Chapter 5

Inflammation and fibrosis as a bistable system

Introduction:

Fibrosis, or excessive scarring, is a medical problem that unites many diseases. It cuts across medicine. In fibrosis, scar tissue replaces healthy tissue and the organ loses function. Fibrosis occurs in the liver, lung, kidney, heart and other organs, and is a major contributor to age-related diseases. It is always preceded by periods of intense inflammation. There is currently no cure for progressive fibrotic diseases other than organ transplant.

In this chapter we will understand inflammation and fibrosis in depth. Our basic question is how a single biological process, tissue repair, can lead to two very different results: healing or fibrosis. We will use this understanding to consider potential avenues for therapy to prevent fibrosis and even to reverse it.

We will use two of our laws in this chapter, all cells come from cells, and biological processes saturate. The third law, cells mutate, will star again in the next chapters.

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

As we know from our childhood injuries, the injured spot gets red, swollen, hot and painful- this is **inflammation**. The wound develops a scar over 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:

$$injury \rightarrow inflammation \rightarrow fibrosis \ or \ healing$$

Thus, the process of tissue repair can lead to two different outcomes, depending on the duration and intensity of the injury. In organs with poor ability to regenerate, like the heart, fibrosis is triggered by almost any injury. In organs that can repair themselves to a certain extent, transient or small injuries lead to healing, but prolonged, repetitive or extensive injuries cause fibrosis.

Fibrosis has an essential physiological function: if there is a pathogen or a foreign object that cannot be removed, the body tries to encapsulate it in fibrous scar tissue rich with collagen. For example, the hepatitis C virus in the liver causes liver fibrosis (cirrhosis). Likewise, a large wound that cannot be quickly healed needs to be filled in to maintain organ integrity. Fibrosis does the job.

But fibrosis has a dark side in aging. Tissues progressively tend to show more fibrosis than healing, as we will soon discuss in part 3 of the book. Fibrosis can cause organs to fail. For example, many types of kidney dysfunction are due to massive scarring of the kidney, and cardiac failure is accompanied by massive scarring of the heart. Alcoholism leads to liver fibrosis, and liver fibrosis also occurs in non-alcoholic fatty liver disease (NAFLD), associated with obesity, which afflicts ~25% of the world population. A fraction of those with NAFLD progress to chronic inflammation and to fibrosis, with loss of liver function. Fibrosis is a risk factor for cancer in many organs. There is currently no treatment for fibrosis except for organ transplant. Treatment for fibrosis is a huge unmet need.

Because inflammation always precedes fibrosis, physicians try to stop inflammation quickly to prevent fibrosis after surgery, stroke, heart-attack and other medical situations. There is usually a **time window** of about two days in which stopping inflammation can avoid fibrosis. If the time window is exceeded, fibrosis is inevitable, even if inflammation is stopped. Why this time-window? In this chapter we will try to find out.

Another intriguing question is the slow timescale of healing and scar formation. Despite the brief time window of days we just discussed, it takes *months 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, which has uncovered a large number of molecular facts. Many signaling molecules activate and inhibit immune cells and fibroblasts, and these cells have many possible states.

We will take a big-picture view, putting the essential facts into a mathematical model that captures the core features. This model has a basic property called **multistability**- the ability to produce two or more different stable steady states. Multistability can shed light on how inflammation can lead either to healing, if the injury is brief, or to fibrosis, if the injury is repetitive or prolonged. This understanding also points to potential strategies to prevent and reduce fibrosis.

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

Injury to a tissue causes cells to release factors that cause **inflammation**. Some of the damaged cells die, and others become large metabolically active cells that stop dividing, called senescent cells. The damaged cells and senescent cells secrete 'alarm' proteins that flow in the blood like IL6, IL1 and TNF that induce inflammation.

The purpose of inflammation is to fight pathogens and to start the repair process. Unlike the T-cells of the previous chapter, which begin to matter only several days after infection, here we are talking about the innate immune system which is much faster and responds in minutes.

Inflammation has four main features, which are easy to remember by a Latin rhyme- rubor, calor, tumor, dolor: redness, heat, swelling and pain. The fifth pillar of inflammation is loss of tissue function.

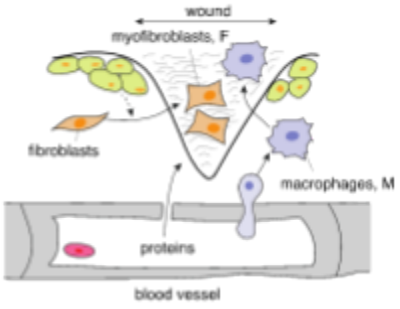

Figure 5.1: Inflammation at an injury involves influx of fluids and macrophages together with differentiation of fibroblasts into myofibroblasts which lay down scar tissue.

Redness, swelling and heat are caused by the dilation of nearby blood vessels, which open up to let immune cells flow into the tissue, together with fluids and proteins that fight pathogens (Fig 5.1). The main immune cells are white blood cells that specialize in fighting bacteria, called neutrophils. With them come blood monocytes that turn into **macrophages** ('big eaters'), cells that can engulf pathogens, dead cells and foreign bodies. They also help to remove the senescent cells, along with other innate immune cells called NK cells. 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 that lays down fibrous material, mainly collagen, to seal up the injury. To do so, damaged cells as well as the incoming macrophages secrete signals including TGF-β that activate a cell type found in every tissue called **fibroblasts** (fiber-forming cells). This signal causes the tissue-resident fibroblasts to proliferate and change their shape to become super-fiber-forming **myofibroblasts** (myo='muscle-bound'), Fig 5.1. Their muscle-like ability to generate force helps to contract and close the wound.

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. Interestingly, F cells also secrete a growth factor for themselves, in an example of an **autocrine loop** (Fig 5.2).

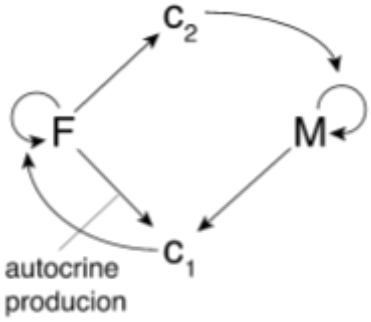

Figure 5.2: macrophages and myofibroblasts secrete growth factors for each other, and myofibroblasts also secrete growth factors for themselves in an autocrine loop.

This circuit of two cell types was characterized in detail by growing the cells together in a plate by Ruslan Medzhitov and colleagues (Zhou et al. 2018) Growing cells in a plate, called an '*in vitro*' approach, allows careful measurement of the parameters and dynamics of the circuit, such as cell growth rates and growth-factor secretion and removal rates. Thus, we have estimates for the rate parameters in this circuit.

The situation in the body, *in vivo*, is certainly more complex than in vitro. Still, *in vitro* studies can provide principles to 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 we do here). Don't expect your friends to know this last term, I invented it for this book.

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

If the injury is repetitive or prolonged, however, or if the tissue can not regenerate (such as heart and brain tissues), M and F populations rise and a permanent scar is formed, made of fibers and cells. This is fibrosis.

Our purpose is to understand the dynamics of the inflammation process and how it can 'decide' to show healing or fibrosis.

Mathematical model for myofibroblasts shows bistability

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 main point is that one equation for myofibroblasts can show two different behaviors, a property called bistability.

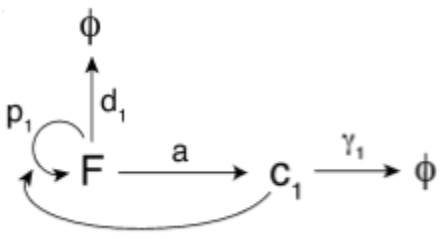

Figure 5.3: Circuit describing how myofibroblasts secrete their own growth factor and are removed.

But first, to clear the mind, let's take a nice deep sigh of relief.

The myofibroblasts produce and secrete a growth factor for themselves, called $PDGFA$, which we will denote c_1 (Fig 5.3). c_1 is degraded at rate γ_1. Thus the rate of change of c_1 is its production minus removal

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

The parameters in these equations, based on in vitro data, are as follows: secretion rates like a are about 100 molecules/cell/min, and degradation half-lives $\ln(2)/\gamma_1$ are hours.

Figure 5.4: The concentration of the growth factor is proportional to the concentration of the F cells that produce it at quasi steady state.

Since the production and removal processes take minutes to hours, and cell division and death take a day or longer, we can use **separation of timescales** as in the previous chapters. On the rapid timescale of hours in which c_1 levels reach their steady state, cell density F hardly changes. Thus, growth factors like c_1 are in quasi-steady-state, which we can compute using $dc_1/dt = 0$. We find that c_1 is proportional to the cells that make it, F (Fig 5.4):

$$(2) \qquad c_1 = \frac{a}{\gamma_1} F$$

We now turn to the equation for the rate of change of F cells, given by cell proliferation (all cells come from cells), which increases with c_1, minus cell removal at rate d_1:

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

Plugging in the quasi-steady-state Eq (2) for c_1 results in a proliferation that rises like F^2

$$\frac{dF}{dt} = \frac{p_1 a}{\gamma} F^2 - d_1 F$$

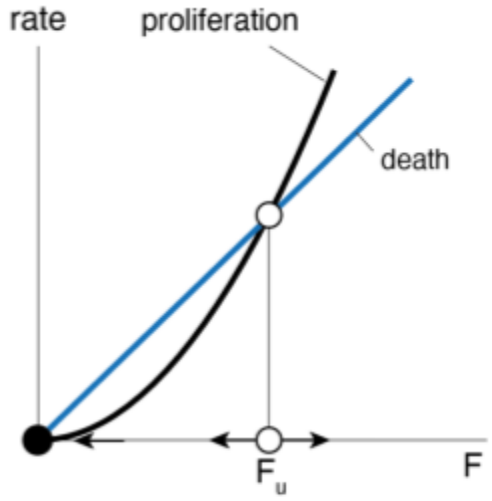

Figure 5.5: The total growth rate of F cells rises quadratically since they make their own growth factor. It intersects the removal rate curve that rises linearly with F at two points, a stable fixed point at zero and a second, unstable fixed point.

Let's find the fixed points where $dF/dt = 0$. For this purpose, we use the **rate plot**, a useful method for equations with one variable, F in this case. We employed the rate plot for beta cells in chapter 2. On the x-axis we plot cell density F and on the y-axis we plot the total cell proliferation $\frac{p_1 a}{\gamma} F^2$ that rises quadratically with F and is thus a parabola (black line in Fig 5.5). We next plot the total cell removal $d_1 F$, a line that rises with F (blue line in Fig 5.5).

The interesting points are where the proliferation and death curves cross. These are the **fixed points**. The curves cross at zero and at a higher point, $F_u = \frac{d_1 \gamma_1}{p_1 a}$. This point is unstable: F rises to infinity if $F > F_u$, because proliferation exceeds removal (the black line is higher than the blue line). More F cells make more of their own growth factor, spiraling out of control.

Such a 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 cells stop dividing when they touch each other. In a tissue they stop growing when they get to a maximal density denoted K. This mechanism prevents fibroblasts from piling up in tissues so as not to gum it up with fibers. The density limit is called a **carrying capacity**. It is an example of the law 2, biological processes saturate.

Carrying capacity is modeled in ecology and biology by reducing the proliferation rate when F comes close to carrying capacity K. Proliferation rate is multiplied by the term (1-F/K). Such a linear reduction term for growth rate, called logistic growth in ecology, is observed experimentally in fibroblasts in vitro (Zhou et al. 2018) by plotting the proliferation rate versus cell number in the plate (blue line in Fig 5.6). Proliferation is measured by a dye that stains cells that replicate their DNA. Indeed, proliferation drops with fibroblast cell density. The intersection of the blue line with the x-axis is the carrying capacity – the cell density that pushes growth rate to zero. Note for future use that macrophages, M, the green data points, seem not to have a measurable carrying capacity.

Thus, our equation with carrying capacity reads

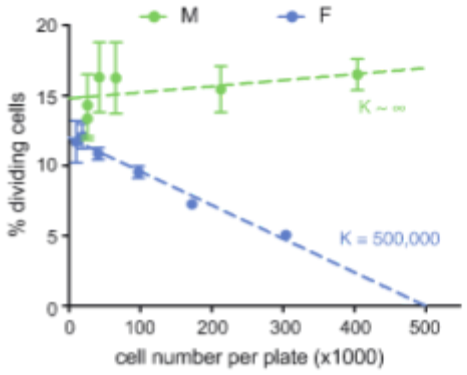

Figure 5.6: Cell carrying capacity can be measured by extrapolating the growth rate as a function of cell concentration to determine the concentration of zero growth. Adapted from (Zhou et al. 2018).

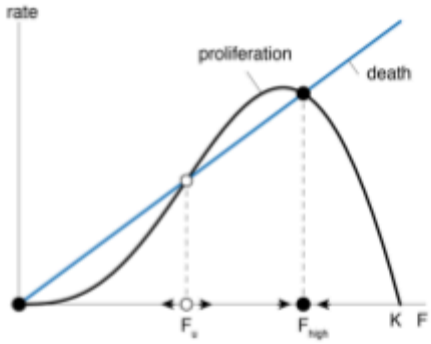

Figure 5.7: With carrying capacity, growth and removal curves can cross three times, generating two stable fixed points with an intermediate unstable fixed point.

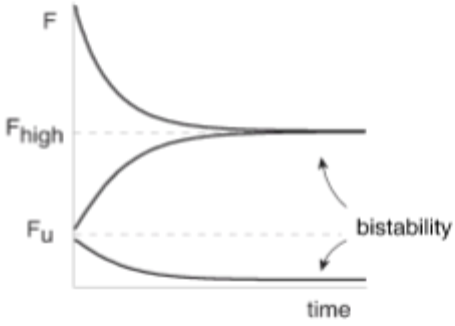

Figure 5.8: Bistability means that the same system has two different stable fixed points. Initial cell concentrations determine whether the high or low fixed point is reached.

$$\frac{dF}{dt} = \frac{p_1 a}{\gamma} F^2(1 - F/K) - d_1 F \qquad (4)$$

Let's find the fixed points using a new rate plot (Fig 5.7). On the x-axis we plot cell density F and on the y-axis we plot the total cell proliferation, which now looks like a hill with a dent on the left. The drop of the hill on the right is due to the carrying capacity term that goes to zero when F=K. The death curve remains a straight line as before.

If death rate d_1 is not too large, the death curve crosses the proliferation curve *three times*: at zero, at a middle concentration and at a high concentration of cells (Fig 5.7). Let's analyze the three fixed points of Fig 5.7. The middle-fixed point is an **unstable fixed** point, F_u. To see this, note that if F is smaller than F_u, the proliferation curve is lower than death and thus 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, 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 5.8): below an initial level of F_u, F crashes to zero; above F_u, F converges to a specific steady-state concentration F_{high} no matter what the starting level was.

Each fixed point has its own **basin of attraction**, defined as the range of initial conditions which flow to that fixed point. Cell density below F_u is in 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.

In vitro, F cells can indeed support themselves at sufficiently high concentrations. The steady-state is an ongoing balance of cells dividing and dying about once per day.

Notably, if the death rate is too high, or proliferation is too low, there is only one solution, at zero, as can be seen in the rate plot in (Fig 5.9). The removal and production curves cross only once. A change of parameters causes the loss of a stable fixed point! We will use this fact when we discuss ways to avoid fibrosis.

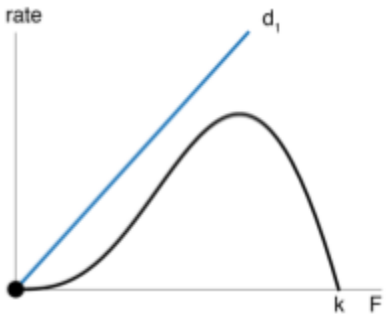

Figure 5.9: Bistability is lost suddenly when parameters change and the high stable fixed point vanishes. This occurs when death rate is high or proliferation rate is low.

This loss of bistability occurs when parameters that remove F cells exceed parameters that favor F cells. One can write a ratio of the 'pro-F' parameters - the carrying capacity K, autocrine growth factor secretion a and F proliferation p_1, relative to factors that are 'anti-F' such as death rate d_1 or growth-factor removal rate γ_1. Loss of bistability occurs when the ratio of these pro-F and anti-F parameters goes below a threshold: $p_1 a K/\gamma d_1 < 4$ (as shown in solved exercise 5.1). Exactly at the threshold, the removal curve touches the proliferation curve at a half-stable point, in addition to the zero fixed point.

The macrophage-myofibroblast circuit provides two fibrosis states and a healing state

We now add macrophages, M. 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 macrophages M pour into the tissue from the circulation during inflammation. Note that we ignore details here such as the existence of multiple states of M cells (called M1 and M2 states, for example), by lumping them together into a single variable M(t). The F cells enhance proliferation of macrophage by secreting an M-specific growth factor (CSF1). Macrophages M support F proliferation by secreting a specific growth factor ($PDGFB$). This is similar to the F autocrine growth factor $PDGFA$ described above, and since the two are secreted at similar rates, we will group the two growth factors together as c_1. Thus, M and F act to increase each other's numbers (Fig 5.10).

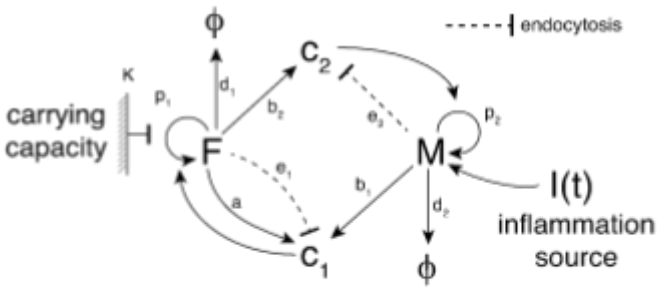

Figure 5.10: The macrophage-fibroblast circuit in which both cell types secrete growth factors for each other and deplete the growth factors by endocytosis. Fibroblasts have a carrying capacity, and secrete their own growth factors in an autocrine loop.

Unlike F cells, the M cells *have a very high carrying capacity*: their numbers can increase by tens of folds when inflammation causes a large influx. They don't approach their very high carrying capacity in most physiological situations (green line in Fig 5.6). Thus M cells require a different mechanism to avoid spiraling out to very high concentrations. This mechanism is a negative feedback loop due to a basic biological process: *the cells that respond to a growth factor also eat it up.* M cells suck in the receptor on their membrane when it binds to the growth factor. They then degrade the growth factor and sometimes the receptor too – a process called **endocytosis** (Fig 5.11). Endocytosis ensures that if there are too many M cells, they eat up their own growth factor and their numbers thus reduce back to steady-state[1].

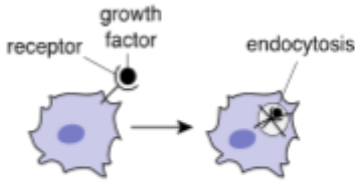

Figure 5.11: Endocytosis is the intake of molecules that bind a receptor, such as growth factors, into the cell resulting in their degradation.

In solved exercise 5.2, we show how to derive the fixed points for this circuit, again using separation of timescales and the useful technique of nullclines.

To understand this system, we use the phase portrait, which as we saw is a convenient way to plot the entire dynamics in a single

[1] Endocytosis also provides a length-scale of about 10-100 microns, or about one to ten cell diameters, in which a secreted molecule diffuses before it is eaten up by its target cells (Oyler-Yaniv et al. 2017). This provides a natural compartment size for cell-cell circuits. The lower the cell density, the longer this range, because there is less endocytosis, ensuring that the secreted molecule reaches its target cells (see exercise 5.5).

picture. The axes are the concentrations of M and F cells. 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. The arrows indicate the direction of flow. It's like a snapshot of the dynamics (Fig 5.12).

The phase portrait can be experimentally measured *in vitro*. To do so, Zhou et al plated cells in many different initial concentrations (initial conditions) in a 96-well plate. They watched how the cell concentrations changed over two days (Zhou et al. 2018), plotting little arrows to indicate the changes. This provided a phase portrait in one fell swoop!

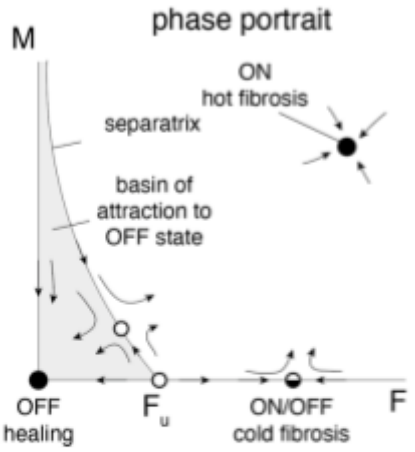

Figure 5.12: The phase portrait shows a stable ON state with both cell types, called hot fibrosis, a stable OFF state with neither, and a semi-stable state with only fibroblasts called cold fibrosis. A separatrix divides the phase portrait into basins of attraction for the two stable states, the ON and OFF states.

The phase portrait (see solved exercise 5.2) reveals two stable fixed points. There is a fixed point at zero cells (F=0, M=0). This is the OFF state. It corresponds to healing, since the myofibroblasts and macrophages are gone.

The other fixed point has high levels of both M and F cells, which sustain each other. We call it the ON state (Fig 5.12). The ON state is a stable fixed point. All arrows in the vicinity flow to it. If a perturbation around the ON state occurs, say that a few extra M arrive, they eat up their own growth factor and cell numbers drop back to steady state. This is how a molecular process, endocytosis, can provide a systems-level effect of stabilizing fixed points. The carrying capacity for F is also essential to stabilize the ON state - without it, both cells would rise indefinitely.

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

To understand fibrosis, we further need to consider the fibers, namely the **extracellular matrix (ECM or E for short)** deposited by F cells. In contrast, M cells produce molecular scissors (matrix metalloproteinases, or MMPs) that cut E up (Fig 5.13). These scissors are also produced at small amounts by the regular cells of the tissue. Thus, E rises with F and drops with M.

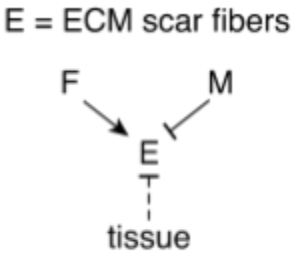

Figure 5.13: Scar fibers E are extracellular matrix proteins secreted by fibroblasts, F . Macrophages M secrete proteins that degrade and remodel the scar.

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, corresponds to 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.

The phase portrait shows another interesting fixed point. This is the ON/OFF state with only fibroblasts. This state can be called 'cold fibrosis' where cold means no immune

cells, M=0. The ON state can be called ‘hot fibrosis’. These are new terms for pathology, borrowed from cancer biology in which hot tumors have more immune cells than cold tumors.

The cold fibrosis fixed point can be stable for certain ranges of the circuit parameters, such as weak secretion by F cells of growth factors for M cells (see exercise 5.6). One can expect that cold fibrosis is ‘worse’ because there is a lack of M cells with their molecular scissors. The ECM is more abundant and stiff than in the ON state. Such stiff and abundant ECM in cold fibrosis is found in end-stage liver cirrhosis called “burnout NASH”.

Examples of both hot and cold fibrosis states can be found in the skin. Dermatology recognizes two main types of scars: keloid scars with abundant macrophages (hot fibrosis), and hyperproliferative scars which eventually lose most of their macrophages (cold fibrosis).

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

To see the dynamics of healing, let’s consider an injury at t=0. There is a small initial number of F and M cells at the injury site.

Inflammation can be modeled as a large influx I(t) of M cells, where I stands for ‘influx’.

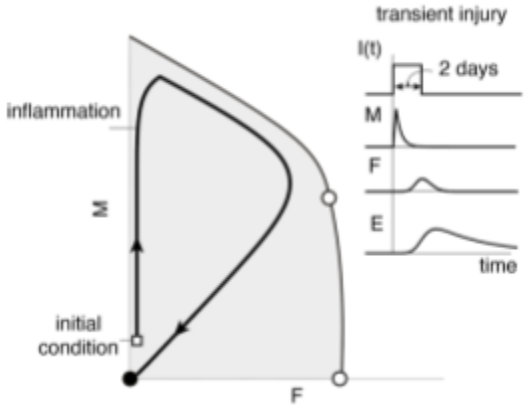

Figure 5.14: A transient injury causes an influx of macrophages and rise of fibroblasts but remains in the basin of attraction of the OFF state so that cell populations decline and the scar is removed.

Consider a two-day pulse of inflammation in which influx 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 c_1. 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 5.14). This trajectory is typical of proper healing. Scar fibers E are 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 5.14). The timescale, using the typical parameters of Table 5.1, is about two weeks.

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 5.15). 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 high steady-state level of fibers E. Thus, a 4-day inflammation event leads to fibrosis.

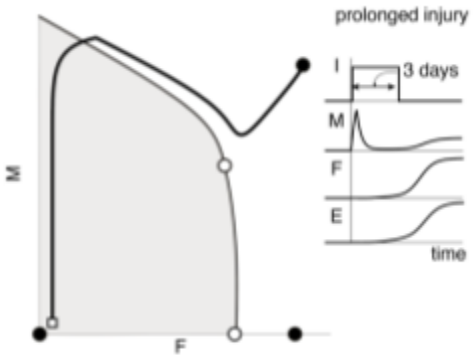

Figure 5.15: A prolonged injury crosses the separatrix into the basin of attraction of the ON state, causing ongoing fibrosis in which fibroblasts and macrophages support each other and continuously turn over.

Similarly, consider a repeated injury. A 2-day inflammation pulse is not sufficient 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 cross the separatrix and go to fibrosis (Fig 5.16).
Thus, the same system can result in either healing or fibrosis, depending on the strength and duration of the inflammation pulse. 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.

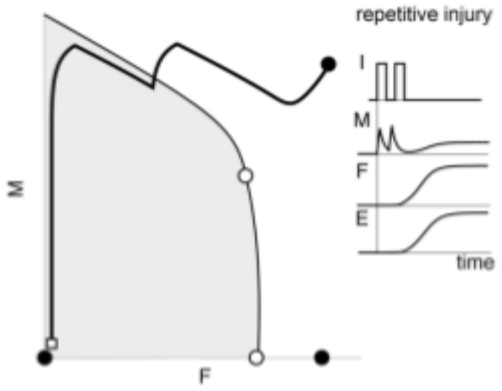

Figure 5.16: recurring or repetitive injury can cross the separatrix and trigger ongoing fibrosis.

The time window for stopping inflammation is due to bistability

We can now understand why it is so urgent to stop inflammation to avoid fibrosis. Let's plot how the duration of the inflammation pulse affects the final (steady state) amount of scar fibers E (Fig 5.17). We see that below a critical duration of inflammation, of about $t_c = 3\ days$, the scar vanishes. Above t_c, M crosses the separatrix and the ON state is inevitable *even if the inflammation stops*.
For example, an inflammation pulse that lasts a bit longer than the critical threshold t_c causes M to cross the separatrix, and F-cells have a bit of time to multiply. When inflammation stops, F cells are not very many and hence M levels sharply drop (Fig. 5.16). But just before they crash to zero, they recover due to the increased F cells that are just enough to support them. Both F and M go up together over weeks to the ON state. Fibrosis occurs even though the inflammatory pulse stopped long ago.

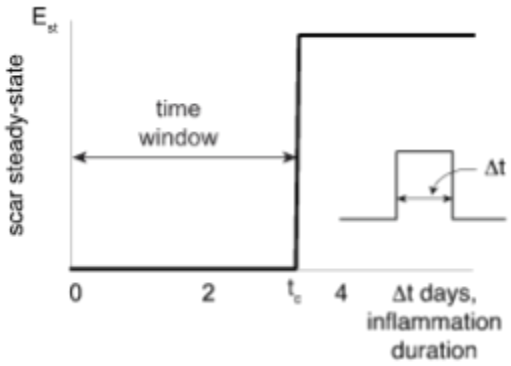

Figure 5.17: A time window for preventing fibrosis: inflammation lasting less than about 3 days avoids fibrosis whereas fibrosis is reached when inflammation lasts longer than three days .

The main circuit property here, bistability, is worth remembering. It can potentially explain other medical situations in which there is a limited time window to prevent irreversible outcomes. Two deadly examples are septic shock and hemorrhagic shock, in which a bacterial infection or hemorrhage causes lowered blood pressure, blood clotting and organ failure. There is a golden hour to treat shock and sepsis, with a turning point: some patients slowly recover whereas others quickly plummet.

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

Scar maturation is a process that unfolds over months; the scar changes until it reaches steady state. This timescale is much slower than the cell turnover time of days. How does the long timescale arise?

The slow timescale found in the model is due to the fact that the dynamics near the separatrix approach an unstable fixed-point. This is the white circle in the middle of the separatrix in Figs 5.14-5.16. By definition, at a fixed point, including an unstable one, the velocity is zero (no change). Thus, the velocity is always slow near a 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 ridge. The ball slows as it approaches the summit, and then speeds up again (Fig 5.18). The summit is an unstable fixed point, any perturbation makes the ball roll away. 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.

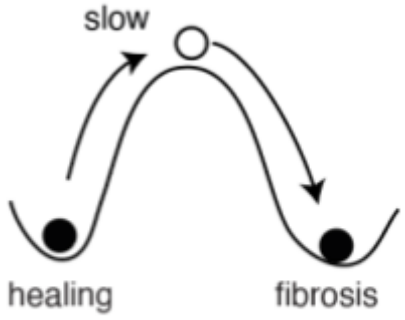

Figure 5.18: Dynamics are slow near an unstable fixed point, in analogy to a ball rolling to the top of a hill.

Strategies for preventing and reversing fibrosis:

A general cure for fibrosis has not yet been achieved, and many attempts have failed. So, let's use what we have learned to explore what future interventions might prevent fibrosis.

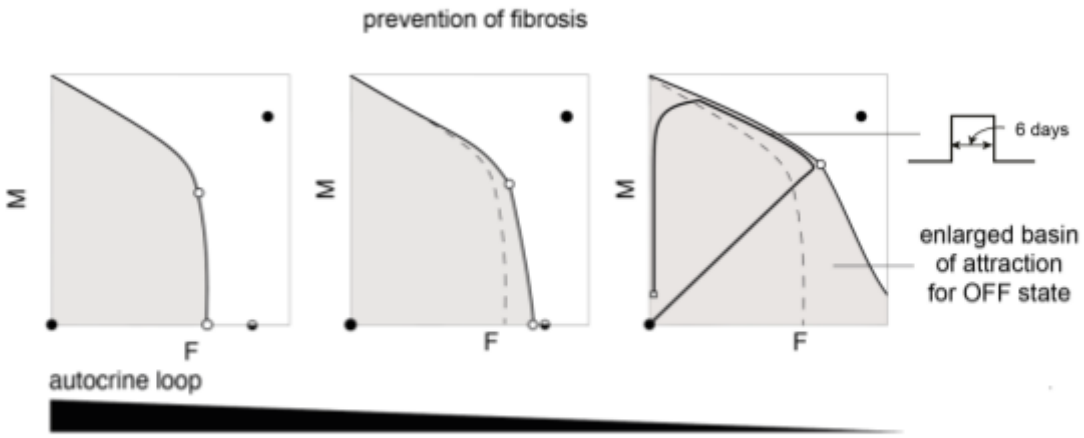

Figure 5.19: Strategy to resolve fibrosis by inhibiting the autocrine loop of fibroblasts. This enlarges the basin of attraction to the healing state.

To prevent fibrosis, we need to enlarge the basin of attraction for the OFF (healing) state. A large basin means that more situations end up resolved without fibrosis (Fig 5.19). We can also explore whether fibrosis can be reversed: can a mature scar in the ON state be made to flow to the OFF state.

Evidence for reversal of long-standing fibrosis has been accumulating, revolutionizing thinking in the field. Fibrosis can vanish in the liver, for example, a few months after successful antiviral treatment of hepatitis C infection. Such reversal of fibrosis depends on the fact that fibrosis is a dynamic steady-state with cell turnover. Similarly, fibrosis seems to be tunable in different biological contexts. Embryos do not show fibrosis after injury, and many mammals regenerate more readily than humans, giving hope that tweaking the circuit can help prevent or abrogate fibrosis.

We can use our circuit model to scan for parameter changes that affect fibrosis. Miri Adler did so and found that prevention and reversal of fibrosis become possible in the model when the ON/OFF fixed-point vanishes, because this greatly expands the basin of attraction of the OFF state. In other words, one must eliminate the fixed point in which F cells support themselves. To do so, as we saw in Fig 5.11, requires a combination of parameters to go below a threshold, the ratio of pro-F to anti-F parameters:

$$p_1 a\, K/\gamma d_1 < 4$$

This parameter group offers several targets against fibrosis. The equation gives hope that one does not need to push a parameter all the way down to zero, which is difficult. Instead, one must merely nudge the system below a threshold in order to collapse fibrosis.

You can see what happens in Fig 5.19 when you reduce this parameter group- the unstable fixed point (white circle on the x axis) moves to higher and higher values, enlarging the basin of attraction to the OFF state. At a certain point, the unstable fixed point collides with the ON/OFF fixed point, and both fixed points annihilate! The basin of attraction is now very large.

The dynamics when this anti-fibrosis condition is met is exemplified in Fig 5.19. A lengthy inflammation pulse of 6 days which would normally lead to fibrosis now flows to the OFF state with no fibrosis. Longer pulses of 8 days can still result in ON-state fibrosis.

One target suggested by the circuit equation is to inhibit the growth factor for F cells which the F cells themselves secrete - the autocrine growth factor for myofibroblasts. This can be achieved by increasing its removal rate γ or reducing its production rate a; both changes push the parameter group down, as required.

Shoval Miyara and Eldad Tzahor took on this challenge, using a well-established model for fibrosis. namely heart attacks. Shoval identified the main autocrine factor of mouse heart myofibroblasts, a growth factor called TIMP1. This growth factor is not expressed in the normal uninjured heart. He injected antibodies against this autocrine factor, thereby inactivating it. Mice showed much less scarring in experimentally induced heart attacks.

Similarly, this theory inspired Shuang Wang in Scott Friedman's lab to inhibit the liver myofibroblast autocrine loop. She achieved a reduction of advanced liver fibrosis in mice, by inhibiting an autocrine receptor for liver myofibroblasts, NTRK3. The prevalence of liver fibrosis is rapidly rising, due to the prevalence of fatty liver that causes liver inflammation known as NASH leading to progressive liver fibrosis.

Thus, this circuit-to-target approach may help to address fibrosis in different organs.

Here we see several benefits of math modeling. It can give hope and guidance. We do not need to kill all the fibroblasts. We just need to push certain rates enough so that they go below a threshold. Another benefit of a good theory is that it can inspire new experiments, as in the experiments on heart and liver. Theory provides new concepts - in

this case, the concepts of hot and cold fibrosis that provide new ways to analyze tissue samples. Their utility was recently demonstrated in the kidney in which hot and cold fibrosis regions can coexist depending on oxygen and inflammation levels
(Setten et al. 2022). This theory also helped to start experiments on the cancer micro-environment, the support system for cancer composed of F and M cells, aiming to collapse this support system and fight cancer. Can't wait to see the results.

Solved Exercises

5.1 Find the condition for bistability in the model for fibroblasts, Eq 4.

Solution: Our equation is $\frac{dF}{dt} = p_1 c_1 F(1 - F/K) - d_1 F$. We use separation of timescales and plug c1 quasi-steady state (Eq 2.) : $\frac{dF}{dt} = \frac{p_1 a}{\gamma} F^2(1 - F/K) - d_1 F$

The fixed points occur at F=0 and at two, one or zero other points determined by whether the removal line $d_1 F$ intersects the proliferation "hill". To solve for those non-zero fixed points, we can set dF/dt=0 and divide by F (since we assume F is nonzero) to find

$$d_1 = \frac{p_1 a}{\gamma} F\left(1 - \frac{F}{K}\right).$$

To simplify things, let's divide and multiply by K, and divide by d_1 so that

$$1 = \left(\frac{p_1 a K}{\gamma d_1}\right)\frac{F}{K}\left(1 - \frac{F}{K}\right).$$

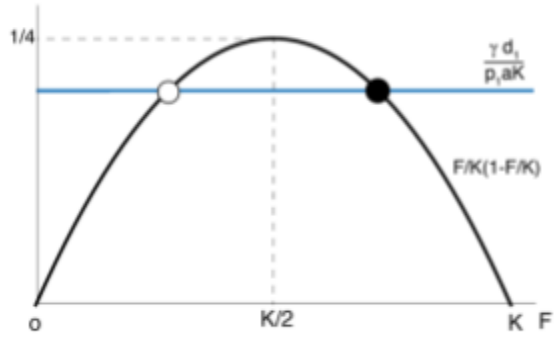

Figure 5.20: Rate plot for the growth and removal rates of fibroblasts shows how the number of fixed points depends on parameters.

We did that because the term $F/K(1 - F/K)$ is easy: it's a symmetric parabola that is zero at $F = 0$ and $F = K$. Its maximum value occurs between the two roots at $F = K/2$, where its height is 1/4 (Fig. 5.20). Thus, the condition for two nonzero fixed points is

$$\frac{p_1 a K}{\gamma d_1} > 4.$$

A single 'half stable' fixed point occurs when this precisely equals 4 (try to analyze this case).

5.2 Find the fixed points of the two-cell circuit composed of fibroblasts and macrophages

Solution: Let's write the equations for this circuit. The F-specific growth factor c_1 is secreted by both M and F cells, and endocytosed by its receivers, F cells:

$$\frac{dc_1}{dt} = aF + b_1 M - e_1 F c_1 - \gamma_1 c_1$$

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

$$\frac{dc_2}{dt} = b_2 F - e_2 M c_2 - \gamma_2 c_2$$

endocytosis rates like e_1, e_2 are about 1000 /cell/minute. Therefore, endocytosis is the main removal mechanism of growth factors unless cell density is very low so that endocytosis can be neglected. Growth factors dynamics have timescales of minutes-hours whereas cell growth is much slower with timescale of days. We thus invoke separation of timescales and compute the quasi-steady-state of the two growth factor concentrations:

$$c_1 = \frac{aF+b_1M}{e_1F+\gamma_1}, \qquad c_2 = \frac{b_2F}{e_2M+\gamma_2}$$

The M cells divide under control of c_2. Unlike F cells, the M cells *are far from their carrying capacity*. Thus, M cells follow the simple equation

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

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

$$\frac{dM}{dt} = M\left(p_2 \frac{b_2F}{e_2M+\gamma_2} - d_2\right)$$

$$\frac{dF}{dt} = F\left(p_1 \frac{a\,F+b_1M}{e_1F+\gamma_1}\left(1 - \frac{F}{K}\right) - d_1\right)$$

Looks a bit complicated... but we can make progress. To understand these equations, we use the method of **nullclines** - a lovely graphical method we've seen in previous chapters. 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 fixed points are where the two nullclines intersect, because at fixed points both cells don't change. It's therefore useful 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 $p_2 \frac{b_2F}{e_2M+\gamma_2} - d_2 = 0$. The latter is a straight-line, $M = \alpha F - \beta$, with an intercept $\beta = \frac{\gamma_2}{e_2}$. The intercept is close to zero because endocytosis dominates degradation and thus $\beta \ll 1$. 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 5.21). The $dF/dt = 0$ nullcline is the y-axis $F = 0$ and the solution to

$$p_1 \frac{aF+b_1M}{e_1F+\gamma_1}(1 - F/K) - d_1 = 0.$$

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

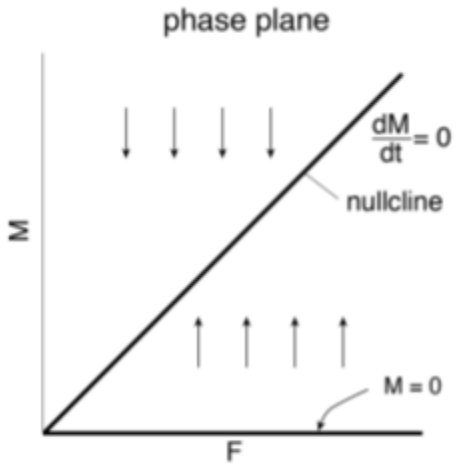

Figure 5.21: Each nullcline dX/dt=0 divides the phase plane into regions where the relevant variable X moves in one direction.

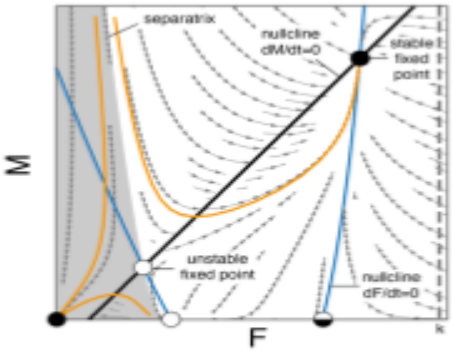

Figure 5.22: Nullclines and phase portrait for the fibroblast-macrophage circuit. Selected trajectories are shown in orange.

is not enough c_1 and c_2 to overcome cell removal, and both cell populations crash.

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 in exercise 1 when we discussed F alone. Plotting the nullcline, that looks like $M \sim (F + \gamma_1/e_1)/(1 - F/K) - a/b_1$F, we see that it has a U-shape which drops through the unstable fixed-point F_u, drops below zero, and rises through the high fixed-point F_{high}, and then climbs up and diverge near the carrying capacity $F = K$ (blue curve in Fig 5.22). The orange curves on the phase portrait show three different trajectories.

The phase portrait indicates that the zero and high stable fixed points are stable (arrows flow into them). An analytical method called linear stability analysis can be used to confirm which points are stable (black dots) and which are unstable (white dots), and which are half-stable (half white half black dots).

Additional exercises

5.1 **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-shaped 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.

5.2 Saturating growth factors 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 its receptor both initiates the signaling that affects proliferation and leads to endocytosis.

5.3 Paradoxical effects of macrophage depletion: Experiments that deplete macrophages show conflicting effects on fibrosis. In some contexts fibrosis is prevented whereas in others it is enhanced (Duffield et al. 2013). Show, using the model of solved exercise 5.2, that the deciding factor is the timing of depletion.

a) What happens when M is set to zero with fibroblasts below their unstable fixed point, $F < Fu$?
b) What happens when M is set to zero with fibroblasts below their unstable fixed point, $F > Fu$?

5.4 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 produced by both macrophages and myofibroblasts.

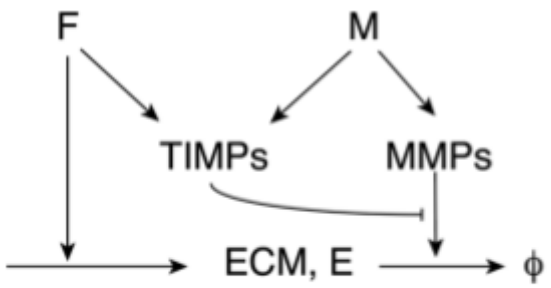

Figure 5.23: Circuit in which fibroblasts F produce extracellular matrix E, which is degraded by MMP proteins secreted by macrophages M. These proteins are inhibited by TIMP proteins secreted by fibroblasts.

(a) Follow the interactions above to explain each term in the 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, rewrite 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 are 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 versus cold fibrosis if you consider that myofibroblasts numbers are approximately the same in the two fibrotic states?

5.5 Diffusion range of growth factors due to endocytosis (Oyler-Yaniv et al, 2017): A growth factor c diffuses with a diffusion coefficient D and is endocytosed (removed) by cells with density F at a rate $e\,F\,c$.
a) How long can the molecule travel on average before being removed? Show that this is approximately $L = \sqrt{\frac{D}{eF}}$. Show that this length-scale is about 100 microns for typical diffusion constants and cell densities.
b) 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?
c) 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?

5.6 Stability of cold fibrosis: Analyze the fibrosis circuit of exercise 5.2, and weaken the secretion rate of secretion of the macrophage growth by myofibroblasts, described by the parameter b_2.

a) At which value of b_2 does the cold fibrosis fixed point become stable?

b) What happens to the hot fibrosis fixed point? Sketch the nullclines and the fixed points.
c) What happens if b_2 =0?
d) What are the implications for considering the macrophage growth factor, or macrophages themselves, as a target for an anti-fibrosis treatment?

Table 5.1

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_i	binding affinity of growth factor c_i	$6x10^8 molecules$
b_2	=1maximal 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}

Table 5.1 Representative parameter values for cell circuits. Adapted from (Adler et al 2018)

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 linearly with c_i as in the text.

Adler, Miri, Avi Mayo, Xu Zhou, Ruth A. Franklin, Jeremy B. Jacox, Ruslan Medzhitov, and Uri Alon. 2018. “Endocytosis as a Stabilizing Mechanism for Tissue Homeostasis.” *Proceedings of the National Academy of Sciences*, February. https://doi.org/10.1073/pnas.1714377115.

Duffield, Jeremy S., Mark Lupher, Victor J. Thannickal, and Thomas A. Wynn. 2013. “Host Responses in Tissue Repair and Fibrosis.” *Annual Review of Pathology* 8 (January): 241–76. https://doi.org/10.1146/annurev-pathol-020712-163930.

Oyler-Yaniv, Alon, Jennifer Oyler-Yaniv, Benjamin M. Whitlock, Zhiduo Liu, Ronald N. Germain, Morgan Huse, Grégoire Altan-Bonnet, and Oleg Krichevsky. 2017. “A Tunable Diffusion-Consumption Mechanism of Cytokine Propagation Enables Plasticity in Cell-to-Cell Communication in the Immune System.” *Immunity* 46 (4): 609–20. https://doi.org/10.1016/j.immuni.2017.03.011.

Zhou, X. Xu Xu, R.A. Ruth A. R.A. Franklin, Miri Adler, J.B. Jeremy B. Jacox, Will Bailis, Justin A. J.A. Shyer, Richard A. R.A. Richard A. R.A. Flavell, Avi Mayo, Uri Alon, and Ruslan Medzhitov. 2018. “Circuit Design Features of a Stable Two-Cell System.” *Cell* 172 (4). https://doi.org/10.1016/j.cell.2018.01.015.

Additional reading References:

Miri Adler, Xu Zhou, Ruth Franklin, Mathew Meizlish, Avi Mayo, Stefan Kallenberger, Ruslan Medzhitov, Uri Alon, iScience 2020

Medzhitov, The spectrum of inflammatory responses , https://www.science.org/doi/full/10.1126/science.abi5200

Gregoire Altan-Bonnet et al (2019), Cytokine-mediated communication: a quantitative appraisal of immune complexity, Nature Reviews Immunology (2019)

Arno, A. I., Gauglitz, G. G., Barret, J. P., & Jeschke, M. G. (2014). Up-to-date approach to manage keloids and hypertrophic scars: A useful guide. Burns. https://doi.org/10.1016/j.burns.2014.02.011

Love, P. B., & Kundu, R. V. (2013). Keloids: An update on medical and surgical treatments. Journal of Drugs in Dermatology.

(Zhou et al. 2022)

(Wang et al. 2023)

(Miyara et al. 2023)