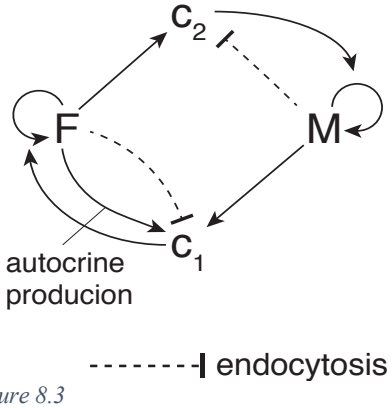

Figure 8.3

This circuit of two cell types has been characterized in detail by growing the cells together in a plate by Ruslan Medzhitov and colleagues (Cell,2018). This plate approach, called 'in-vitro', allows careful measurement of the parameters and dynamics of the circuit, such as cell growth rates, secretion and endocytosis rates. Thus, we have estimates for the rate parameters in this circuit. The situation in the body, 'in-vivo', is no doubt more complex than in vitro. Still, the in vitro information can provide guidelines to the principles that can help us understand the in vivo process. Generally, in systems medicine there are four approaches: *in vivo*, *in vitro*, *in silico* (computer simulation) and *in envelopo* (back of the envelope calculations, like in this course). Don't expect your friends to know this last term, we invented it for this course.

If the injury is transient, inflammation is resolved, M and F populations shrink (die by programmed cell death, called apoptosis) and vanish. The small scar formed is removed. The tissue cells, such as epithelial cells, divide, and the injury is healed.

If the injury is repetitive or prolonged, however, M and F populations rise and form a permanent scar. The fibrotic scar is thus made of fibers and cells. Our purpose will be to understand the dynamics of the inflammation process and how it can 'decide' to show healing or fibrosis.

Mathematical model for myofibroblasts can show bistability: an ON and an OFF state.

Let's begin by considering only the myofibroblasts, F. This will help us explain the equations, and will be useful soon when we add in the macrophages. The myofibroblasts produce a growth factor for themselves, called $PDGF\alpha$, which we will denote c_1 (Fig 8.4). They also eat up c_1 by endocytosis at rate e_1, and c_1 can also be removed by degradation, at rate γ_1. Thus

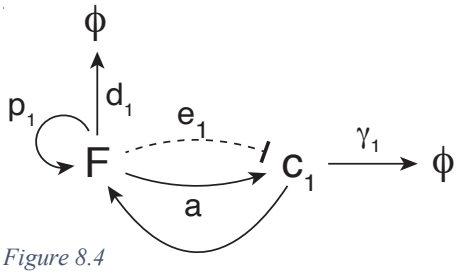

Figure 8.4

$$\frac{dc_1}{dt} = a\,F - e_1 F c_1 - \gamma_1 c_1 \qquad (1)$$

The parameters in these equations, based on cells grown in a plate (in vitro), are as follows: secretion rates like a are about 100 molecules/cell/min, endocytosis rates like e1 are about 1000 /cell/minute, and degradation half-lives like γ_1 are hours. Thus, endocytosis is the main removal mechanism of growth factors like c1, unless cell density is very low so that endocytosis can be neglected[1].

Since the production and removal processes take minutes to hours, and cell divisions and death rates are about 1/day, we can use separation of timescales as in the other lectures. We assume that on the timescale of minutes to hours in which c_1 levels reach their steady state, cell levels hardly change. Thus, growth factors like c_1 are in quasi-steady-state. Solving for $dc_1/dt = 0$, we find that c_1 rises with F and then saturates at a balance of production and endocytosis (Fig 8.5):

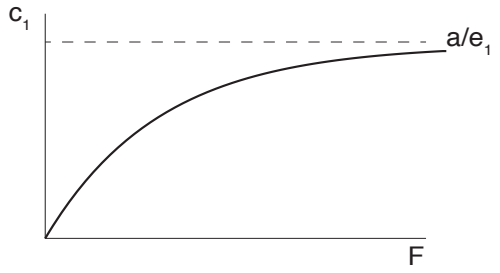

Figure 8.5

$$c_1 = \frac{aF}{e_1 F + \gamma_1} \quad (2)$$

The equation for the rate of change of F cells is the difference between cell division which increases with c_1, and cell death:

$$\frac{dF}{dt} = p_1 F c_1 - d_1 F \quad (3)$$

Plugging in Eq (2) for c_1 results in

$$\frac{dF}{dt} = p_1 \frac{aF}{eF + \gamma} F - d_1 F$$

Let's find the fixed points where $dF/dt = 0$. For this purpose, we can use a **rate plot**, a useful method for equations with one variable, such as F in this case. On the x axis we plot cell density F, on the y-axis we plot the total cell proliferation $p_1 \frac{aF}{eF+\gamma} F$ that starts out quadratically like F^2 and then, when F is large, continues linearly (Fig 8.6). We next plot the total cell death $d_1 F$, a line that rises with F (red line in Fig 8.6). The interesting points are where the proliferation and death curves cross, because these are the **fixed points**. The lines cross at zero and at a higher point, approximately $F = \frac{d_1 e_1}{p_1 a} = r_1$. This point is unstable: F rises to infinity if $F > r_1$, because F make their own

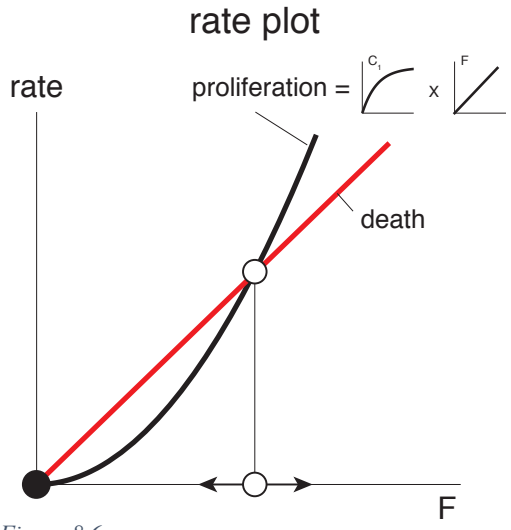

Figure 8.6

[1] Endocytosis also provides a natural length scale of about 100um, or about ten cell diameters, in which a secreted molecule of c_1 diffuses before it is eaten up by its target cells. This provides a natural 'compartment' size for cell-cell circuits. The lower the cell density, the longer this range, similar to screening effects in physics, so that the range is 'self-tuning'.

growth factor. This rise to infinity is not biologically feasible. We need something to control F levels.

To resolve this, we use the fact that fibroblasts can sense the density of other fibroblasts, and stop growing when they are too dense. In a plate, for example, F stop dividing when they touch each other. In a tissue they stop growing when they get to a maximal density of K. This mechanism prevents fibroblasts form piling up in tissues so as not to gum it up with fibers. This density limit is called a **carrying capacity**.

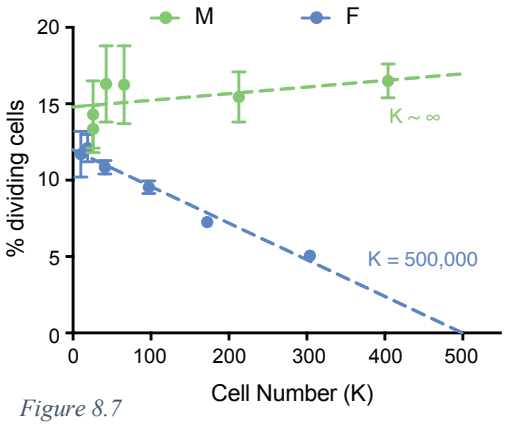

Figure 8.7

Carrying capacity is modelled in ecology and biology by a reduction of proliferation when F comes close to carrying capacity K, by multiplying division rate by the term 1-F/K. Such a linear reduction term for growth rate is observed experimentally in fibroblasts in vitro [Zhou, Cell 2018], by plotting the proliferation rate versus cell density, given by the total cell number in the plate (Fig 8.7). We can normalize F concentration by it carrying capacity K, so that the carrying capacity term is $1-F$. Thus, our equation with carrying capacity reads, after rescaling parameters:

$$\frac{dF}{dt} = p_1 \frac{aF}{eF+\gamma} F(1-F) - d_1 F \qquad (4)$$

Let's find the fixed points where $dF/dt = 0$ using a new rate plot (Fig 8.8). On the x axis we plot cell density F, on the y axis we plot the total cell proliferation, which this time looks like a hill with a dent on the left- the drop of the hill is due to the carrying capacity term that goes to zero when F=K. The death curve is a line as before. If death rate d_1 is not too large, the death curve crosses the proliferation curve *three times*: at zero, at mid concentrations and at high concentrations (Fig 8.8).

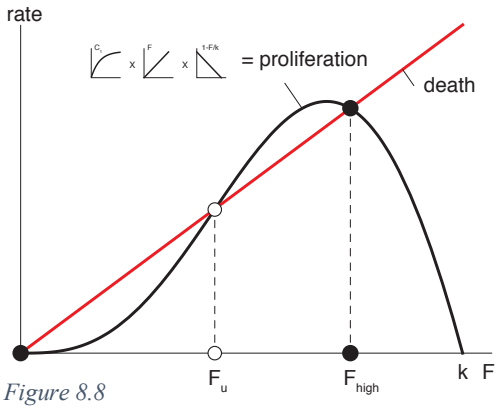

Figure 8.8

Let's analyze the three fixed points of Fig 8.8. The middle-fixed point is an **unstable fixed** point, at a position we will call F_u. To see this, note that if F is smaller than F_u, proliferation is smaller than death and F flows to zero. If F is larger than F_u, F levels flow to the high fixed point, F_{high}. Thus $F = 0$ and $F = F_{high}$ are two **stable fixed points**.

This feature of two stable fixed points for the same equation is called **bistability**. Depending on initial conditions, the system flows to one of two possible stable states. This can be seen in a plot of F versus time for different initial conditions (Fig 8.9): below an initial level of F_u, F crashes to zero; above F_u, F goes to a specific steady state concentration

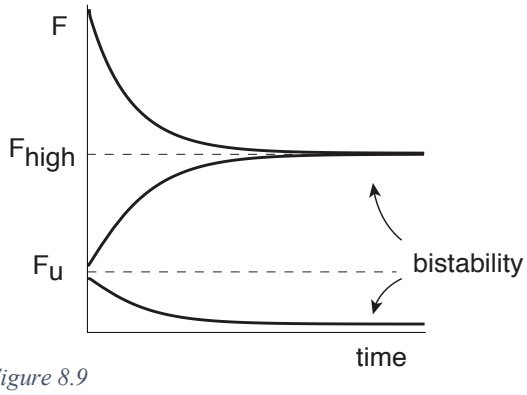

Figure 8.9

F_{high} no matter what the starting level was. There is thus a **basin of attraction** for each fixed point. Below F_u is the basin of attraction to the zero fixed point, called the OFF state; above F_u is the basin for the high fixed point, called the ON state.

Experimentally F cells can indeed support themselves in a plate at sufficiently high concentrations (Fig 8.10).

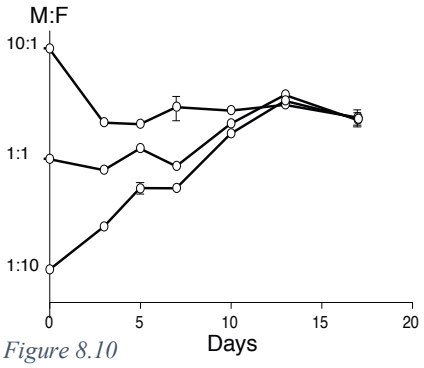

Figure 8.10

Notably, if death is high, or the hill-shaped curve is low, there is only one solution, at zero, as can be seen in the rate plot in (Fig 8.11). We will use this fact when we discuss ways to avoid fibrosis. The loss of bistability occurs if parameters that favor F cells are low, namely the autocrine secretion a and proliferation p_1, or if factors that are anti-F are high such as death d1 or endocytosis e_1. Loss of bistability occurs when a parameter combination which is the ratio of these anti and pro-F parameters exceeds one $\mathrm{d_1\ e_1}/a\ \mathrm{p_1}\ > 1$

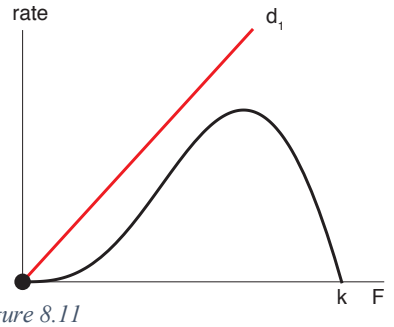

Figure 8.11

Macrophage-myofibroblast circuit provides two fibrosis states and a healing state

We now add macrophages, M, and see that the two-cell types together form a circuit that generates bistability, with an OFF state of healing and an ON state of fibrosis. They even have an additional ON/OFF state which is a second kind of fibrosis.

The monocyte-derived macrophages M pour into the tissue from the circulation during inflammation. Note that there are details here such as several states for M cells (called M1 and M2 states, for example) which we lump together into a single variable M(t). Macrophages M support F proliferation by secreting a specific growth factor ($PDGF\beta$), which F cells sense and also degrade by endocytosis. This is similar to the autocrine growth factor described above, and we will group the two growth factors together as c_1. The F cells support M cell division by secreting an M-specific growth factor (CSF1). Thus, M and F act to increase each other's numbers (Fig 8.12).

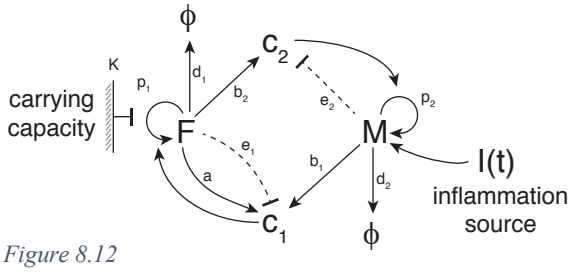

Figure 8.12

The F-specific growth factor c_1 is now secreted by both cells, and endocytosed by F cells:

$$\frac{dc_1}{dt} = aF + b_1M - e_1Fc_1 - \gamma_1 c_1$$

where b_1 is production rate per M cell. The M-specific growth factor, c_2, is produced by F and endocytosed by M

$$\frac{dc_2}{dt} = b_2F - e_2Mc_2 - \gamma_2 c_2$$

So that at quasi-steady-state

$$c_1 = \frac{aF + b_1 M}{e_1 F + \gamma_1}$$
$$c_2 = \frac{b_2 F}{e_2 M + \gamma_2}$$

The M cells divide under control of c_2. Unlike F cells, the M cells *have no carrying capacity*: their numbers can increase by tens of folds when inflammation causes a large influx. More precisely, they never approach their very high carrying capacity in physiological situations (Fig 8.7). Thus, M follows the simple equation

$$\frac{dM}{dt} = p_2 M\, c_2 - d_2 \mathrm{M}$$

The F-cell equation is as above, Eq 8.4.

Plugging in the quasi steady-state values for c_1 and c_2, we arrive at the cell equations on the scale of cell turnover (days)

$$\frac{dM}{dt} = M(p_2 \frac{b_2 F}{e_2 M + \gamma_2} - d_2)$$
$$\frac{dF}{dt} = F(p_1 \frac{aF + b_1 M}{e_1 F + \gamma_1}(1 - F) - d_1)$$

Looks a bit complicated... but we can make progress. To understand these equations, we use the method of **nullclines** - a lovely graphic method. Nullclines are the extension of the rate plot approach. Whereas rate plots work well for a single variable, nullcline are helpful for systems of equations with two variables such as M(t) and F(t).

Nullclines are curves in which one of the two cell concentrations does not change. One nullcline is $dM/dt = 0$, and the other is $dF/dt = 0$. The points to watch are the points where the two nullclines intersect. These are fixed points, since both cells don't change. It's therefore nice to draw both nullclines on the **phase plane**, whose axes are F and M cell concentrations, and study the intersection points.

The $dM/dt = 0$ nullcline is composed of the x-axis, $M = 0$ and of the solution to $p_2 \frac{b_2 F}{e_2 M + \gamma_2} - d_2 = 0$. The latter is a straight-line, $M = \alpha\, F - \beta$, with an intercept close to zero, $\beta = \frac{d_2 \gamma_2}{p_2 e_2}$. Plotting this line separates the phase plane into two regions, a top region in which M drops and a bottom region in which M rises (Fig 8.13).

The $dF/dt = 0$ nullcline is the y-axis $F = 0$ and the solution to $p_1 \frac{(aF + b_1 M)}{e_1 F + \gamma_1}(1 - F) - d_1 = 0$. The $F = 0$ and $M = 0$ nullclines intersect at zero, which is the OFF state. Zero cells is a stable state since at very low cell numbers there is not enough c_1 and c_2 to overcome cell death, and both cell populations crash.

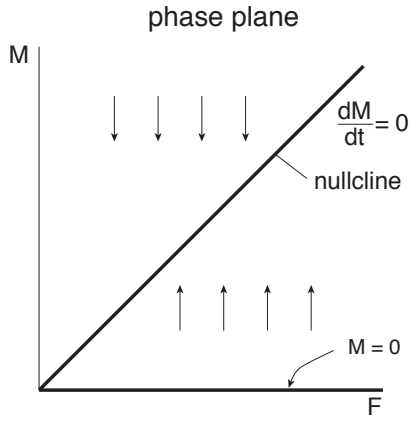

Figure 8.13

The more complicated F-nullcline equation can be understood if we look at the $M = 0$ line. There, we have the three fixed points we saw above when we discussed F alone. Plotting the nullcline, that looks like $M \sim (F + \beta)/(1 - F) - a/b_1$F, we see that it has a U-shape which drops through the unstable fixed-point F_u and rises through the high fixed-point F_{high}, and then climbs up and diverge near the carrying capacity $F = 1$ (Fig 8.14).

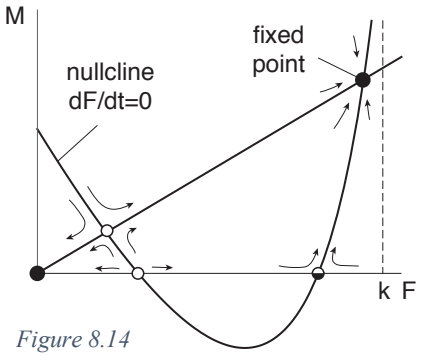

Figure 8.14

At each point in the phase plane, we can plot a little arrow showing where M and F flow to if they start at that point. Nullclines can help because they separate the plane into regions in which M and F move in a given direction (exercise 8.1). We can fill in arrows for the motion using the nullclines, and get a sketch of the **phase portrait**, in which arrows indicate the directing of flow. It's like a snapshot of the dynamics (Fig 8.15). The phase portrait was experimentally measured by using many different initial cell conditions in a 96-well plate, and watching where they move over two days [Cell 2018].

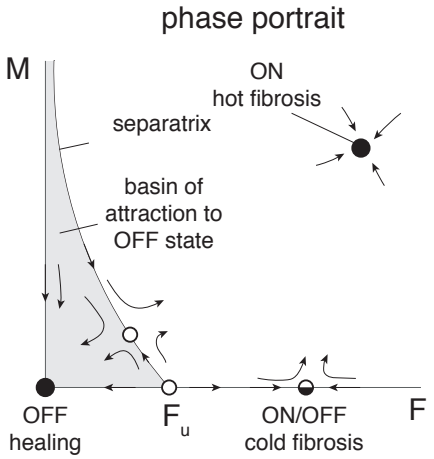

Figure 8.15

Near the carrying capacity the F-nullcline intersects the rising M nullcline. This is our final fixed point, in which both F and M are high and sustain each other. We call it the ON state (Fig 8.15). The ON state is a stable fixed point. All arrows in the vicinity flow to it. If a perturbation around the ON state occurs in which a bit of M is added, for example, M eats up its own growth factor and drops back to steady state. Similarly, a drop in F is stabilized by reduced endocytosis by F of its specific growth factor c1, and hence more growth factor and more F divisions. This is how a molecular feature, endocytosis, can provide a systems-level effect of stabilizing fixed points. The carrying capacity for F is also essential - without it, both cells would rise indefinitely.

The general condition for stability of such two-cell circuits was defined by Miri Adler et al [PNAS 2018]: either (i) both cell types have a carrying capacity or (ii) one cell type has a carrying capacity and the other a negative feedback on its growth factor such as endocytosis. The latter condition applies to the current situation.

To understand fibrosis, we need to consider also the fibers, namely the **extra-cellular matrix (ECM)** deposited by F cells, denoted E. The F cells produce E, and M cells produce molecular scissors (MMPs) that cut E up (Fig 8.16). These scissors are also produced at small amounts by the regular cells of the tissue. Thus, E rises with F and drops with M.

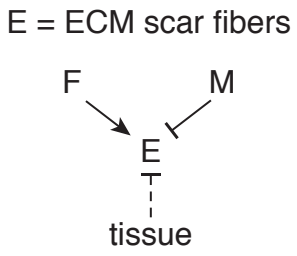

Figure 8.16

The OFF state, in which $F = M = 0$, is the healing state. The fibers E go to zero. The ON state in which both cell types are at a high steady state concentration is fibrosis. The fibers E

reach a high steady state concentration, continually made by F and degraded by M. The fibrotic scar is a living tissue.

Injury and inflammation can be modelled by a transient influx of M cells

To see the dynamics of healing, let's consider an injury. At t=0, there is a hole in the tissue. There is a small number of F and M cells at the injury site. Inflammation can be modelled as a large influx I(t) of M cells. Adding a source-term I(t) to the M equation results in

$$\frac{dM}{dt} = I(t) + M\ (p1\ c2 - d1)$$

Consider a two-day pulse of inflammation in which I(t) is high for two days and then returns to zero. We again use the phase portrait, but this time in log scale so that we can more easily see the region of low cell concentrations. M levels rise sharply, and produce c1. As a result, F cells begin to divide. If the dynamics stay within the basin of attraction to the OFF states, M levels fall, and with them F levels, until F=0, M=0 is reached (Fig 8.17). This trajectory is typical of proper healing. Scar E is deposited by F cells, and when the F cells are gone, scar is degraded by the tissue. Scar E(t) rises and then vanishes (Fig 8.17). The timescale, using the typical parameters of Table 8.1, is about two weeks.

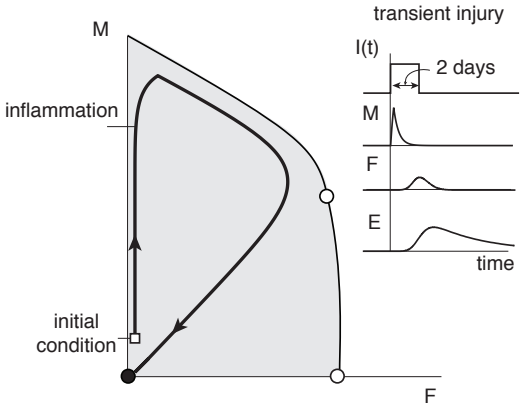

Figure 8.17

Now consider a longer pulse of inflammation which lasts for 4 days. M levels rise sharply, and cross the boundary to the basin of attraction to the ON state (Fig 8.18). This boundary is called the **separatrix.** Now there are enough F and M cells to support each other. The cells flow to the ON state. They create a scar tissue with constant turnover of M and F cells, and a steady state level of fibers E.

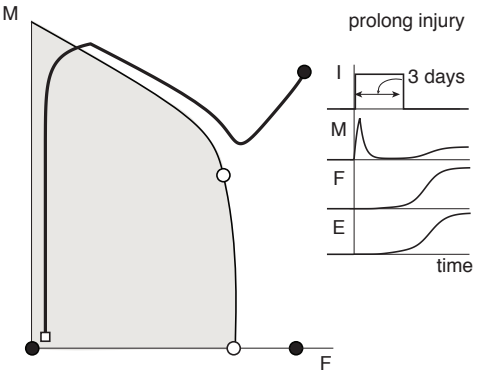

Figure 8.18

Similarly, consider a repeated injury. A 2-day inflammation pulse is insufficient to cross the separatrix, but if another 2-day pulse occurs after a week, there are enough M and F cells left from the first injury to make the dynamics cross the separatrix and go to fibrosis (Fig 8.19)

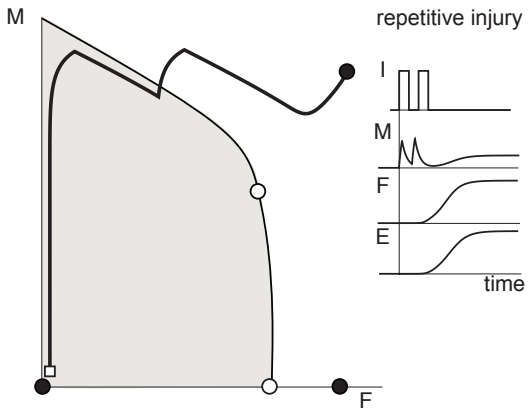

Figure 8.19

Thus, the same system can result in either healing or fibrosis, depending on the strength and duration of the inflammation pulse. To sum up, the system has a healing state with zero M and F cells and no scar. It has a fibrosis state with lots of F and M cells and permanent scars. It also has a third stable state, with only F cells, called the ON/OFF state. Since there are no M cells to degrade fibers, this ON/OFF fibrosis state has the most scarring fibers of all. In analogy to tumors, which are called hot or cold according to whether they have immune cells in them, we can call the ON state a 'hot fibrosis' because it has immune cells (M cells), and the ON/OFF state 'cold fibrosis' because it has no macrophages.

Examples of hot and cold fibrosis states can be found in the skin. Dermatology recognizes two main types of scars: keloids with abundant macrophages (hot fibrosis), and hyperproliferative scars which have fewer and fewer macrophages with time (cold fibrosis).

The time window for stopping inflammation is due to bistability

We can now understand why stopping inflammation within a brief time window is so urgent. Let's plot how the duration of the inflammation pulse affects the steady-state amount of scar fibers E (Fig 8.20). We see that below a critical duration of inflammation, $t_c = 3\ days$, the scar vanishes. Above t_c, M cross the separatrix and the ON state is inevitable *even if the inflammation pulse stops*!

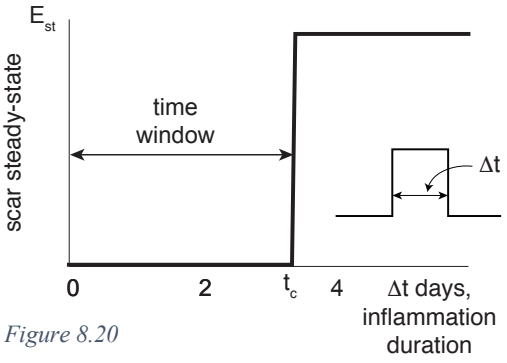

Figure 8.20

For example, an inflammation pulse just above t_c causes M to cross the separatrix, and F-cells have a bit of time to multiply. When inflammation stops, however, F cells are not very many and hence M levels sharply drop (Fig 8.18). But just before they crash to zero, they recover due to the increased F cells that are just enough to support them, and both F and M go up together over weeks to the ON state. Fibrosis occurs even though the inflammatory pulse stopped long ago.

The long timescale for scar maturation and healing is due to the slowdown near an unstable fixed point

Scar maturation is the process that unfolds over months to years in which the scar changes until it reaches steady state. This timescale is much slower than the cell turnover time of

days. The slow timescale found in the model is due to the fact that the dynamics near the separatrix approach an unstable fixed-point in the middle of the separatix (white circle in the separatrix line in Figs 8.16-8.18). By definition, at a fixed point, including an unstable one, the velocity is zero (no change). Thus, the velocity is slow near the fixed point, causing a slowdown phenomenon.

Intuitively, the slowdown is similar to a ball trying to climb out of a valley and go over a hill. He ball slows as it approaches the summit, and then speeds up again (Fig 8.21). The same applies to the healing process, which dawdles around the unstable fixed point and takes about two weeks to resolve back to the OFF state.

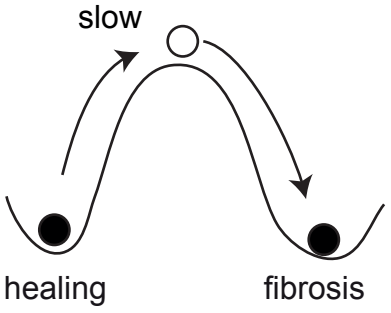

Figure 8.21

Aging and fibrosis

As we saw in the last two lectures, aging involves inflammaging - low-grade chronic inflammation. This is caused in part by the rise in SnC in many tissues. SnC secrete inflammatory molecules like IL6, IL1 and $TNF\alpha$. The SnC therefore supply, effectively, a prolonged inflammation signal.

If fibroblasts are triggered to differentiate into myofibroblasts in a given tissue region, due to a micro-injury or infection, these pro-inflammatory factors make the system more likely to cross the separatrix and result in fibrosis. A given injury that would be healed in the young develops into fibrosis in the old. Thus, aging increases the risk of fibrotic diseases. Aging can also supply the injury itself, as we saw in the case of IPF and OA where tissues approach a collapse in susceptible individuals due to slowdown of stem cell proliferation.

Strategies for preventing and reversing fibrosis:

Finally, we can explore what interventions might, in principle, prevent fibrosis. To prevent fibrosis, we need to enlarge the basin of attraction for the OFF (healing) state. Such enlargement means that more situations will end up resolved without fibrosis (Fig 8.22).

We can also explore whether fibrosis can be reversed, in the sense that a mature scar in the ON state can be made to flow to the OFF state. This can be achieved by depleting macrophages, provided that conditions are created such that myofibroblasts can no longer proliferate in the absence of macrophages, and must flow to the OFF state no matter how many myofibroblasts are present. Such reversal of fibrosis depends on the fact that fibrosis is a dynamic steady-state with cell turnover.

We find that both prevention and reversal of fibrosis become possible in the model when the ON/OFF fixed-point vanishes. This occurs when the unstable fixed-point Fu, in the case of only F cells, vanishes from the x axis (rises in the phase plane).

Both enlarging the basin and providing hope for reversing fibrosis can be achieved in the model by eliminating the fixed point in which F support themselves. To do so, we saw in Fig 8.11 that we need to lower the hump-shaped proliferation curve or increase the death of F cells. To so this, requires a combination of parameters exceeds one, the ratio of anti-F to pro-F parameters:

$$C = \frac{e_1}{a}\frac{d_1}{p_1} > 1$$

Thus, raising endocytosis rate of c1 by F cells, or F cell death rate can work. Similarly, decreasing a, the autocrine secretion of c1 by F, or decreasing F proliferation rate, will also work. Approaches suggested by this analysis thus include using small-molecule inhibitors of autocrine secretion or of endocytosis of the c1 receptors.

The dynamics when this condition is met ($C > 1$) are shown in Fig 8.22. A lengthy immune pulse of 6 days which would lead to fibrosis with wild-type parameters, now flows to the OFF state with no fibrosis. The ON state can still be reached after a pulse of 8 days.

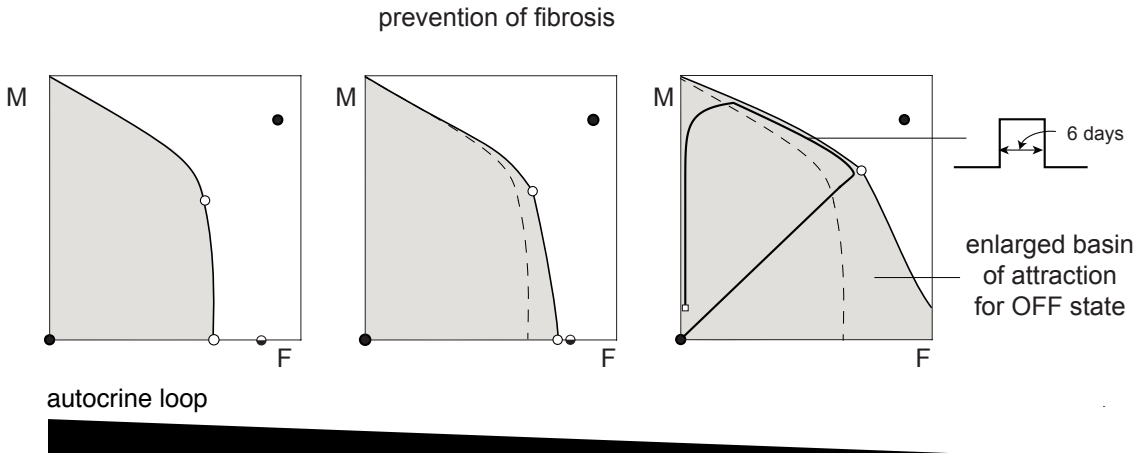

Figure 8.22

Removing the unstable fixed-point Fu can potentially also contribute to **fibrosis reversal** if macrophages can be depleted. Due to the absence of the ON/OFF fixed point, even if the ON state is reached, depletion of macrophages after 50 days of fibrosis leads to the OFF state, signifying fibrosis reversal (Fig 8.23). Depleting macrophages makes the dynamics go into the basin of attraction of the OFF state, because F-cells cannot support themselves with too few M-cells.

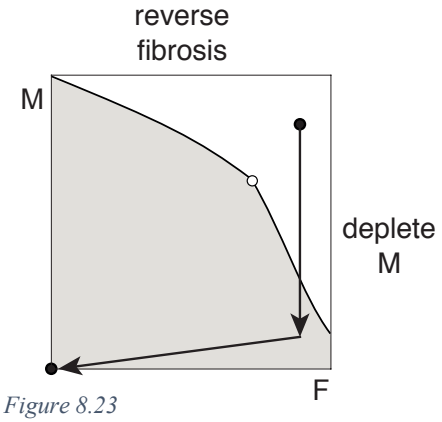

Figure 8.23

Such insight can explain certain medical treatments for the skin scars mentioned above, called **keloids**. Keloids are characterized by high densities of macrophages, as in hot fibrosis, and inflammation persisting in a constant state over years (Santucci et al., Virchows Arch 2001). Keloids are thus living tissue, and when you cut most of them out of the skin by surgery, they typically regrow (Love et al., J Drugs Dermatol 2013). One can interpret this by the resilience of the macrophage-plus-myofibroblast tissue at the ON state, such that even if a tiny volume remains with high cell concentrations after surgery, it can regrow.

Keloids can be treated by anti-proliferative therapies such as local injection of cortisol, cryotherapy, radiotherapy or topical application of cytostatic drugs such as bleomycin (Arno et al., Burns 2014), followed by surgery. The anti-proliferative therapy can reduce the proliferation rate of the fibroblast. This reduction in proliferation can act to remove the needed fixed point. The treatment also kills macrophages which are usually more sensitive

than F cells. Now surgery works well, because if a tiny amount of scar with mainly F cells is left, it cannot support itself, and flows to the OFF state.

A general cure for fibrosis has not yet been achieved, and many attempts have failed. To give hope for the plasticity and potential to reduce fibrosis, it is noteworthy that fibrosis is avoided in some biological situations. For example, embryos do not get scars or fibrosis if injured. In embryos there is no inflammation, fibroblasts do not differentiate into myofibroblasts and healing is mediated mainly by regeneration of epithelial cells rather than forming a scar. This makes sense because embryos are in a protected environment of the womb, where there is no danger of pathogens or foreign material entering the wound. Similarly, some mammals avoid fibrosis and regenerate more organs than humans do; mice regenerate their heart for example in the first week of life, and lose this ability thereafter. Thus, the healing circuit can apparently be tuned to avoid fibrosis in physiological settings.

Exercises

8.1 **Nullclines and directions of motion:** The nullcline $dM/dt = 0$ is the line where M does not change. On one side of the nullcline in phase plane, $dM/dt > 0$ which means that M grows, and on the other side $dM/dt < 0$ which means that M shrinks

(a) Why is this statement true?

(b) Which side of the nullcline corresponds to $dM/dt > 0$ and which to $dM/dt < 0$?

(c) Repeat for the $dF/dt = 0$ nullcline. Explain why this U shape nullcline separates the phase plane to a middle region where F flows to higher levels, and regions at low and high F where F flows to lower levels.

(d) Use these results to sketch the arrows in the phase portrait and to explain the stability of the fixed points.

8.2 Repeat the calculation when c_1 and c_2 act on F and M in a Michaelis-Menten way $c_1/(k_1 + c_1)$, $c_2/(k_2 + c_2)$. The same terms appear in the endocytosis term, because binding of growth factor to receptor both initiates the signaling that affects proliferation, and leads to endocytosis.

8.3 paradoxical effect of macrophage depletion:

Experiments have shown that depleting macrophages at different timepoints after an injury can result in improved healing or excessive fibrosis. Explain using the phase portrait.

8.4 ECM accumulation in tissue repair and fibrosis:

ECM is produced by myofibroblasts. ECM degradation is controlled by proteins called MMPs and TIMPs, where MMPs enhance the degradation of ECM and TIMPs inhibit the degradation of ECM. MMPs are produced mainly by macrophages apart from a small baseline level that is produced by the tissue. TIMPs are produces by both macrophages and myofibroblasts.

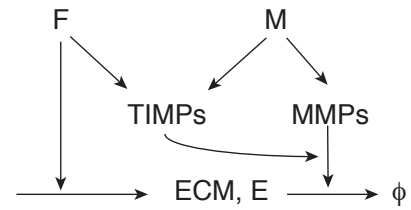

Figure 8.24

(a) Follow the interactions above to explain each term in following set of differential equations for MMPs, TIMPs and ECM.

$$\frac{dMMP}{dt} = \epsilon + a\,M - \alpha_1 MMP$$

$$\frac{dTIMP}{dt} = b\,M + c\,F - \alpha_2 TIMP$$

$$\frac{dECM}{dt} = d\,F\, - \alpha_3 \frac{MMP}{TIMP + k} ECM$$

(b) Assuming that the factors that control ECM degradation reach steady state faster than ECM, re-write the equation for ECM with the steady states of MMPs and TIMPs.

(c) Solve the steady state of ECM and describe its dependence on the number of myofibroblasts and macrophages.

(d) What is ECM steady states in healing, hot fibrosis, and cold fibrosis (don't solve the cells steady state, just use steady states notation such as F-hot for myofibroblasts level in hot fibrosis)? What can you say about the dependence of the scar size on F in hot

fibrosis Vs cold fibrosis if you consider that myofibroblasts numbers are approximately the same in the two fibrotic states?

8.5 Diffusion range of growth factors due to endocytosis: A growth factor c diffuses with diffusion coefficient D and is endocytosed (removed) by cells with density F at a rate $e\ F\ c$.

How long can the molecule travel on average before being removed? Show that this is approximately $L = \sqrt{(D/e\ F)}$. Show that this length-scale is about 100 microns for typical diffusion constants and cell densities.

Suppose the density of target cells F is low. How does this affect the range? What are the consequences for biological regulation of cell circuits?

Suppose that two micro-injuries of diameter 50 micron are made in a tissue at a distance of r from each other. Intuitively guess how the response would differ if r is much larger than L or similar to L?

Parameter	Biological meaning	Value
p_1	maximal proliferation rate of myofibroblasts	$0.9\ day^{-1}$
p_2	maximal proliferation rate of macrophages	$0.8\ day^{-1}$
d_i	removal rate of the cells	$0.3\ day^{-1}$
K	carrying capacity of myofibroblasts	10^6 cells ($\sim 10^{-3}\ \frac{cell}{\mu m^3}$)
k_{ij}	binding affinity (K_d) of growth factor C_{ij}	$6x10^8 molecules$
b_2	maximal secretion rate of CSF1 by myofibroblasts	$470\ \frac{molecules}{cell\ min}$
b_1	maximal secretion rate of PDGF by macrophages	$70\ \frac{molecules}{cell\ min}$
a	maximal secretion rate of PDGF by myofibroblasts	$240\ \frac{molecules}{cell\ min}$
e_2	maximal endocytosis rate of CSF1 by macrophages	$940\ \frac{molecules}{cell\ min}$
e_1	maximal endocytosis rate of PDGF by myofibroblasts	$510\ \frac{molecules}{cell\ min}$
γ	degradation rate of growth factors	$2\ day^{-1}$

This table uses the more accurate parameters for Michalis Menten functions for endocytosis and proliferation, which go as $c_i/(k_i + c_i)$ instead of as lnearly with c_i as in the text.