



Healing by numbers: mathematics meets medicine

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The molecular biology revolution is providing vast quantities of information about the cell biology and biochemistry underlying clinical medicine. How can the pieces of this jigsaw puzzle be put together? This is currently a major biological challenge, and help is at hand from an unlikely source – mathematics.

When Sir Alan Hodgkin and Sir Andrew Huxley performed their Nobel prize winning research on nerve signalling in the 1950s, their experiments were supplemented by mathematical equations, which predicted how ion concentrations would vary along the nerve axon. This approach, which was extremely unusual at the time, has now fully come of age. Experimental laboratories are generating new information about chemicals involved in human disease at a staggering rate. But this information does not, on its own, provide answers to the questions of clinical medicine. The sheer complexity of human physiology prevents that – each chemical interacts with many others, and each cell type performs multiple functions. The traditional biological approach to this difficulty is *in vitro* experiments, in which one uses simpler laboratory systems to understand different aspects of, say, the growth of a tumour or the healing of a wound. But mathematical modelling is now emerging as an alternative approach, which can in some cases be more efficient and informative.

Shallow wounds

Shallow wounds on the skin, such as blisters, affect just the outer epidermal layer of the skin. An understanding of their healing is not of direct clinical importance, but it provides key information that is clinically significant in

other contexts, such as the repair of damaged corneal epithelium, and the growth of cultured epidermal autografts to treat chronic burn wounds. Since they involve a single cell population, shallow skin wounds provide a simple case to begin considering mathematical models.

The skin responds to a shallow wound in two main ways: the cells at the wound edge start to crawl into the wound, and the surrounding cells begin to divide rapidly, providing a new population of cells to replace those that have been removed. Both of these processes depend on biochemical signals, and the aim of mathematical modelling is to understand the details of this chemical control. The key part of the model is an equation of the form:

$$\text{Net increase of cells} = \text{change due to cell movement} + \text{change due to cell division} - \text{change due to cell death}.$$

This equation is like a census for cells: by combining information on births, deaths, immigration and emigration, we can calculate the change in the cell population. Using biological data, one can accurately represent the effects of biochemical regulators on these terms. Many such chemicals are produced by the epidermal cells themselves; recognising that the number of cells around them is lower, these cells attempt to change things by producing growth factors that will make them and their neighbours divide more quickly. These chemicals also increase cell



Figure 6. Martin Hall sifting through soil from a burial site for evidence of maggots and puparia.

awareness of the potential of forensic entomology in Police Forces, especially in Scenes-of-Crime Managers. A good foundation of knowledge on insect involvement as decomposers has been laid, but there is still much work needed to improve methods of ageing maggots and other developmental stages of those species we know a lot about, and to begin to gather data on those species we know little about (Figure 7). In addition, it will be important to establish a database of cases of suspicious death (past, present and future), both involving and not involving insects, so that we can begin to have a database that can be interrogated in relation, for



Figure 7. Sarah Donovan checking a laboratory culture of bluebot-tle blowflies, maintained for research on the development of maggots.

example, to the question: 'How long does it take for a body in a fifth-floor flat to become infested in the summer, compared to a ground floor flat and what species of flies are involved?' The uniqueness of each forensic case can make it extremely difficult to assess what is often scanty information with great accuracy. Nevertheless, continued high quality research and data gathering, as indicated above, will continue to enhance forensic entomology as a genuine quantitative science, with a greater analytical and lesser empirical element, thereby improving the quality of the conclusions that it makes.

Acknowledgement

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Further reading

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Websites

www.forensic-entomology.com/

An excellent site, especially good for practical aspects of forensic entomology, such as collecting samples from a crime scene.

http://folk.uio.no/mostarke/forens_ent/forensic_entomology.html

An excellent introductory site full of valuable information gathered from the literature on all aspects of forensic entomology.

www.missouri.edu/~agwww/entomology/index.html

Official site of the American Board of Forensic Entomology, with introductory information to forensic entomology and details of the professional status of forensic entomologists in the USA.

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migration, and dose-response curves for both effects have been found experimentally. With these data incorporated in our mathematical equation, we can use computer simulations (Figure 1) to predict the effect of the growth factor on the wound healing process.

Unsurprisingly, work of this type predicts that such growth factors promote wound healing, and that adding more chemical – placed on a wound dressing, for example – can speed up the repair process. But intuition alone cannot tell us the optimal level at which to add a particular growth factor, because it depends on a complex combination of dose-response curves for cell movement and cell division, both of which are non-linear. The mathematical model rapidly converts these data into the dose-response curve of real clinical interest – the speed of repair as a function of growth factor addition.

Cell-cell interactions in cancer

Interactions between different cell types are central to many areas of medicine. One example is in solid tumours, which contain a range of cell types, including cancer cells

From words to equations

To create mathematical models, word equations such as that on the previous page for a cell population must be converted into mathematics. For each term, we require a mathematical representation, in terms of the cell density n , which is a function of space x and of time t since wounding. The rate of increase of cell density is denoted mathematically by $\partial n/\partial t$. Cell movement is, in this case, predominantly unbiased, so that a diffusion term $D\nabla^2 n$ is appropriate, where D is a positive constant. In many other biological situations, cells tend to move up gradients of regulatory chemicals, so that more complex terms are required.

The key term in this equation is that for cell division. This will depend on the cell density, with lower division rates as cells become closer packed. But cell division is also controlled by growth factors, and in this simple model it is reasonable to assume a single generic growth factor with concentration c , again varying with space x and time t . Thus, the overall division rate per cell is $f(c) \cdot g(n)$, where f is an increasing function and g decreasing; this must be further multiplied by n to give the total division rate in the population. Experimental data, mainly from *in vitro* studies, can be used to determine the appropriate forms for the functions f and g .

Finally, the cell death rate can be represented as kn , where k is a positive constant. This gives the overall equation:

$$\partial n/\partial t = D\nabla^2 n + f(c) \cdot g(n) - kn$$

Before this can be solved, one must go through a similar process to derive an equation for c , which will involve n . Thus we have a system of two coupled partial differential equations, which represent the dynamics of shallow wound healing. The simulations in Figure 1 were obtained by solving this system of equations numerically. Analysis of the equations is also possible using relatively standard methods for partial differential equations; for example, a formula can be derived for the speed of healing as a function of the concentration of exogenously applied growth factor.

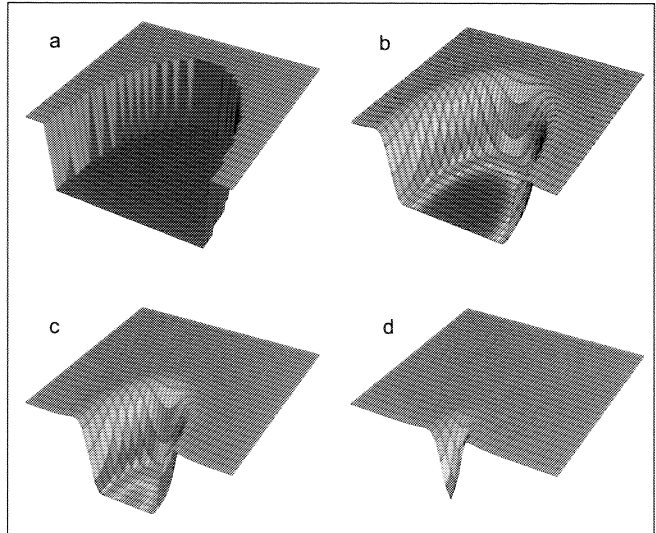


Figure 1: A computer simulation of the healing of a shallow wound. Cell density is plotted for half of a circular wound, at four times during healing. The colours represent the growth factor concentration (red = low, blue = high). The growth factor level is moderate in the tissue around the wound, and higher at the wound edge, where the cells produce the growth factor as a response to being at the edge. Within the wound, the growth factor level is low because there are no cells in this region, and it is the cells that produce the growth factor.

and macrophages, a type of white blood cell. Macrophages are able to recognise tumour cells as foreign and then to selectively kill them – but not very effectively, which is why we still get cancers. Experimental research *in vitro* provides a wide range of data about the way in which macrophages and tumour cells interact. For instance, macrophages aggregate in growing tumours because of biochemical signals produced by the tumour cells; and macrophages are initially passive, requiring activation by another chemical before they are able to recognise and kill the tumour cells.

This is an ideal problem for mathematical modelling: a large availability of information at the single cell level, but

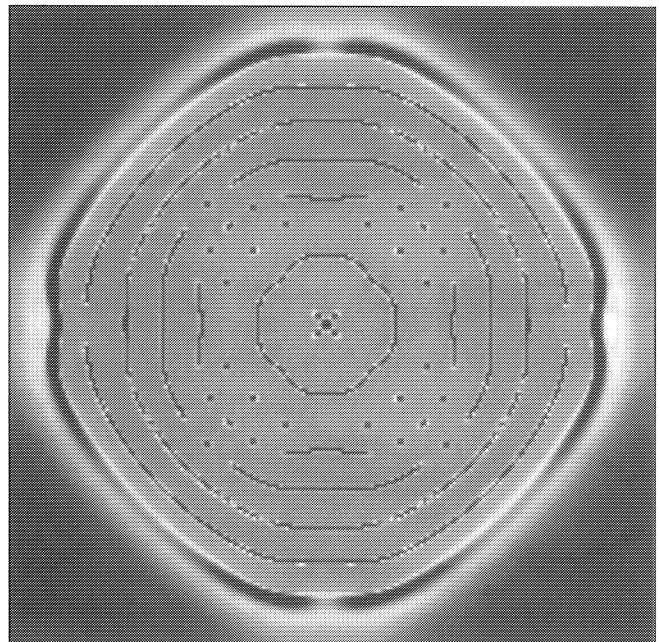


Figure 2: A computer simulation of the early growth of a solid tumour, showing the formation of spatial heterogeneity in macrophage distribution. The density of macrophage cells is plotted at a single time point, in a cross-section through the tumour (red = low, green = high).

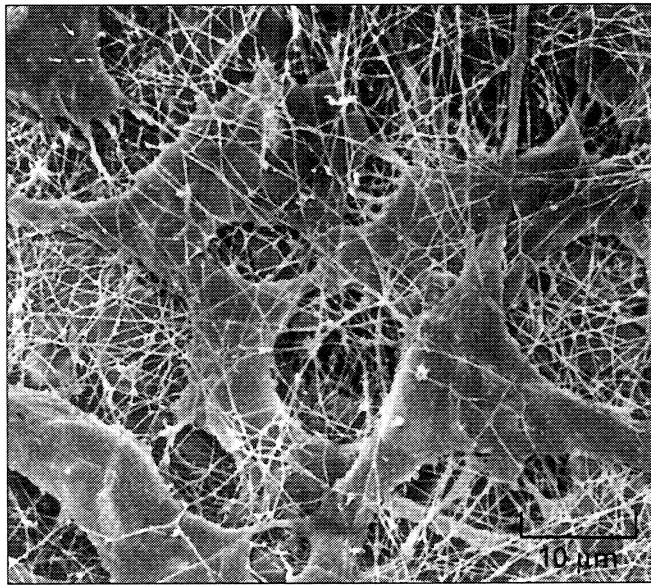


Figure 3. Scanning electron micrograph of connective tissue, showing fibroblast cells surrounded by a network of collagen fibres. The tissue is from the cornea of a rat. (Reproduced from Nishida et al. (1988), *Invest in Ophthalmol Vis Sci*, **29**, 1887–1890, with permission from the Association for Research in Vision and Ophthalmology).

no clear understanding of what these data imply for the large interacting populations of cells within a growing tumour. Markus Owen (Department of Mathematical Sciences, Loughborough University) and I studied this problem (Owen & Sherratt, 1997). Our model is based on the same principles as those for shallow wound healing described above, but this model has more terms to reflect the more complex biology, such as cells moving up gradients of regulatory chemicals, and the killing of one cell type by another. The model makes a surprising prediction: that the presence of macrophages during early tumour growth can initiate spatial heterogeneity. Specifically, the macrophages do not distribute uniformly throughout the tumour mass, but rather, the tumour develops with macrophage-free regions, which are ‘hot spots’ of tumour cell density (Figure 2). Such spatial heterogeneity is

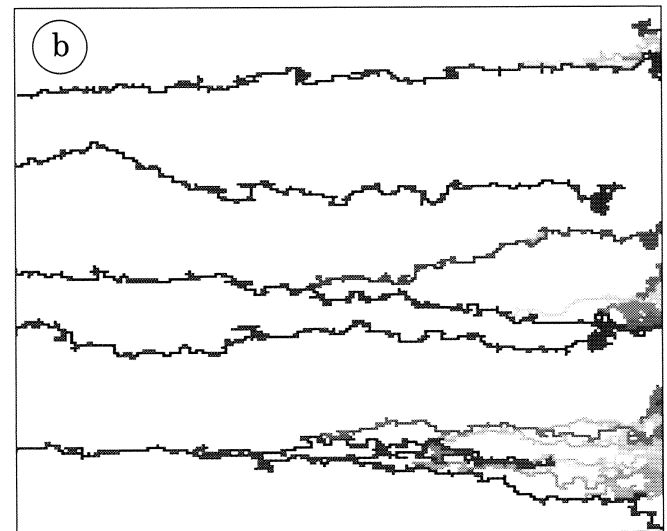
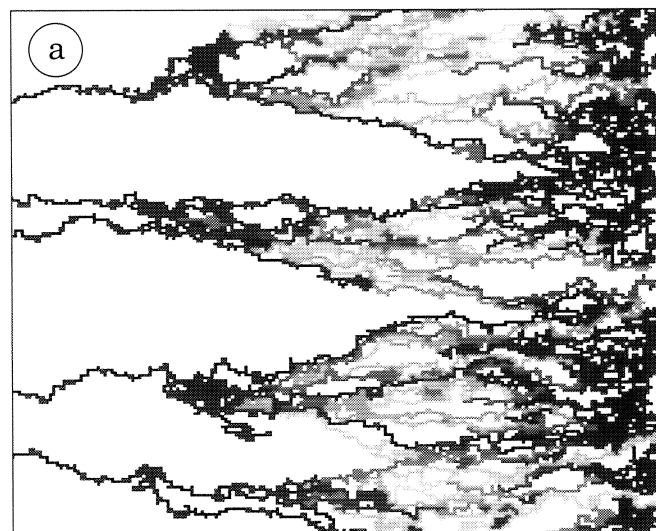


Figure 4. Prediction of the capillary network near a solid tumour. The capillaries develop because the tumour (right of picture) produces chemicals that attract endothelial cells from a nearby blood vessel (left of picture). (a) A simulation of the normal angiogenesis process, in which the individual capillary branches become intertwined near the tumour. (b) The corresponding prediction when the tendency of the cells to move up gradients of extracellular matrix is removed. The intertwined nature of the capillary network near the tumour then disappears almost completely. The colours show the separate capillary branches, so that when a new branch forms, it is assigned a new colour. This figure is courtesy of A R A Anderson, University of Dundee.

observed in many types of human tumours, and can be important as a prognostic indicator; mathematical modelling predicts a mechanism for its formation that would have been very hard to identify experimentally.

Cells and their environment

The main supportive tissues in the body are known as connective tissues; these include tendons, bone, cartilage, and the dermal layer of the skin. In connective tissues, cells are not directly joined to one another, but rather lie within a meshwork of fibres formed from the protein collagen (Figure 3).

This is in contrast to the epidermis of the skin, for example, where cells are in direct contact. The interplay between cells and the fibrous extracellular matrix around them raises many challenges for mathematical modelling and is a major focus for current work. One situation in which cell-matrix interactions are fundamental is the development of a capillary network around solid tumours – a process known as tumour angiogenesis. In their early growth, the cells of solid tumours obtain nutrients in a passive way from the surrounding tissue. However, when the tumour reaches the size of a millimetre or two across, this form of nutrient supply becomes inadequate and the tumour becomes quiescent – no longer growing, but still present and viable. Further growth usually requires the tumour to acquire its own blood supply, and this occurs by the tumour cells producing chemicals known as tumour angiogenic factors. These chemicals diffuse out to a nearby blood vessel, and some of the cells that line this vessel move towards the tumour, attracted by the angiogenic factors, forming new capillaries as they move. The angiogenic process is complex, but a detailed understanding is important because of the possibility of anti-angiogenesis therapies, which are currently under development.

The new vascular network starts at a nearby blood vessel as two or three separate capillaries. One of the intriguing aspects of angiogenesis is that the network becomes more and more intertwined and branched as it approaches the tumour, and this is central to the regulation of blood flow within the new capillaries. Using mathematical modelling, Sandy Anderson and Mark Chaplain (Department of

Mathematics, University of Dundee) have shown that cell-matrix interactions are key to this process (Anderson and Chaplain, 1998). The cells that line blood vessels are known as endothelial cells, and it is these cells that migrate towards the tumour, initially as two or three separate streams of cells. As they move, the endothelial cells produce enzymes that break down the extracellular matrix around them. Consequently, the matrix is depleted along the cell streams, but not between them. One of the complexities of cell-matrix interactions is that cells will tend to move in directions of higher matrix density, and this causes cell streams to come together, forming loops. This happens more and more as the new capillaries advance, forming an increasingly intertwined network (Figure 4a).

One of the powers of a mathematical model is the ease with which one can perform 'mathematical experiments' with cell behaviour. Having demonstrated the process of capillary looping in their model, Anderson and Chaplain then removed the term in their model representing the movement of cells up gradients of extracellular matrix. The intertwined nature of the capillary network near the tumour disappears almost completely (Figure 4b), confirming this as the key underlying mechanism.

Understanding scar formation

In angiogenesis, the fibrous nature of the extracellular matrix does not play a major role. However, in some other contexts it is crucial, and one such case is the formation of scars following wounding. Although scar formation is an everyday phenomenon, an understanding of its biology has only started to emerge recently. In particular, the reason why a scar looks different from the normal skin around it has recently been shown to lie in the orientation of the extracellular matrix fibres. In normal skin, these fibres have a haphazard, basket-weave appearance, whereas in

scar tissue they have a single predominant direction. This makes a scar weaker than normal tissue, as well as changing its appearance.

In collaboration with John Dallon (Brigham Young University), Philip Maini (University of Oxford) and Mark Ferguson (University of Manchester), I have been using mathematical models to study the way in which fibre orientation in a scar is controlled (Dallon *et al.*, 1999). This modelling builds on the ground-breaking experimental work performed by Mark Ferguson's laboratory in Manchester, which has shown that the fibre orientation, and hence the degree of scarring, can be controlled by changing the balance of chemical regulators in a healing wound. This has raised the possibility of anti-scarring therapies, which are now in early stage clinical trials. The aim of our modelling work is to understand in more detail the fundamental biology of scar formation, which we anticipate will lead to refinements in the anti-scarring therapies.

The basic process of scar formation begins with the blood clot, which is composed of disordered fibres made from the protein, fibrin. The upper portion of the clot dries out and becomes the scab, which covers a wound during healing, but the lower part is the building block for scar formation. Fibroblast cells from surrounding skin move into the blood clot, break it down, and produce collagen fibres to replace it. Collagen is also the main constituent of the protein fibres in surrounding skin – the difference lies in the fibre orientation.

The orientation of matrix fibres significantly affects the direction of cell motion, because cells tend to move along individual fibres. But as they move, the cells change the fibre orientation, aligning existing fibres with their direction of motion, and producing new fibres that trace out their recent path. These complex interactions can be studied using a mathematical model that follows the progress of fibroblast cells as they break down a blood clot and replace it with a collagen network. This model is based on *in vitro* experimental data on cell movement. The model predicts the replacement of the blood clot by protein fibres with a single predominant direction, exactly as observed in scar tissue (Figure 5a).

Using a mathematical model to predict something known is, of course, useless. The point of modelling is to predict something unknown, and in this case our objective was to understand which part of the complex biology of fibroblast cells was key to the observed pattern of fibres in scar tissue. To study this, we varied separately a wide range of fibroblast properties – such as proliferation rate and their ability to realign collagen fibres – and used the model to determine the effect that this has on the resulting scar formation. This type of mathematical experiment predicted that one aspect of fibroblast behaviour was much more important than all others; namely, the frequency with which the cells changed their direction of movement. By altering this single aspect of cell behaviour, model solutions show a significantly more disordered collagen fibre network (Figure 5b), which is more like normal skin, and corresponds to reduced scarring. The chemicals that form the basis for the anti-scarring therapies now on trial have many different effects on fibroblast cells. This type of modelling work highlights which of these effects is the important one, enabling attention to be focused on more specifically targeted therapies.

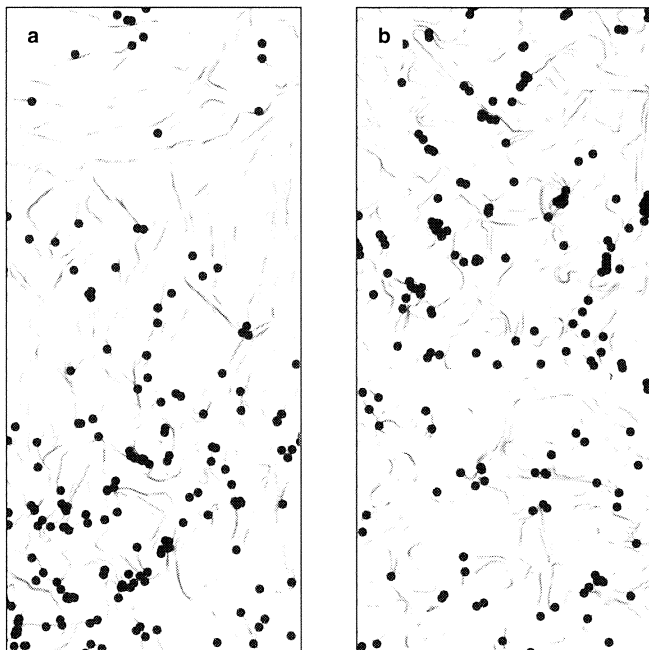


Figure 5. Prediction of the collagen fibre network in scar tissue. (a) The normal repair process, which produces a collagen fibre network with one predominant orientation. (b) The effect of increasing the frequency with which fibroblast cells change direction: this results in a more disordered fibre network, corresponding to reduced scarring. The colours show collagen density (red=high, blue=low) and the dots show fibroblast positions (only 10% shown, for clarity). The plots are five days after wounding, with skin surface at the top of the figure.

The future

The rapid emergence of new cellular and biochemical data on processes underlying human disease means that the areas of medicine to which mathematics can be usefully applied are increasing rapidly. Within the UK alone, recent modelling work covers a wide range of new application areas. In

January of this year, David Gammack and Helen Byrne (Nottingham), working with Claire Lewis from the University of Sheffield Medical School, published a model for the development of tumour cell populations in response to changing oxygen levels. Their model highlights the key role played by hypoxia in regulating the growth of a sub-population of cells with mutations in the p53 tumour suppresser gene. In February, Jaroslav Stark (UCL) and colleagues published the first mathematical model for preimplantation embryo development (Hardy *et al.*, 2001). Their model predicted details of the dynamics of cell division and death in the early embryo, which were then confirmed by experimental collaborators at Imperial College. This work suggests a new direction for research aimed at improving *in vitro* fertilisation success rates. These exciting developments are examples of the increasingly wide span of mathematical modelling applied to medicine. At the time of writing, mathematical models are being developed in the UK alone to study conditions as varied as brain tumours, kidney disease, psoriasis, bacterial infections, atherosclerosis, hepatitis C, and many others. In the coming years, the interplay between mathematical prediction and biological experiments will play an increasingly important role in the conversion of basic biological data into new therapeutic approaches.

Acknowledgements.

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www.ma.hw.ac.uk/maths/medicine/

The home page of Heriot-Watt's Centre for Theoretical Modelling in Medicine, which contains more information on the material in this article, including movies and a layman's introduction to mathematics in medicine. Relevant scientific papers are also available.

www.mcs.dundee.ac.uk/~sanderso/research.html

Information on mathematical modelling of tumour angiogenesis and invasion at Dundee's SIMBIOS Centre. This includes a well-illustrated account of tumour angiogenesis, with movies of computer simulated blood vessel growth.

www.smb.org/

The Society for Mathematical Biology, a good source for meetings on the applications of mathematics to biology and useful links.

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