Systems Medicine 2021 Lecture 5

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BE333

Inflammation and fibrosis as a bistable system

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

Introduction:

Fibrosis, or excess scarring, is a medical problem that unites many diseases. It cuts across medicine. The problem is that scar tissue replaces functional 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. There is currently no cure for progressive fibrosis diseases other than organ transplant.

In this lecture we will understand inflammation and fibrosis in more 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.

In this lecture we use two of our laws, all cells come from cells, and all biological processes saturate, and leave the third law, cells mutate, to the next lectures.

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:

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

Thus, 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. The scar seals up the damaged tissue and prevents holes in the organ that can be lethal. In organs that can repair themselves to a certain extent, fibrosis is caused by injury which is prolonged, repetitive or extensive. Transient or small injuries lead to healing.

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 tissue rich with collagen- the scar. For example, the hepatis 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.

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 course. 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. Fibrosis also occurs in non-age-related diseases. Alcoholism leads to liver fibrosis, and liver fibrosis also occurs in non-alcoholic fatty liver disease (NAFLD), associated with obesity, which afflicts ~25% of world population. A fraction of those with NAFLD progress to chronic inflammation and to fibrosis, with loss of liver function.

Because inflammation always precedes fibrosis, physicians try to stop inflammation quickly to prevent fibrosis after surgery, stroke and heart-attack, as well as in other medical situations. There is usually a **limited 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 lecture 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 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 focused on 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 main and indisputable facts into 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 repetitive or prolonged. This understanding can also point 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 the cells of the tissue to release factors that cause **inflammation**. These factors include 'alarm' proteins that flow in the blood like IL6, IL1 and TNFa. The purpose of inflammation is to fight pathogens, and to start the repair process. Unlike the T-cells of the previous lecture, 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 seconds.

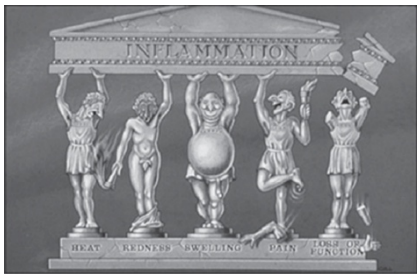

Figure 5.1

Inflammation has four main features, which are easy to remember by the Latin rhyme rubor calor tumor dolor: redness, heat, swelling and pain. The fifth pillar of inflammation is loss of tissue function (Fig 5.1).

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 fluids and proteins that fight pathogens (Fig 5.2). The

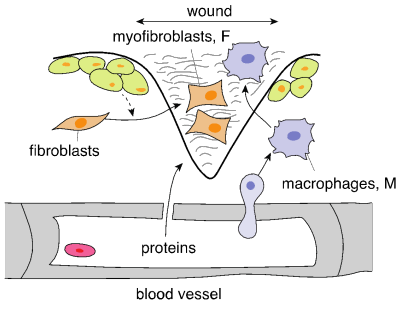

Figure 5.2

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. 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 $TGF\beta$) 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 5.2. Their muscle-like ability helps 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 the growth factor for themselves, in an example of an **autocrine loop** (Fig 5.3).

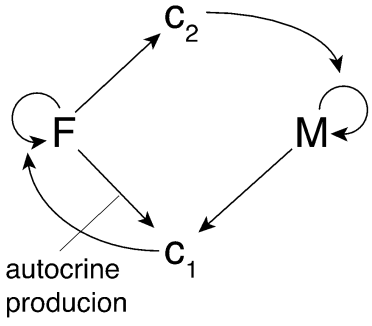

Figure 5.3

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). This plate approach, called '*in-vitro*', 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 in this course). Don't expect your friends to know this last term, I invented it for this course.

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, M and F populations rise and a permanent scar is formed, made of fibers and cells. This is fibrosis. 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 main point is that

one equation for myofibroblasts can show two different behaviors, a property called bistability.

The myofibroblasts produce and secrete a growth factor for themselves, called $PDGF$, which we will denote c_1 (Fig 5.4). c_1 diffuses in the tissue, and is degraded at rate γ_1. Thus the rate of change of c_1 is its production minus removal

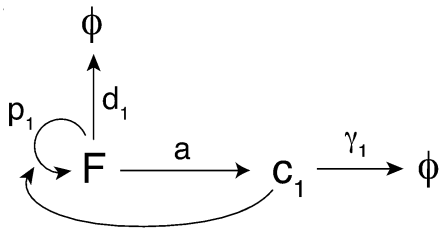

Figure 5.4

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

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 like γ_1 are hours.

Since the production and removal processes take minutes to hours, and cell division and death take about a day or longer, we can use **separation of timescales** as in the previous 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, which we can compute using $dc_1/dt = 0$. We find that c_1 is proportional to the cells that make it, F (Fig 5.5):

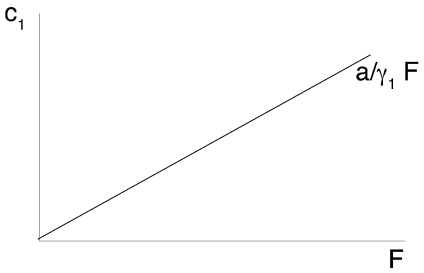

Figure 5.5

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

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

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

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

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

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, such as F in this case. We employed the rate plot for beta cells in lecture 2. On the x-axis we plot cell density F, 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 5.6). We next plot the total cell removal $d_1 F$, a line that rises with F (red line in Fig 5.6).

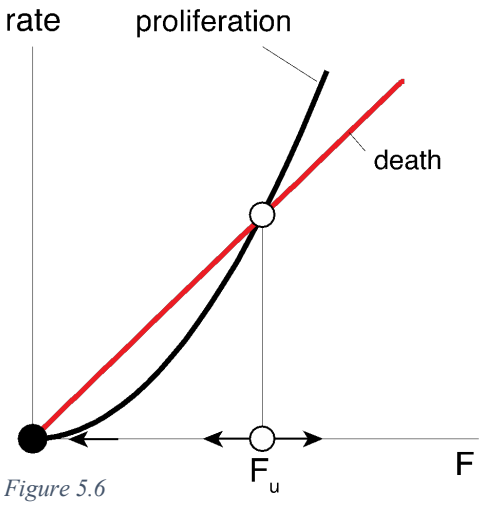

Figure 5.6

The interesting points are where the proliferation and death curves cross. These are the **fixed points**. The lines 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 red 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 'all biological processes saturate'.

Carrying capacity is modelled in ecology and biology by a 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 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 5.7). Proliferation is measured by a die that stains cells that replicate their DNA. The intersect of the line with the x-axis is the carrying capacity.

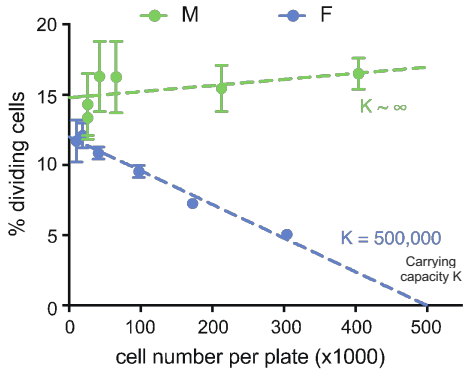

Figure 5.7

Thus, our equation with carrying capacity reads

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

Let's find the fixed points using a new rate plot (Fig 5.8). On the x-axis we plot cell density F, 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 is a line as before.

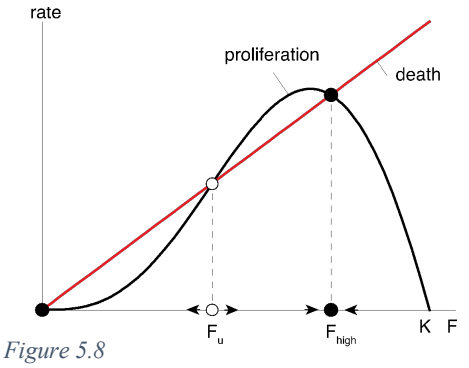

Figure 5.8

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 5.8). Let's analyze the three fixed points of Fig 5.5. 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 smaller 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 5.9): below an initial level of F_u, F crashes to zero; above F_u, F goes to a specific steady-state concentration F_{high} no matter what the starting level was.

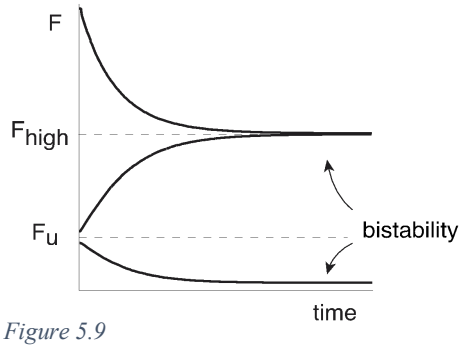

Figure 5.9

Each fixed point has its own **basin of attraction**, defiend as the initial condirons 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.

Experimentally F cells can indeed support themselves in a plate at sufficiently high concentrations (Fig 5.10). The steady-state is an ongoing balance of cells dividing and dying about once per day.

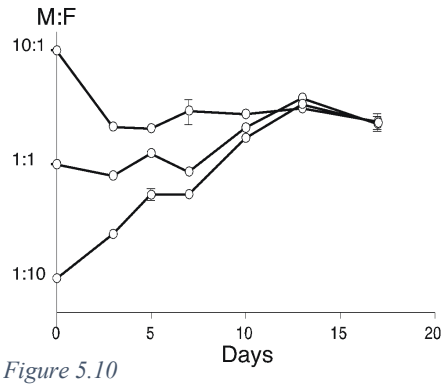

Figure 5.10

Notably, if death rate is high, or proliferation is low, there is only one solution, at zero, as can be seen in the rate plot in (Fig 5.11). We will use this fact when we discuss ways to avoid fibrosis. The loss of bistability occurs if parameters that favor F cells are lower than parameters that remove F cells. These are the 'pro-F' parameters of 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 d1 or growth-factor removal rate γ_1. Loss of bistability occurs when a parameter combination which is the ratio of these pro-F and anti-F parameters goes below a threshold: $p_1 a\, K / \gamma d_1 < 4$ (derived in solved exercise 5.1). Exactly at the threshold, the removal curve kisses the proliferation hill at one half-stable point, in addition to the zero fixed point.

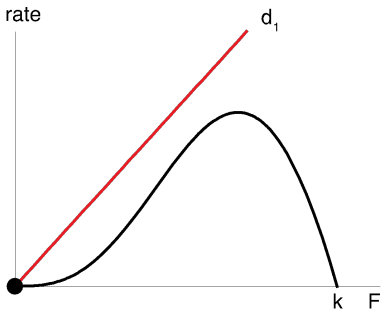

Figure 5.11

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 several states for 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 ($PDGF_\beta$). This is similar to the F autocrine growth factor $PDGF_\alpha$ described above, and we will group the two growth factors together as c_1. Thus, M and F act to increase each other's numbers (Fig 5.12).

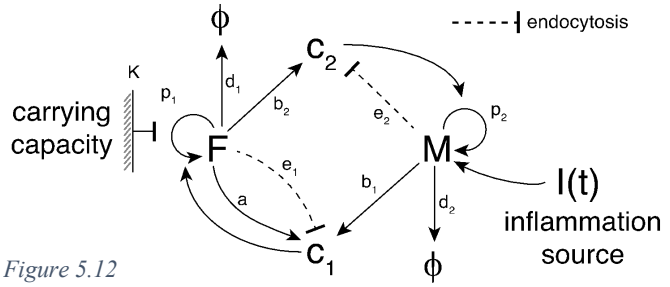

Figure 5.12

Unlike F cells, the M cells *have no carrying capacity (or at least a very high one)*: 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 5.7). Thus M cells require a different mechanism to avoid spiraling out to infinity. 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 5.13). 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].

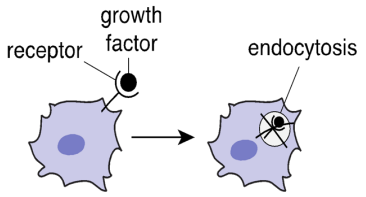

Figure 5.13

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 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 5.14). The phase portrait was experimentally measured *in-vitro* by using many different initial cell conditions in a 96-well plate, and watching how the cell concentrations change over two days (Zhou et al., 2018).

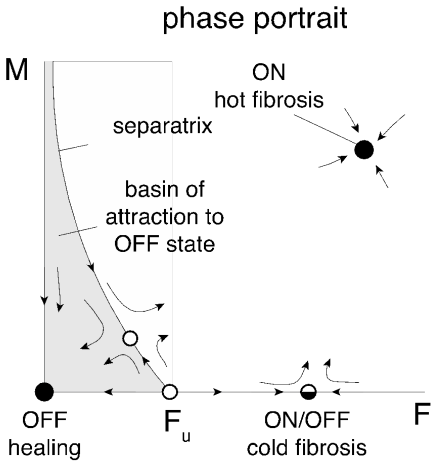

Figure 5.14

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 5.14). 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 bit of extra M arrive, M eats up its own growth factor and cell numbers drops back to steady state. 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 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 such as endocytosis. The latter applies to the current situation.

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[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. This provides a natural 'compartment' size for cell-cell circuits. The lower the cell density, the longer this range, because there is less endocytosis, similar to screening effects in physics. The range is thus 'self-tuning', ensuring that the secreted molecule reaches their target cells (Oyler-Yaniv et al Immunity, 2017, Altan-Bonnet 2019 et al https://doi.org/10.1038/s41577-019-0131-x).

To understand fibrosis, we further need to consider the fibers, namely the **extra-cellular matrix (ECM)** deposited by F cells. We denote the ECM by E for short. In contrast, M cells produce molecular scissors (MMPs) that cut E up (Fig 5.15). These scissors are also produced at small amounts by the regular cells of the tissue. Thus, E rises with F and drops with M.

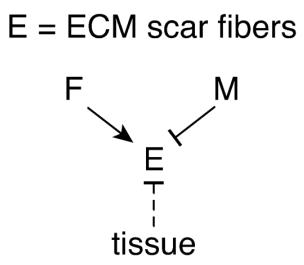

Figure 5.15

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 calld 'cold fibrosis' where cold means no immune cells M. The ON state can be called 'hot fibrosis'. These are new terms for pathology, borrowed form cancer biology in which hot tumors have more immune cells than cold tumors. 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 stiffer than in the ON state. Such stiff and abundant ECM in cold fibrosis is found in end stange 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: keloids with abundant macrophages (hot fibrosis), and hyperproliferative scars which eventually lose most of their macrophages (cold fibrosis).

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 small initial number of F and M cells at the injury site.

Inflammation can be modelled as a large influx I(t) of M cells, where I stands for 'influx'.

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 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 5.16). This trajectory is typical of proper healing. Scar fibers E are deposited by F cells, and when the F cells are

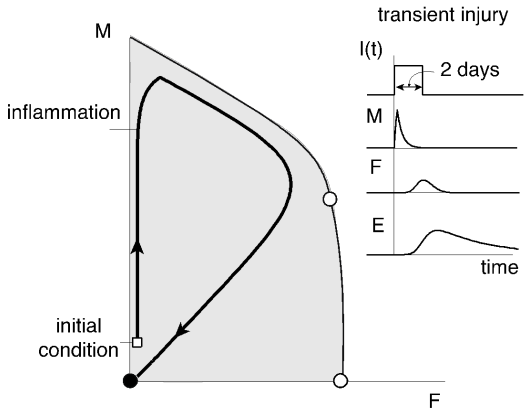

Figure 5.16

gone, scar is degraded by the tissue. Scar E(t) rises and then vanishes (Fig 5.16). 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 5.17). 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.

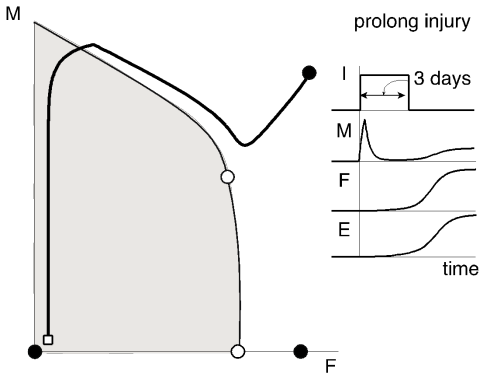

Figure 5.17

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 make the dynamics cross the separatrix and go to fibrosis (Fig 5.18).

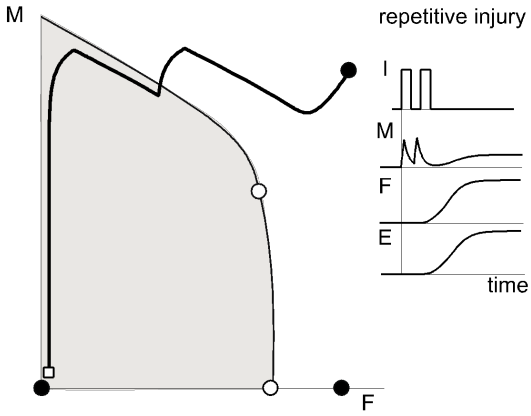

Figure 5.18

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.

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 5.19). We see that below a critical duration of inflammation, of about $t_c = 3\ days$, the scar vanishes. Above t_c, M cross the separatrix and the ON state is inevitable *even if the inflammation stops*.

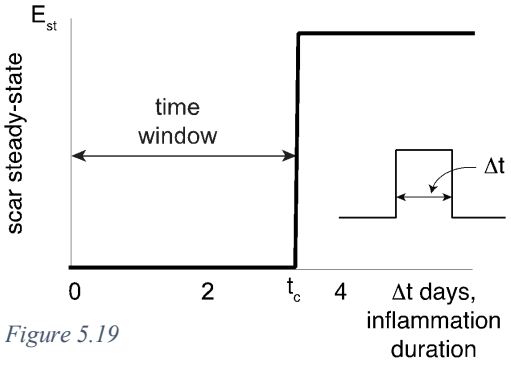

Figure 5.19

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 5.18). 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.

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 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 5.16-5.18). 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 ridge. The ball slows as it approaches the summit, and then speeds up again (Fig 5.20). 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.

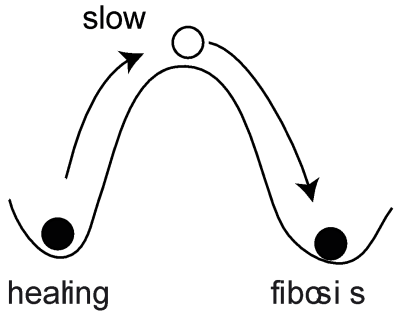

Figure 5.20

Strategies for preventing and reversing fibrosis:

Let's use what we learned to explore what interventions might, in principle, prevent fibrosis. 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 5.21).

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. Recently evidence of reversal of long-standing fibrosis has been accumulating and revolutionizing thinking on the field. Fibrosis can vanish in the liver, for example, a few months after successful antiviral treatment of hepatitis C. Such reversal of fibrosis depends on the fact that fibrosis is a dynamic steady-state with cell turnover.

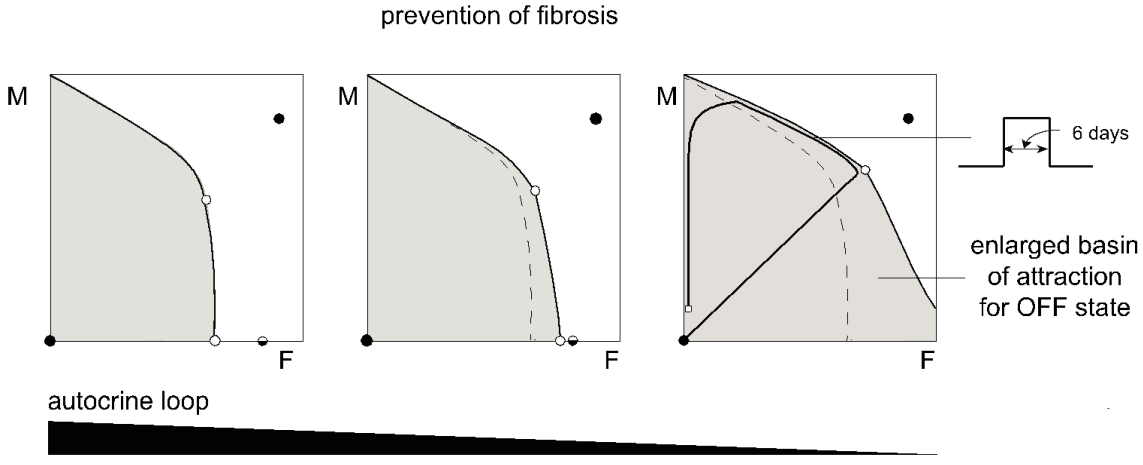

Figure 5.21

Both 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. This means eliminating the fixed point in which F support themselves. To do so, we saw in Fig 5.11 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 C exceeds one, the ratio of anti-F to pro-F parameters (this time with endocytosis by both M and F cells, exercise 5.2):

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

Thus, raising the endocytosis rate of c_1 by F cells, or F cell death rate can work. Similarly, decreasing a, the autocrine secretion of c_1 by F, or decreasing F proliferation rate p_1, will also work. Approaches suggested by this analysis thus include using inhibitors of autocrine secretion or of endocytosis of the c_1 receptors.

The dynamics when the anti-fibrosis condition is met ($C > threshold$) are shown in Fig 5.21. 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.

Here we see a superpower of math modelling. It can give hope and guidance. We do not need to kill all the fibroblasts. We just need to push certain rates slightly so that they go below a threshold. It's like in COVID- no need to prevent its spread completely, only to get replication number below 1. This gives hope to treat fibrosis, and a direction of which parameters to target. Another superpower of a good theory is that it can inspire new experiments. Indeed, this theory has started three different labs collaborating with us to identify the autocrine loop and develop antibodies against them as a therapy – in heart, liver and the cancer microenvironment. Can't wait to see the results.

Removing the unstable fixed-point F_u can potentially 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 a long period of fibrosis leads to the OFF state, signifying fibrosis reversal (Fig 5.22). Depleting macrophages makes the dynamics go into the basin of attraction of the OFF state, because F-cells cannot support themselves.

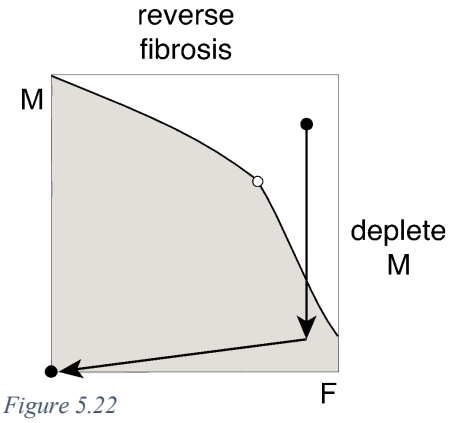

Figure 5.22

Such insight is relevant to 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 over years (Santucci, Borgognoni, Reali, & Gabbiani, 2001). Keloids are thus living tissue. When you cut most of them out of the skin by surgery, they typically regrow (Love & Kundu, 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 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, Gauglitz, Barret, & Jeschke, 2014) followed by surgery. The anti-proliferative therapy reduces 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 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 protected in the womb, with little danger of pathogens or foreign material entering the wound. Similarly, some mammals avoid fibrosis and regenerate better than humans do; for example mice can regenerate their heart in the first week of life, and lose this ability thereafter. Thus, the healing circuit has the potential to avoid fibrosis in certain physiological settings.

The main property we described, bistability, is worth remembering. It can explain a large number of medical situations, in which there is a limited time window to prevent irreversible outcomes. Two deadly examples are sepsis and 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.

Inflammation again / Music: Let it be, Beatles

When I find my self in times of swelling,
Swelling redness heat and pain
There will be an answer: Inflammation again.
And though it may be itchy
Its just my body trying to defend
There will be an answer: Inflammation again.

Inflammation again x2
There will be an answer: Inflammation again.

And when inflammations over,
Theres a little scar that shines on me
There will be an answer: let it be
And if I can reduce the autocrin loop
I might be fibrosis free
There will be an answer: let it be

Let it be x4
There will be an answer: may you be fibrosis free

Words: welcome, breath, review, fibrosis, inflammation, sequence, toime window, damage, senescencem macrophages, fibroblasts, in vitro circuit, in envelopo, growth factor, autocrine, separation of timescales, bisatbility, carrying capacity, basin of attraction, parameter group, MF phase portrait, ON, ON/OFF,

Solved Exercises

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

Solution: The fixed points occur at F=0 and at two, one or zero other points determined by whether the death line $d_1 F$ intersects the proliferation "hill". To solve for those non-zero fixed points, we can set dF/dt=0 in Eq. 4 and divide by F to find

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

Let's now divide and multiply by K, and divide by d_1 so that

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

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. 5.23). Thus, the condition for two nonzero fixed points is

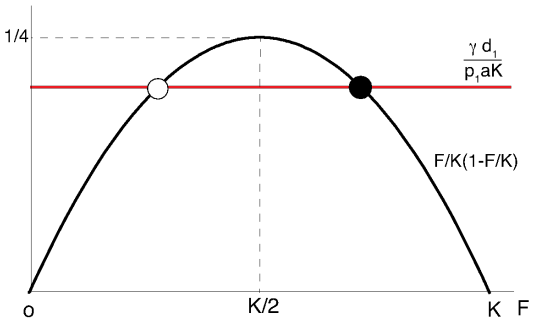

Figure 5.23

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

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

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

Solution: Lets first 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_2 are about 1000 /cell/minute. Therefore, endocytosis is the main removal mechanism of growth factors like c_2, unless cell density is very low so that endocytosis can be neglected. Growth factors have timescales of minutes-hours, and cells are much slower with timescale of days. We thus invoke separation of timescales, we compute the quasi-steady-state these two growth factor concentrations:

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

The M cells divide under control of c_2. Unlike F cells, the M cells *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. 5.4. 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)

$$\frac{dM}{dt} = M\left(p_2 \frac{b_2 F}{e_2 M + \gamma_2} - d_2\right)$$

$$\frac{dF}{dt} = F\left(p_1 \frac{aF + b_1 M}{e_1 F + \gamma_1}(1 - F) - d_1\right)$$

Looks a bit complicated... but we can make progress. To understand these equations, we use the method of **nullclines** - a lovely graphical method weve seen in previous lectures. 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 useful to draw both nullclines on the **phase plane**, whose axes are F and M cell concentrations, and study the intersection points.

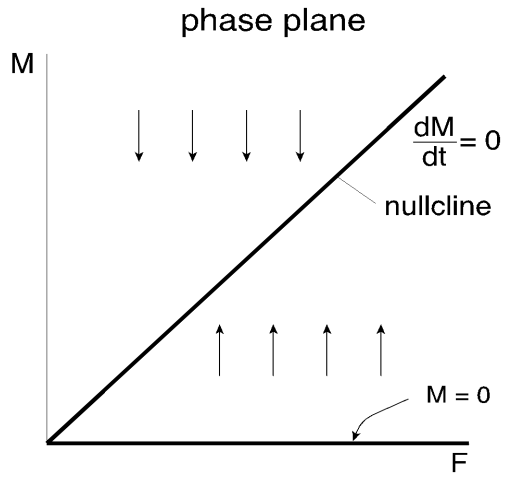

Figure 5.24

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, $\beta = \frac{d_2 \gamma_2}{p_2 e_2}$. The intercept is close to zero because endocytosis dominates degradation and thus $\frac{d_2 \gamma_2}{p_2 e_2} \ll 1$. 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 5.24).

The $dF/dt = 0$ nullcline is the y-axis $F = 0$ and the solution to

$$p_1 \frac{aF + b_1 M}{e_1 F + \gamma_1}(1 - F/K) - d_1 = 0.$$

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 is not enough c_1 and c_2 to overcome cell death, 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 above when we discussed F alone. Plotting the nullcline, that looks like $M \sim (F + \beta)/(1 - F/K) - a/b_1 F$, 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 $F = K$ (Fig 5.25).

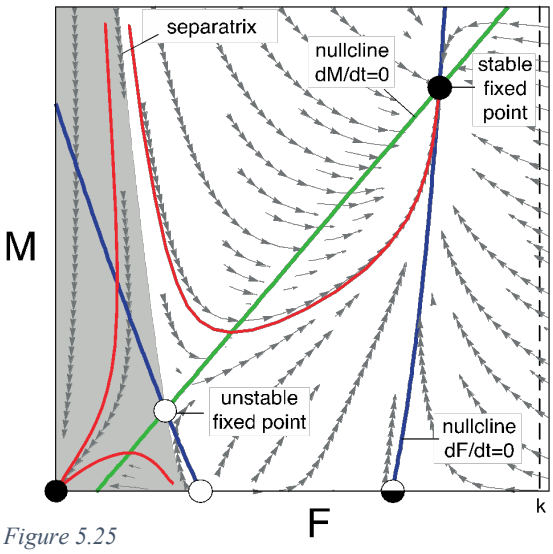

Figure 5.25

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).

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 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.

5.2 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.

5.3 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.

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 produces by both macrophages and myofibroblasts.

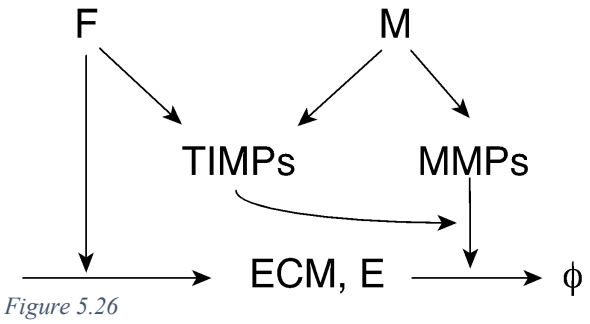

Figure 5.26

(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?

5.5 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?

Table 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	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.

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