Bottom-up and top-down in 3D rock shapes.
Two forms of Portland Quarry Rock Art. Left is a partly exposed fossil ammonite (150 million years old). Right is a sculptured 'hat and hands' (*Tout Quarry Sculptors, Dorset*). The ammonite was always there, buried in rock which is being chipped away (arrows). It is a discovered beauty. The hat never existed until the artist imagined it and chipped it *into* the rock. The sculptor fabricated the hat *bottom-up*, from basics. Chipping the ammonite out is a *top-down* process, revealing what was there to be found (see 'Veselius').

1 Which Tissue Engineering Tribe Are You From?

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1.1 Why do we need to engineer tissues at all?

As we are frequently reminded, tissue engineering and regenerative medicine (collectively called TERM) are new disciplines. Tissue engineering is widely considered to have its origins at the point of collaboration between (bio)materials scientists, cell biologists, surgeons and physical scientists/engineers (or any combination of these) towards generating therapeutic/tissue technologies. This, we hope, is moving towards the distant dream of true therapeutic tissue *regeneration*. Regeneration is the key word here, and we shall be getting under the skin of its real implications later in this book, along with its near neighbours: repair, replacement, scarring and amphibian-limbs.

The target of initiatives in the two fields of tissue engineering and regenerative medicine was originally to produce successor treatments for both prosthetic (synthetic) implants and living tissue grafts or cadaveric transplants. Implantable prosthetic devices have had, and continue to have,

Extreme Tissue Engineering: Concepts and Strategies for Tissue Fabrication, First Edition. Robert A. Brown.

an immensely successful history in many clinical and reconstructive surgical disciplines. Despite their many advantages, however, they still suffer the key limitation of never being more than a temporary substitute. They never work better than the day they are implanted; they are always foreign, artificial devices which the body tolerates – for a while – until they wear out or clog up.

Living tissue grafts and transplants – from heart and liver to skin, cornea and tendon – have all the advantages of natural systems which are missing in prosthetics, but these advantages also come with serious costs. Autografts, taken from one part of a patient's body and used to reconstruct another part, are not rejected and cannot infect the patient. They are used across the spectrum of plastic and reconstructive surgery, from rebuilding seriously injured or burned patients through to cosmetic body reshaping, but these approaches are also flawed. Relying on a single – usually injured – individual as the sole source of tissue is always a problem, as the available tissue pool tends to be unsuitable, insufficient or of poor quality. Worse still, the idea of adding *intentional* 'donor-site injuries' onto already severely injured patients (e.g. children, old people, burns victims) is clearly less than attractive.

Transplants or tissue allografts which get around this by being taken from donor individuals can be therapeutically excellent, as in the cases of kidney, heart or liver transplantation. However, donors are typically relatives, unknown or deceased persons, the tissues *will* be rejected without drugs[2] and they carry the risk of life-threatening infections. Needless to say, all of these are also in chronically short supply donor tissues.

The key shared feature of all these existing techniques is that, no matter how hard we work to improve them, they will always retain these same basic drawbacks. In fact, we now are finding 'worst scenario' examples, in which the *more successful* the procedure is, the worse their problems become. For example, as kidney and heart transplants became successful and immune-suppression becomes better managed, the waiting lists for donors became inexorably longer. As we live longer and age better, suitable donors become ever more scarce – and this only gets *worse*.

Another example of a success-driven time-bomb can be found in the story of the prosthetic hip replacement. This is such a successful and long-lived operation that more and more patients across an increasing age range have been demanding it. As a result, the cumulative number of people (a) with steel and polymer hips and (b) living longer active lives has been spiralling up for many years. This would be fine, except for the base problem that no matter how well these prosthetics are made, they will *always eventually wear out* and fail. Consequently, there is now a parallel spiral in the number of patients needing much more complex, but much less successful, 'revision surgery' to remove and replace the worn implants. This represents a *major* healthcare-generated cost and problem which governments would prefer not to feed any longer than necessary.

1.1.1 *Will the* real *tissue engineering and regenerative medicine please stand up?*

How should we define tissue engineering and regenerative medicine?

It is customary, at a starting point such as this, to put forward a definition which captures the goals of the discipline or which lays out a theme that will recur through the book. Many short definitions have been proposed to sum up the targets and technological approaches involved in tissue engineering or regenerative medicine, and some examples of these are given at the end of this chapter as a guide to current concepts (see Annexes 1 & 2). However, this is not a simple or routine task. The next sections in this chapter will discuss why it is non-trivial, not least because an understanding of the paradoxes also provides essential insight into the nature of tissue engineering. So keep faith – definitions *will* emerge.

This section starts with an analysis of why it is perhaps unrealistic to expect a single, crisp

[2]Rejection is almost certain without the lifelong use of immunosuppressive drugs, which themselves can carry severe health side effects.

'definition', in its traditional sense, which we can really trust. The key factor here is that, while tissue engineering and regenerative medicine are two *new* subjects, they encompass several other *well-established* disciplines, all of which, by definition, are moving in their own independent directions. The big question, then, becomes: who can we trust to provide a sufficiently balanced perspective? In other words, however careful one author or another may be, (s)he will also be from one of the component *tribes* of tissue engineering and will tend to see the new discipline of tissue engineering as a derivative of their own speciality. Yet the idea that tissue engineering is just a branch of biomaterials, surgery, bioengineering or cell biology is probably the least acceptable of all options.

The theme of this book is to peek under the concealing conventions and to glimpse around the bend into the less well visited parts of the tissue engineering territory. We may as well start right at the beginning, then, by asking, "Why do we have *so much* trouble with definitions in *TERM*?"

1.1.2 Other people's definitions

There have been many formal attempts to define tissue engineering. Perhaps the fairest approach would be to go with the originator of the term itself. Fairness, though, is not necessarily a close acquaintance of 'useful'. The difficulty is that the most widely accepted (defining) feature of tissue engineering is that it is cross- or inter-disciplinary. This means that each discipline will have its own viewpoint on the subject. In particular, each will tend to consider, quite reasonably, their own discipline to be *the* critical and core essence of tissue engineering. This will include ideas on where the subject is going to and where it came from. Definitions with different starting points, perspectives and viewpoints tend to have patchy histories.

Defining concepts from different standpoints/disciplines can be highly problematic, but the practice is far from unique. Aside from the scientific world, most of the current 300 million US citizens utilize volume measures based on 'the gallon'. Most of their northern and Southern neighbours are obliged to convert these measures to litres, one US gallon being defined as 3.785 litres.

However, the gallon was originally a British measure. It seems though, that some spillage may have occurred on those early transatlantic voyages to America, as UK gallons are 4.546 litres – 1.2 times bigger! Now, that is a serious perspective-dependent shift for a definition. But, despite some disappointments among British visitors to US beer-houses, it did work reasonably well over 2.5 centuries of transatlantic trade (see Box 1.1 for a more accurate historical analysis). The system seems to have been made to work by the simple expedient of nomenclature-sub-division, which resulted in the persistence of the 'US gallon' and the increasingly rare 'Imperial gallon' into 21st century life. This 'name sub-division' may be what is happening with tissue engineering and regenerative medicine, though hopefully it will not take 250 years to bring clarity!

While this may sound like gentle avoidance of the hard question, it is not. The key point here is to understand *why* definitions in this field only ever get us into the foothills of the mountain range. Foothills, of course, are fine, so long as we do not mistake a gentle information hump for journey's end and a peak in the Sierras (more of mountain analogies later). Another way of getting a realistic initial taste is to be obviously reductionist about our definitions. Thus, one foothill-walking approach is to stick literally to the words we have in the subject titles.

What, then, is literally the meaning of '*tissue engineering*'? Perhaps, in reductionist terms, we should be happy with the idea that this describes activities aimed at the engineering of living tissues – but there is a small ambiguity here within the term 'engineering'. As a verb, it could be used to signify either fabrication/construction of new structures from basic elements, *or* modification/alteration of pre-existing structures. In more conventional terms, this might be seen as the difference between designing, testing and fabricating a completely new model of, say, Land Rover, as opposed to engineering an existing petrol-fuelled Land Rover model in order to allow it to run on liquid gas (LPG). The special challenge of our definition, however, comes when

Text Box 1.1 Gallons and gallons

Just to illustrate more fully the confusion that can follow when definitions are not really definitions but viewpoints, let us look a little deeper into the many guises of 'the gallon'. Bear in mind that this is supposed to be a unit of measure whose main claim to utility is its constancy and predictability between people (merchant, sailor, scientist, clinician). It is essential to know precisely what is being offered or demanded in such a measure, and there will clearly be tears if the definition shifts, depending on what substance the volume refers to and where or when it is used.

The real story of 'gallons' is, in fact, more surprising and informative than we implied earlier. At the time of the American War of Independence, both sides recognized no less than *three* forms of gallon, used for different substances. These were the *corn gallon*, for measurement of dry materials (i.e. the dry gallon, of around 4.41 litres), the *wine gallon* (also quaintly, though unhelpfully, known as Queen Anne's gallon – approx. 3.8 litres) and the *ale gallon*, which was around 4.62 litres (perhaps reflecting its greater water content).

Not prone to tinker with a perfectly functional system, the thrifty Americans basically stuck with both the dry and the liquid gallons. Consequently, the present-day US (along with a number of Central and South American Republics) measure petrol and cola in US gallons, which approximate to the old British 'wine gallon' (happily, few of us need to barter in US corn, so do not have to wrestle with dry to liquid gallon conversions). Meanwhile, in 1824, the less conservative British Parliament succumbed to a wave of decisiveness and drained off all except the ale gallon (i.e. *not* the version used in the USA – relationships were a little prickly at the time). This was renamed the 'Imperial gallon' and, true to that name, it was used liberally over the British Empire, including Canada and a number of Caribbean islands. Not surprisingly this has caused the Canadians some difficulties and, in the 20th century, after briefly flirting with their own 'gallon' redefinition, they sensibly opted to switch to litres.

After more than 250 years, though, these jelly definitions may finally be resolving. With the UK now pumping and drinking metric volumes, only a few Caribbean states retain the dilemma of the flexi-gallon and the rest of us talk *either* litres or gallons.

we try to team this activity with that most biological of terms, '*tissues*', and all that it implies about hierarchical, biological structure, sub-structure and molecular interplay*.

* To biologists, the word 'tissue' is very specific. It is not synonymous with either 'cellular' structures at one extreme or 'organs' at the other. These are each distinct entities, with different levels of organisation and hierarchy.

1.1.3 Defining our tissue engineering: fixing where we are on the scale-hierarchy

It clearly will not be possible to achieve fabricated structures at one level of scale without first selecting where we want to be on the scale-hierarchy spectrum. This runs from cells up to organs. In other words, what *scale* do we need to focus on to engineer our tissue of choice?

In biology (arguably more than anywhere else), hierarchical levels of structures and systems are the source of much of the famous 'bio-complexity' and are notoriously difficult to view in isolation. For example:

1. Molecular and atomic level forces are critical to the specificity of binding between larger biomolecules (i.e. at the sub-nano to nano scale). These are essential to the shape – and so the function – of proteins and genomic DNA, providing the exquisitely complex molecular recognition patterns which drive aggregations from:
   (i) nucleotide base-pairing to the DNA double helix and gene folding; or,
   (ii) cell surface receptor proteins (integrin-subunits) to physical connection of the internal cell skeleton (cytoskeleton) with its surrounding 3D extracellular matrix;
   (iii) antibody-antigen recognition in the immune system.

2. However, examples (i) and (ii) also merge beyond this into the next layers of the scale-hierarchy (i.e. meso- and micro-scale). For example:
   (i) Nucleotide base-pair binding operates throughout the structure of genes, then chromosomes and up to the complete nuclear structure. This is most obvious during cell division (mitosis), when all of the nuclear gene content is perfectly duplicated and then pulled apart into two identical halves, one for each daughter cell. Nucleus and cell division processes most definitely operate at the micron scale, yet are still governed by atomic level (nano) forces.
   (ii) The same hierarchical continuity is present in the protein-protein recognition between receptor and substrate molecule surfaces (i.e. nano-scale), but hundreds of thousands of the same interactions will allow a multi-micron diameter cell to move millimetres through its tissue matrix.
3. Finally, we can follow the example of the organisation of the extracellular matrix. The most important tensional load-bearing protein here is collagen, a protein consisting of three chains held in a triple helix spiral by millions of the same nano-scale bonds. However, to generate functional mechanical properties in our connective tissues (skin, bone, tendon, eye), this humble molecule is packed together in countless repetitions of 3D spirals and the same exquisite bond-recognition patterns (Figure 1.1). The longest linear tissue dimension of the largest living creature we know is probably the skin of a blue whale (the largest animal ever). From lip to fluke tip, this can reportedly approach 40–50 m (0.05 km). So, in this case, we potentially have a functional structure at the sub-km scale, made up of repeating nanometre-scale structures, all assembled in interdependent hierarchies. For collagen, then, these functionally inseparable hierarchies (i.e. they are all *physically joined*) nominally span 11 orders of size-magnitude, from ≈0.5 to 50,000,000,000 nm (100 billion:1).

This is important, as it means that when biological members of the tissue engineering community come

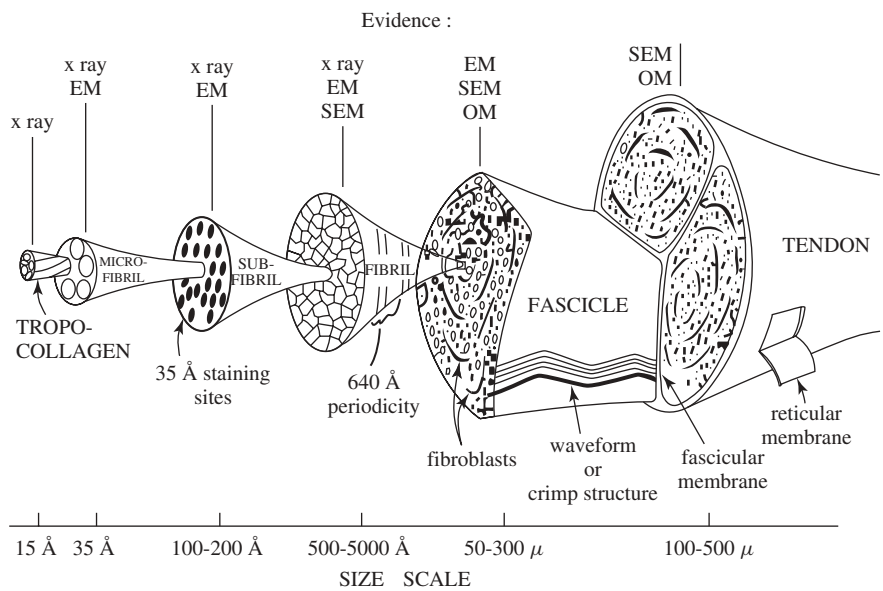


Figure 1.1 Collagen size-scale figure/drawing from tendon. Reproduced from J. Kastelic, A. Galeski, and E. Baer 'The Multicomposite Structure of Tendon', Connective Tissue Research, 1978, Vol. 6, pp. 11–23.

to discuss (i) cellular or (ii) organ engineering, they mean two different but completely interdependent things. We have created a bit of an artificial conundrum, because the design of vertebrate biology, based on natural evolution, means that *only* the whole, intact organism is capable of sustained, independent existence. So, in nature, the individual is the *de facto* functional unit. The conflict comes because, in the lab, we can now keep and manipulate isolated organs, tissues cells and even sub-cellular organelles and protein-systems.

In order to maintain their sanity and to make rational progress, bio-scientists over the centuries have described or invented numerous hierarchical levels or classifications. In the past, these have been largely based on microscopic structures of cells and tissues, and more recently on cell expression of proteins and gene-based classifications.

Examples of these include the identification on structural grounds of cells in different tissue layers of major arteries, in the skin and in the nerves. Differentiation hierarchies of stem and progenitor cells in the marrow (i.e. hematopoietic cells, generating blood-borne immune cells) are well understood. But the functional understanding of haematopoietic stem cells which underpins bone marrow transplantation was not enough to prepare biology for the shock of adult (stem) cell plasticity or reprogramming. Similarly, the treatment of haemophilia with factor VIII was worked out based on an understanding of the coagulation cascade (another protein hierarchy), but few other protein-replacement treatments have been as simple or successful. The problem is, we are only just finding out which bio-hierarchies are and are not *functionally* valuable divisions, where they are oversimplified or exaggerated.

So, what scale (or hierarchical level) of new body parts should we focus on making? Do we aim really high on the complexity spectrum for (say) a whole, beating heart, a factory-fabricated (ready to inflate) lung or a 4 kg mass of hot, living liver? The alternative, further down the hierarchical line, is to fabricate smaller and simpler spare parts, such as muscle strips, hollow tubes for nerve guides and blood vessels, tough sheets or rods to rebuild and refurbish

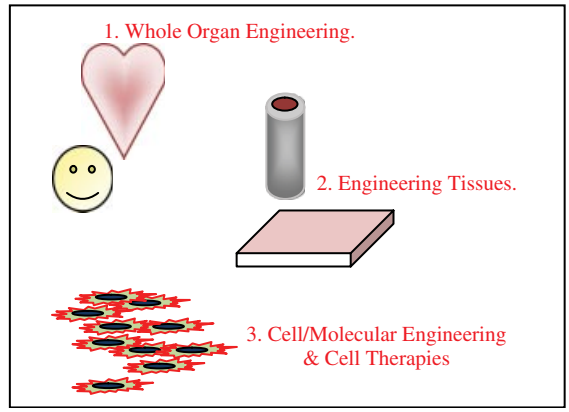


Figure 1.2 Three 'levels' of tissue and cell engineering. We can call them size scales, hierarchies or levels of complexity – but they are different.

more complex structures (Figure 1.2). This would be analogous to patching up and refitting your car's wing mirror, after an accident, with small parts such as a mirror, plastic casing and wires, rather than acquiring a complete new factory-sealed mirror unit, or even a complete new door and mirror.

Down at the really minimal end, we might aim to deliver as little as a small bolus of special cells (with suitable control factors) by injection to the injury site. The aim in this case is that the cells would be pre-programmed with all the information and vigour needed to regenerate a new body part completely. Examples might include: injecting articular cartilage cells – chondrocytes – into joint defects; corneal stem cell and keratinocyte therapies; or injection of olfactory ensheathing cells (OECs) into spinal lesions. This can be reduced and summarized (Figure 1.2) into (1) organ engineering, (2) tissue engineering and (3) cellular engineering and cell therapies.

The various merits and choices represented by this huge simplification will come up again and again as we progress. Since there tend to be few hard answers available to this question at present, we tend to work, pragmatically, across many such levels, from sub-cell and cellular to tissue and organ engineering. Yet, for convenience, we stick with the same language and classifications to describe what

we are doing, and here we generate misunderstanding. By analogy, perhaps, we are not yet specifying whether we are using US or Imperial gallons.

To illustrate this, when we design and fabricate engines for transport, we look for input from mechanical, chemical and electrical engineering specialities to bring together bearing surfaces, hydrocarbon mixtures and ignition control systems. In addition, though, it is essential to understand the detailed function of that engine – specifically, will it be used in the automotive, shipping, rail, aircraft or toy industries? So, with engineered tissues designed to correct failing cardiac function (note, the target here is a *function*), should we put all efforts to fabricate an entire heart, as it is logically a 'unit' muscle with very specific application and function? Or, alternatively, should we try to adapt or multiply the functions of many lesser small muscles, as engineers might adapt an array of automotive diesel engines to power a small ship or adapt an aviation turbine for use in a train.

Members of the 'biology tribe' of tissue engineering might tend towards the perfection of the whole heart. In contrast, the 'engineering tribe' could argue the case for flexibility. After all, developing a small, generic muscle (able to be combined in all sorts of multiples) has the potential of solving many more problems than 'just' cardiac dysfunction (e.g. finger and arm movement, eye turning, sphincter (valve) control in the digestive/urogenital systems, or even breathing).

So what is so special and difficult (the engineers may be fidgeting here) about engineering '*a tissue*'? Just because it contains living, self-replicating structures (cells), why can it not simply be assembled, like anything else?[3] Indeed, this is a basic question we shall return to repeatedly. The equally simple answer (from the biological side) comes in two parts. They are special because:

- they *are* living and dynamic (and we should not make light of what that implies);
- when replacing a bio-component at *any* (hierarchical) level, we do not control the removal and re-fitting processes as we do in engineering.

1.2 Bio-integration as a fundamental component of engineering tissues

The second of the two points made at the end of the last section is so fundamental to us that it is easy to miss its significance. We ourselves, are, after all, living beings, and we take that for granted. We cannot unscrew or unplug a discrete 'unit' of a biological organism, for example one layer of the hierarchy. The surgeon *cuts out* what was once part of a structural-functional continuum when he removes one hierarchical part of the patient's body unit – hopefully, the defective part of a tendon, skin-patch or vein. This is clear, because, once the piece is removed, the patient's wound margins bleed, give pain (nerves are cut) and often physically retract. In the reverse direction, surgeons generally cannot 'clip in' a new bio-spare part. They must offer it into the host site in such a way that it might 'grow' into the existing biological structures and hierarchies. This in-growth and reconnection is an immensely complex, poorly understood and variable process, collectively termed 'integration'.

Integration comprises *vascular, neural* and *mechanical* (marginal) attachment into the rest of the body system. Superficially, it resembles the reattachment of the oil/fuel/water lines and the electrical cables and then bolting down a replacement engine into your car after a major refit. But these engineering steps are only equivalent to the surgeon's use of suture threads, screws, wires and glue to 'fit' the bio-implant into position. In automotive engineering fitting/implantation is the end of the process. *The car drives away*. For the patient, it is only the start, as bio-integration can *only* occur at the cell level with participation of the surrounding (wound margin) tissue surfaces. After all, it is the intimacy of this integration-linkage that

[3] In UK bio-science, this is called a 'Mrs. Lincoln question'. At the exit to Ford's theatre after that tragic performance of April 1865, a journalist caught Mrs Abraham Lincoln with the question, "Aside from that, Mrs L., how was the play?" This is a question where the caveat is so big that it becomes irrelevant, even embarrassing.

made it necessary for the surgeon to cut (rather than unclip) the tissue 'unit' from its hierarchical position in the patient.

In other words, the tissue in question never *was* a 'unit' as we normally consider the term in engineering. Consequentially, the concept of fabricating a replacement 'unit' is somewhat flawed from the outset. In particular, we cannot yet escape a heavy reliance on the natural tissue repair processes for integration. The integration process (hopefully) restores our engineered body part into its place in the hierarchy of the real functional unit, i.e. the patient.

Since we do not fully understand how some of these processes work (and especially how they work *together*), direct engineering or assembly of tissues starts to look daunting. This hurdle becomes clearer (and more scary) to the engineers and physical scientists as they begin to ask their questions about, even basically, *what exactly is it that we are being asked to fabricate*. Characteristically, the answers start to come back with what sound like enormous caveats, variations and unquantifiable flexibilities. It is here that the fresh-entry engineer learns the *real* meaning of 'cross-disciplinary working'. Despite the huge leaps in understanding of biological mechanisms in recent times, biological mechanisms rarely come with the precision, reproducibility and limits of tolerance that engineers and physical scientists take for granted.

1.2.1 Bio-scientists and physical scientists/engineers: understanding diversity in TERM

We are painting a very real intellectual chasm between the biological and the engineering tribes of tissue engineering. In essence, this hinges on the need for engineers to define almost all points that they touch (making fine control possible) and, as far as possible, avoiding those points where precision is not possible. The modern biologist, in contrast, has evolved to cope with the opposite, particularly in multi-cell systems. Biomedical scientists of all shades would make absolutely no progress at all if they avoided the indefinable.

Biotechnology to date has been very effective in simple cell systems, where the potential for systems interactions can be limited and so conditions can be controlled to some extent. However, in complex, multi-component or 3D systems (e.g. beyond fermentation-like processing), the potential to increase the permutations and system complexities increases exponentially. Such systems in nature seem to be controlled by innate cell-to-environment 3D feedback regulation, which is presently understood only loosely. The source of exponential complexity in tissue engineering is implicit and built into the need to put cells, often of different types, into three-dimensional, hierarchical structures such as layers or zones. 3D structure with multiple cell types is at the core of 'tissue' function, and so is a largely unavoidable source of control complexity.

Despite the prickly, scary nature of these points, it is a helpful analysis to make as it leaves us with clear concepts of the size of the problem. While it may, at first, seem daunting, it is essential for any rational strategy that we map out the key drivers and blocks.

For example, it would seem like basic good practice for us to work out rationally (and before we start) whether our clinical problem is best tackled using a cell, tissue or organ engineering approach (or a composite). From this, we can hope to identify:

(i) which clinical/non-clinical applications really can be solved, simply and incrementally by techniques we currently have;
(ii) which targets are just too far ahead at present, as these will demand that we first answer intermediate or even basic questions nearer to our real position;
(iii) which technologies and approaches really are 'too simple' to help with the larger problems, or have already been used to deliver just about all the useful applications they can.

To biological scientists, there is a tendency to interpret every compromise and simplification of nature as likely to lead to 'poor' function. In engineering science, natural systems are impossibly

Text Box 1.2 Teaching and learning tissue engineering is especially tricky

It is likely, even this far into the chapter, that you have been struck by the way some parts of the story seem to sound overly simple (or, dare we say, boring). This is *the* difficulty which is inherent in any text trying to describe the basics of tissue engineering, for reasons which are obvious (once pointed out). Researchers, from aspiring undergraduates to grizzled post-docs, tend to have a specialist's training and background within one of the single disciplines, the *tribes*, of TERM.

Also, and let us be clear about this, we are not talking about close relatives, such as cell biology and genetics or biochemistry on one hand, or mechanical with electrical or process engineering on the other. These are linkages between major cultural and philosophical divides, with seriously different *approaches*, as we have glimpsed already. Students trained in physical sciences tend to rely on precision and mathematical predictability (formulae) which are unnerving to biologists. However, they suffer similar insecurities as they grapple the seemingly infinite complexity, imprecision and variability of the biomedical sciences. Since the characteristic feature of TERM states that it *only* exists where traditional disciplines are crossed, then you, the reader, must be aspiring to cross those boundaries.

It then becomes inevitable that some parts of the story will sound very simple, even naive, to some readers. The problem is that these 'obvious, boring bits' are essential and not at all obvious to other pools of readers, such as no less genuine aspiring tissue engineers, who are coming from a different discipline. It is a truism that it is the 'simple and obvious' which divides and so retards good tissue engineers, as these are the parts of our own specialities that we are *least* likely to clarify in discussion.

So, where you start to feel bored and fidgety, console yourself in the knowledge that there is no alternative. In this case, *all* of the basics have to go into a single book. In other words, explanations of stress, strain and stiffness will appear, even though it is clear that some of you are mechanical engineers. If it is any consolation, though, the cell biologists will be treated to distinctions between epithelial and stromal cells and between tissues and organs.

It is fascinating how many biological colleagues believe that it must be easier for physical scientists to learn the descriptive world of biology than it is for biologists to cope with the mathematics in engineering and physics. Yet, in the very next room, you can meet as many engineers no less convinced that biology consists of an impenetrable range of fact-cliffs and concept-mountains – give them a good, long computer model any day. The truth is that it is difficult in both directions, and we just have to learn to live with 'going over the basics' as it is not basic to everyone. This chapter is only doing its job if the reader at one point feels bored and then at another suddenly anxious and informed. Clearly, though, preparing a book which is guaranteed to bore most of its readers at some point is itself a teaching challenge.

Exercise: Try swapping reading materials with colleagues from other disciplines (e.g. cell biologists with engineers, surgery and repair biology with materials scientists) and check out what sections *they* have highlighted, compared to you. The chances are that you will learn valuable lessons about the locations of your respective comfort and uncertainty zones. Identifying the location of your collaborator's uncertainty zones is a key part of learning to be a good tissue engineer.

complex to copy in the detail they seem to have. In blending these two cultures, it is inevitable that the tracks ahead will twist and turn from (i) to (iii) above, and so must be under continuous review. Paradoxically, there is a strong argument for working hard to preserve this process of oscillation, although it can seem to some like instability or indecision.

The pragmatic value of the oscillation here[4] comes because the systems *are* so complex and we *cannot* know, for any given application and

[4] As with life by the ocean; when you adapt to working with slow, powerful oscillations, you learn to be *very* certain when the *turning points* are coming (and equally confident in the tide-chart tools you use!).

technology, whether an apparently very simple solution will be sufficient for function (i). Conversely, how do members of the engineering tribes know when a technology is just too simple to work and stop trying to apply it (iii)? On the other side of the coin, there is a perfectly reasonable and correct default that in such complex bio-systems: 'we always need more basic knowledge' (ii: the bio-medical tribes).

Since this can easily become an open-ended problem with indefinite timelines, it needs to be tested to identify when such 'enabling' research is just too ambitious to be currently practical. The result may well be that we identify an effective system for progress without fully understanding why in the first place. After all, we have many examples of simple approaches that worked before we knew how they worked, because they happened to tap into natural bio-controls. When this happens, it can bring rapid and inexpensive solutions. Historical examples can be seen in immunization against viral infection or sunlight therapies for rickets. It might also come to include the injection of naked, cultured cells into tissue lesions (i.e. simple cell therapies).

However, some equally important successes have depended on long developments, involving many complex, knowledge-based technologies, more similar to fabricating complex tissues in 3D tissue-bioreactors.

1.3 What are the 'tribes' of tissue engineering?

One thing about tissue engineering which almost every author agrees on is that it is interdisciplinary (sometimes cross-disciplinary or multi-disciplinary). It is important to scratch the surface of that statement to find out exactly what each particular author really means by the term, but nevertheless this is a consensus that we can work from (more of interdisciplinarity later).

The practical truth of this was evident in the very earliest years of tissue engineering, when the subject was brand new, full of exciting possibilities and, above all, highly fundable. Suddenly, there were many tissue engineers at meetings, publishing and securing grants. These many scientists were legitimate members of a growing community, and yet there had been no route to become officially trained in TERM, nor a reasonable lag period where expertise of the new field might have been gained.

The reason for this was simple. The community of tissue engineers (at least in Europe) had sprouted *directly* from expertise in its component disciplines. If you were a biomaterials scientist, a bio-mechanical engineer, a tissue repair or a cell biologist, or a surgeon with special interests in engineering a tissue, you could reasonably claim to be a tissue engineer. All of the traditional learned societies (to which the new tissue engineers also belonged) sprouted sessions on TERM, and then complete conferences focused on the subject. These societies still routinely have either tissue engineering or regenerative medicine as a default topic-for-invited-papers at their annual symposia. Importantly, each of these scientific and engineering communities tend to tackle and consider the TERM field in their own special way and from their own particular standpoint.

Working in the TERM field, then, feels like being within a loose federation of tribes. It is intellectually rich and behaviourally diverse, but it also can be slow to progress and prone to misunderstanding – even naivety. To the newcomer or trainee tissue engineer, the effect can be bewildering until this clarity emerges (Text Box 1.2).

In more familiar terms, its nature can be understood from parallels with the Scottish clans or the communities of the early settlements in New England. Before the advent of easy, rapid transport, the clans or extended family groups that lived near to the coast would prosper by fishing, boat-building, smuggling, etc. Those in the mountains would forage off wild game, cut timber or mug lost travellers, while others, living by rivers in the lowlands, would grow crops, establish banks, build roads or sell fraudulent maps for travellers going to the mountains. Each group would bring their own views and technical expertise to events which demanded common effort or joint defence against outside elements. That is, they would also work together for the wider

'national' needs and common interests of Scotland or New England.

Smaller and larger versions of this pooling of diverse experience can be found in the histories of China, Switzerland and modern South Africa. Examples of the opposite of this analogy (i.e. non-cooperation and competition at every scale) might be represented in the medieval city-states of Italy or the recent history of the Balkan states. In effect, the great strength of such cooperation springs from the very diversity of experience which makes the 'tribes' different. So it is with tissue engineering. Challenging as the habits of the different tribes/disciplines may seem (especially the smuggling, mugging and fraud), they are the source of joint progress.

Perhaps, then, homogeneity or consensus should be viewed as the main *enemy* of TERM. The key to cooperative success in such systems lies in the tension between opposing targets. On the one side is the need to get closer and to promote useful cooperation. On the other is the imperative to maintain the specialist tribal skills and contributions by keeping separate. In some cases, this tension can resolve into a balance between the demands of competition and cooperation. Because this is so central to the understanding of tissue engineering, yet so rarely analysed, it is a recurrent theme of this first chapter.

To understand a little of how the scientific and engineering specialities contribute to successful tissue engineering, we need to identify who they (the tribes) are. Indeed, this can be subtly very helpful for analysing the messages of lectures and papers in the field. Throughout the 1990s, many talks on tissue engineering started with a slide of the speaker's perception of its component disciplines (Figure 1.3a). In fact, the speaker's selection of these disciplines

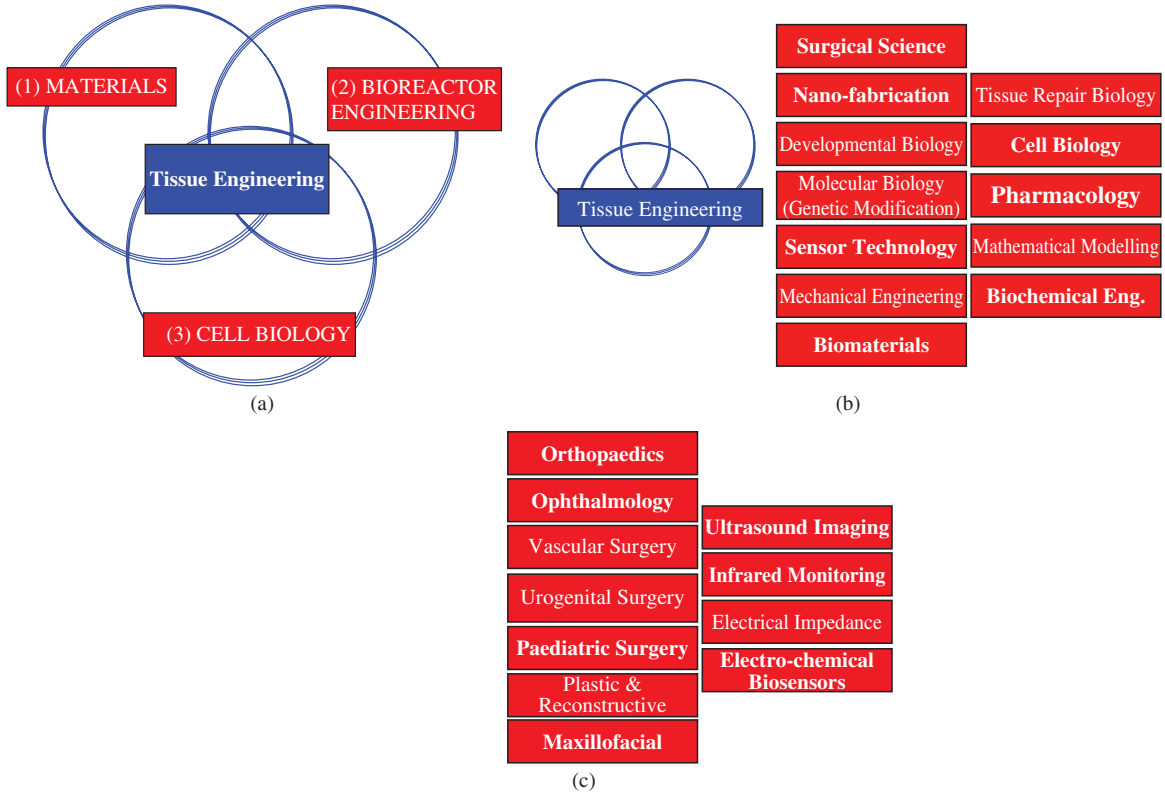


Figure 1.3 (a) One typical depiction of the essential intersecting quality of contributing TERM subjects. (b) Some other major component disciplines of TERM. (c) Some sub-disciplines possible within *only* surgical science and monitoring technology.

was actually more informative of his or her origins and intellectual approach than they would like us to know. It was, and still is, important to listen *very* closely to this introduction. It should, indeed, form the basis of any critical, academic analysis of the technology and scientific interpretations that follow, in the body of the lecture or review.

There are several major science and engineering disciplines (and many, many sub-specialties) which could contribute to the overlap which makes tissue engineering exist. Figure 1.3a shows one example, with tissue engineering being that part of the intersecting rings where at least two, and ideally all, are overlapping. Clearly, where there is no overlap, there is no reason to think that the subject is anything more or less than the traditional discipline itself. Equally, it is possible to have as many intersecting rings as you choose, but here correctness and increasing division has to be balanced by clarity and brevity. It is important, then, to ask the question, 'why did they make *this* particular choice of disciplines?'

Analysis of the example in Figure 1.3a indicates that the three example components put forward are not on an equal size-footing. Two are broadly based general disciplines (biomaterials and cell biology), while the third is more specific, that is, a sub-specialty. This indicates (correctly in this case) that the talk being introduced was going to draw on aspects of cell biology and biomaterials as they applied to the design, construction or operation (i.e. the engineering) of bioreactors.

Now we come to the scary bit. Can we make a list of those disciplines that tissue engineering should cross? The answer of course must be 'Yes – but . . . '. Figure 1.3b gives an illustration of what are, in fact, fairly major subjects found in real examples of tissue engineering research. By the time we have set out from surgical science and tissue repair biology and we reach mathematical modelling and biochemical engineering, it is becoming clear that this could include most of biomedicine and a sizeable chunk of physical sciences and engineering.

Where the component disciplines are not particularly diverse, it may be that the work fits better into a more traditional field and may not really be tissue engineering at all. For example, a main discipline of development biology teamed with cell culture technology and optical imaging might, in fact, be better described in terms of conventional research into mechanisms of embryo development. It is sometimes suggested that research themes are 'tissue engineering', based on a possibility that it *could* lead to important, if serendipitous, findings. This cannot be an acceptable criterion, as it could be said of almost any activity, but it erodes away the basic logic of the field. For example, research into polymer chemistry would be considered primarily *polymer chemistry* rather than biomaterials in nature, until a viable tissue engineering biomaterial is likely to be prepared. It is not the common convention to classify research fields based on their *possible* long-term outcomes.

Understanding which sub-disciplines are involved in any given tissue engineering approach can be helpful. It partially indicates where the work and its ideas are coming from, and it helps to inform on where particular logics, technologies or concept will be strong (and where others may be usefully inserted). For example, we may want to review work on the follow-up of the fate of clinical implants (say, engineered large vessel grafts) by use of new approaches in minimally invasive sensor technology. This would be a collaboration involving the overlap of surgical science, biomaterials and sensor physics. But what type of sensors? Electrical field, mechanical or optical sensor technologies would each give their own distinct set of approaches and capabilities. However, the application of, say, ultrasound or magnetic imaging technologies would carry completely different implications and limitations. In effect:

- Which parameters of implant performance are measured (e.g. vessel wall structure, blood flow rates, clot formation)?
- How often can measurements be made (infrequent for heavy, costly equipment versus regular for indwelling sensors)?
- What is the data quality (e.g. resolution limits for identifying fine features of the vessel wall or micro-thrombi formation; tendency for signals to

be obscured by overlying tissues; poor relevance of the data as a measure of implant performance)?
- What are the dangers to the patient, damage to the implant or surrounding tissues (e.g. heating effects of some ultrasound treatments or the need for injection of disclosing agents to the patient to make structures visible)?
- How much work is needed to adapt the monitoring technology to the demands of the implant system (i.e. how much research is needed)?

If the problem of spiralling numbers of permutations needs any reinforcing, then the addition of sub-disciplines will help. Figure 1.3c indicates some possible sub-discipline pairings which would be expected for tissue engineering based on *only* the primary disciplines of surgical science and monitoring technology. Clearly these lists can, and do, go on and on, with more examples of new subject-matches being added to the literature every year. It is equally clear that these are not useful as a basis for definitions, and that they most definitely are not for predicting future successful combinations. They simply form a historic record, indicating the extent to which ever greater diversity of approaches and discipline combinations can prove useful.

Despite the diversity, then, can we put a name to the *chief* tribes of tissue engineering? It would certainly be an unusual tissue engineering application, for example, if there were no cell or developmental biologists, surgical or tissue repair specialists, biomaterials scientists, (perhaps pharmacologists), biomechanical engineers or optical physicists/imaging specialists. So, while hard and fast rules continue to be rare members of the TERM community, we do have a group of familiar suspects who turn up regularly. To continue the Scottish clan analogy, while you might not expect to see *all* possible Highland names at the Town Fling (the Oliphants or Rosses might be out of town), it would be reasonable to suspect a phoney event if there were absolutely no Campbells, McGregors, MacIntyres or McDougalls.

So, perhaps we should conclude at this early stage that the subject of tissue engineering is not only enriched by the contribution of many differing specialities, but indeed that it cannot really exist (at least as 'tissue engineering') without the contribution of at least two of the more traditional component specialties. After all, there must be a tangible reason why a topic can be distinguished as tissue engineering rather than, for example, biomaterial science or optical bio-monitoring.

1.3.1 Special needs for special characteristics: why is networking essential for TERM?

The 'accepted wisdom', of at least a decade, implies that you can hardly be a real scientist unless you work across disciplines. However, since it is now hardly possible to challenge this idea, it is also becoming more difficult to understand what different specialists *mean* by it. Despite the obvious advantages to novelty and scientific vigour, it is alarming that there is so little critical examination of the costs, more particularly the downsides, to interdisciplinary collaboration. After all, it is only politicians who suggest that we have such a thing as 'cost-free benefit'. So, where is the downside to being multi-disciplinarity?

One clue to this can be found by looking closely at why, for so many years, career scientists (particularly bio-medical) chose to focus their life's work on a pinhead specialization. As an entomologist, already pretty specialized in the study of insect biology, it has been possible to make a major reputation in *just* moth migration (British only), or in the distribution of a particular group of parasitic wasp. We might look, then, at what good things the specialist must give up to become truly cross-disciplinary. This question does assume that the cross-discipline gap we are talking about is more along the lines of 'entomologist to aeronautical engineer' than 'moth to beetle biology'. A similar story can be seen in the clinical specialities. Plastic surgeons, for example (famously expected to operate in almost any body space), will rapidly find a special niche. This may not even just be in hand, nerve or breast reconstruction, but often (s)he will become known for a particular surgical technique, sometimes associated with a new stitch type.

The potential for loss here is that you become less excellent (less knowledgeable, expert, up to date) as you get further from your sphere of expertise. The risk of leaving that focused niche is that of making naive mistakes. In other words, the perceived danger is *loss of intellectual safety*.

Loss of intellectual safety has been a huge factor in traditional subjects, acting against the obvious benefits of working across big subject discipline gaps. In effect, remaining an 'expert' (excellent at an international level) requires a huge depth of understanding of the published literature in all of the topics surrounding 'your speciality'. Given the depth and complexity of the modern literature, this can be an immense task. While it is possible to read and critique the annual output of published material in (say) hand reconstruction or the secret life of Antarctic whales, the equation rapidly becomes impossible if it has to be blended with advances in tendon gene abnormalities or satellite tracking. And actually, these examples are relatively modest in modern tissue engineering collaborations. To help to understand this, Figure 1.3 illustrates how multi-disciplines can turn into a real nightmare for the would-be tissue engineer.

As you look through the basic texts in tissue engineering, you may notice how common it is for a research group or institute to describe itself as being within a 'type' of tissue engineering (see tribes and identities, above). This normally takes the form of a prefix to the group name, to designate their tissue focus. There can be bone, cartilage, skin, nerve, muscle. cardiac, liver, blood vessel (small and large) and bladder/urogenital tissue-engineers to name a few. Figure 1.4a lists only four examples of these in our matrix of expertise.

It is then also useful to focus on the engineering problems of a particular tissue. Even within one tissue group, there are usually many sub-groups or forms of tissue. There may, for example, be different clinical needs at different stages of tissue formation (e.g. for childhood disorders) or in different body sites. Cartilages, for example, are needed for joint repair, but also for reconstruction of facial tissues (nose and ears) or for reformation of trachea and inter-vertebral discs. Even for the replacement of joint surfaces (i.e. articular or 'hyaline' cartilage), the tissues which are needed can vary between joints and according to patient age and disease, while in some cases (e.g. meniscus) a quite different 'fibrocartilage' is thought to be required. Consequently, engineering even single tissue types really can merit such levels of specialization.

However, contributing research knowledge to the pathology and physiology of your tissue (cartilage) is the job of the appropriate biomedical scientist – *not* the tissue engineer (a caveat to this comes later). Tissue engineers should be special, and so distinct from a tissue specialist, because they aspire to *fabricate* their specialist tissue. Consequently, they must also understand the elements of the main platform technologies which they would be expected to use to achieve that characteristic 'fabrication' aspiration. These may, for example, include 'cell

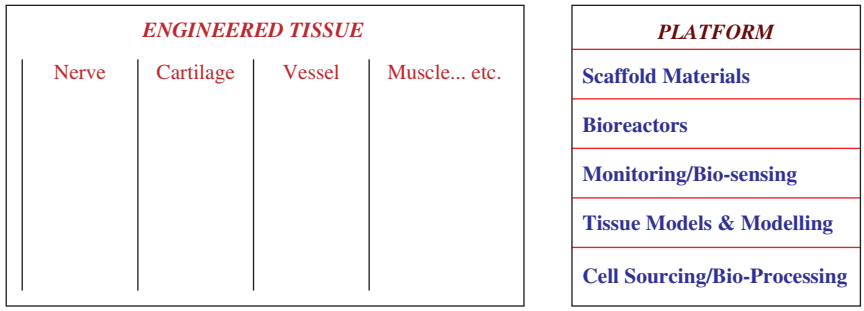
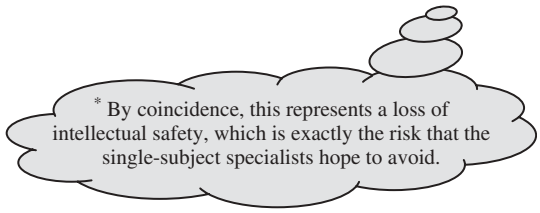


Figure 1.4 (a) Short example-list of tissue engineering 'tissue' specialities. (b) Short example-list of tissue engineering enabling or platform technologies.

sourcing', 'scaffold materials' or 'monitoring and bio-sensing'. A few of the many possible examples of these platforms are given in Figure 1.4b.

The special problem which comes inevitably with the *engineering* of tissues is that the plethora of platform technologies which are used across the range of tissues can have different levels of success (often surprising) *in different tissues*. For example, a breakthrough in polymer scaffold 'design' or 'controllability', as applied to cartilage tissue engineering, may well have enormous advantages for skin and vessel engineering. Similarly, if neural engineers identify a revolutionary way to process and monitor tube structures or promote cell guidance, then vascular or urothelial tissue engineers will also stand to benefit. The problem is that the research advantage goes to the group who pick up on the key enabling innovation *first*. Those who do not look across the wider tissue engineering spectrum will see it late, and so may end up appearing 'naive'*.



In fact, in the special case of tissue engineering, their very diligence in the use of traditional focus and specialization onto a narrow tissue-base can become a *major weakness*. This is illustrated in the exemplar matrix of cross-interests in Table 1.1. Clearly, the breadth of cross-over here between apparently distinct tissues and the enabling areas (often engineering) makes traditional monitoring of research progress inadequate. This effect is amplified by the tendency for tissue engineering tribes to publish in their favourite 'tribal', rather than TERM, journals.

For example, if your primary area is cell biology, how often do you scan titles such as *Advanced Functional Materials* or *The Journal of Biophysics*? Equally, if your work is primarily in scaffold biomaterials, would you catch original work from journals such as *Gut* or *Cartilage and Osteoarthritis*, where you might learn of specialist surgical and repair innovations, or from *Development* for new thinking on tissue regeneration. The telling factor here is that these are all successful, high-impact journals – attractive honey pots for excellent researchers *in their respective specializations*.

So, the central problem of identifying relevant innovations in time remains. It is implausible to cover the volume of knowledge and out-of-field innovation needed through literature scanning or by attending specialist conferences. This, then, is the source of the idea that tissue engineering, like no other discipline, depends on aggressive, continuous *networking*. Effective networking allows participants across very diverse areas to pick up early hints and indications of cross-disciplinary excitement or innovation which normally would be slow to traverse the divides.

There are now numerous organisations which perform this role, from the international tissue engineering and regenerative medicine societies (with acronyms like TERMIS and ICCE) to continental, national and even regional network organisations.

Table 1.1 Example of a full knowledge-matrix for tissue engineering, using only 4 × 5 tissue/platforms; derived from Figures 1.4a and 1.4b.

Platforms	Tissue 1	Tissue 2	Tissue 3	Tissue 4
	Nerve	Cartilage	Vessel	Muscle
Scaffold materials				
Bioreactor engineering				
Monitoring and sensing				
Tissue models and modelling				
Cell sourcing/bio-processing				

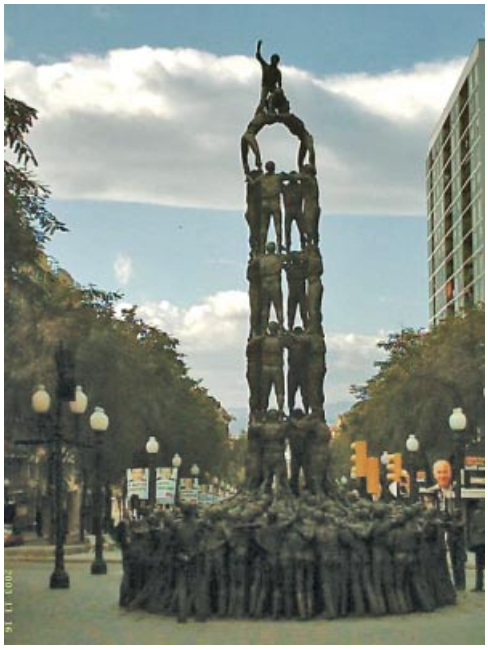


Figure 1.5 Bronze sculpture of a human tower (Tarragona, Spain), illustrating the principle of network cooperation. Note the many figures needed to support the base.

The nature and logic of many tissue engineering networks can be illustrated through analogy with the bronze sculpture shown in Figure 1.5. This depicts a human tower traditionally formed in some parts of Spain (this case celebrates a record-breaking tower from Tarragona). Notice that this tower supports at its summit a small boy. Members of the tower-layers may take turns to enjoy the position and its view. However, the key point is that the weight of the tower itself has to be supported by a surprisingly large number of collaborators pressing inwards to hold up its base. In other words, a few people at any one time can, in turn, get a clear long view from the tower because of the concerted support efforts of the 'many' participants.

For reasons which are mainly cultural and political, the European Union probably has the best example of integration and networking between groups. Figure 1.6 illustrates approximately the spread and distribution of major networking groups active in tissue engineering. Around the year 1999, this could be drawn as an approximate north-south corridor (roughly the red box). Ten years later, and with considerable central EU support, the geographical (and intellectual) spread of this has expanded dramatically to fill two linked shapes (blue triangle plus crescent), covering western Europe, including the northern Mediterranean rim.

Interestingly, networking activities tend to be a balance between those collaborative activities which participant groupings consider to be beneficial and the natural tendency to consider other groups as competitors. In other words, there is commonly a tension between networking (sharing) and perfectly healthy competitive activities.

The very time and effort required for network formation and collaboration can be seen as potential loss of competitive edge, and it is important to understand this balance when comparing activities in different continental zones. For example, there has been a sustained and conscious effort to promote (and fund) collaborative and networking activities across the European Union (EU). This has given a characteristic style to cross-disciplinary EU science, which is arguably less well developed in the same fields in North America or Asia. Here, there may currently be a greater tendency to adopt competitive models, at least over wider geographical areas. The jury will be out for some time over the question of which of these models is closer to being the most effective balance.

The 'take home concept' from this section is that we have identified the first (of a series) of the tissue engineering tensions – between cross-disciplinary cooperation and essential competition. The key point, though, is that the existence of both elements of the tension is necessary for success in tissue engineering. There will be no Utopia using only one or the other, so our task must be to discover ways to 'ride the tension' – to find effective balance points, not to eliminate the tension.

1.4 Surprises from tissue engineering (Veselius to Vacanti)

It is probably true that followers (evangelists?) from almost every new wave of research, from

Figure 1.6 Scheme showing the approximate geographical growth and distribution of major tissue engineering groups around Europe. Key: ≈1999: red corridor (NW-SE central/western EU). ≈2010: contained in two blue areas. © iStockphoto.com/Pingebat.

biochemistry and molecular biology to nanomedicine and synthetic biology, have claimed that their emerging perspective will bring with it new revolutions and scientific dawns. Of course, tissue engineering and regenerative medicine is no exception.

Some of these are admittedly small dawns, but we can never tell in advance. The fact that a book such as this inevitably has just this bias does not mean we can avoid the idea that there really is something revolutionary about tissue engineering. The first stage must be to identify what this 'special feature' might be. Only then can we ask if it really is *so* special.

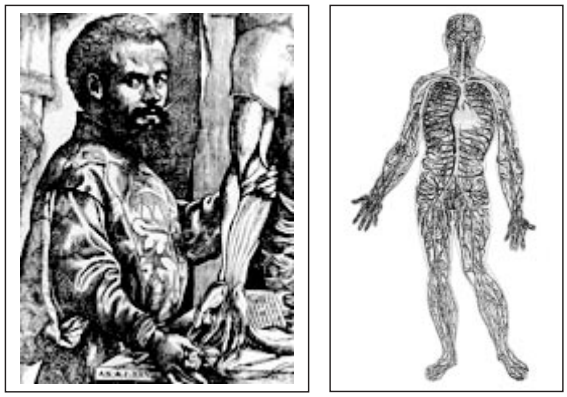
The proposition here is that something very new and surprising is already happening. In fact, the ability to fabricate basic biomimetic structures (simple tissues) is turning upside-down the fundamental, traditional approaches used for centuries in biological scientific research.

To understand where this view comes from, it is necessary to appreciate that biological sciences and scientists attempt to understand some of the most seriously complex systems anywhere. This is perhaps not surprising, after a thousand million years of evolution refining the complexity of biosystems. Complexity, versatility and diversity are, in fact, their defining characteristics. An equally characteristic approach to research in the field has developed over its long history, quite distinct from that in the physical sciences and engineering. This might reasonably be described as:

1. breaking down of the intact complex system into component parts;
2. description and classification of the parts; and then
3. hypothesis-driven deduction and analysis of how the parts *might* have functioned in the intact system or organism.

This has proved to be hugely successful over many hundreds of years, from the early anatomists such as Vesalius (Figure 1.7) to the present day.

Andreas Vesalius (1514–1564) was a Flemish surgeon living in Brussels. He insisted on basing the anatomy he taught to his students on original, systematic dissections of human cadavers, as opposed to animal dissections or artistic interpretations. Although this was initially illegal, a helpful judge in the Italian city of Padova eventually eased his task by supplying the cadavers of executed criminals. From such systematic dismantling of intact body structures, Vesalius was able to describe in detail the layout of both the nervous and blood systems. It is not unreasonable then to consider that this descriptive work in part enabled the British physician William Harvey (1578–1657; Figure 1.8) to deduce and demonstrate the mechanisms by which the heart pumped blood through a discrete circulatory system. This is a classic example of the approach of dissect-describe-hypothesize, spread over a long period.



(a) (b)

Figure 1.7 Andreas Vesalius and his description of the human system of blood vessels. (a) Reproduced with permission © Getty Images; (b) Reproduced with permission © Royal Society of Medicine.

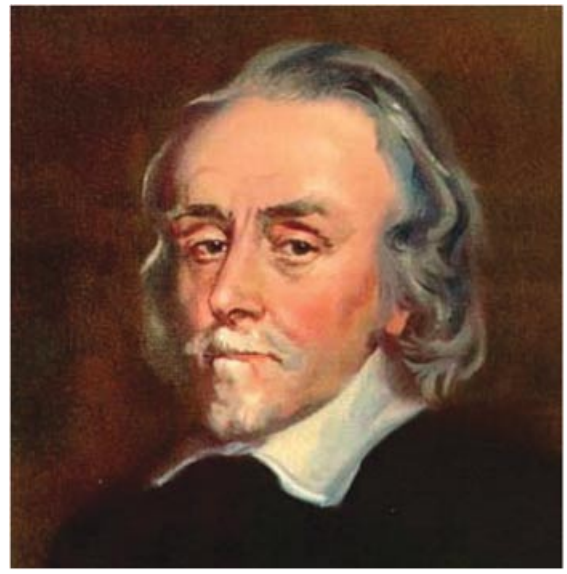


Figure 1.8 Portrait of William Harvey (1578–1657), who is credited with the first detailed description of the blood circulation system. Harvey was a physician at St Bartholomew's Hospital, London and 'physician in ordinary' to King Charles I. The latter got him into significant trouble with Cromwell's Parliamentary (anti-monarchy) troops, and his great discovery did little to help Charles in the end.

The Swedish naturalist Carl Linnaeus (1707–1778; Figure 1.9) is considered to be the father of systematic taxonomy (classification of species). The work of Linnaeus and many other descriptive biologists after him provided the basic interrelationships between animals and plants which have enabled all branches of bio-science to deduce, test and refine our understanding of key biological mechanisms, from Kreb's cycle and respiration biochemistry to modern genetic shift and inheritance.

This same basic pattern of 'break down, describe and reassemble' is even visible in the present day, with the solving of the human genome. First, with genomics the human genetic code was discovered, broken down and progressively described. However, close on the heels of the full genome description came the predictable quest to understand the mechanisms by which these coded proteins operate, leading to proteomics. The remarkable constancy of approach is clear – though, interestingly, the time

Figure 1.9 Carl Linnaeus (1707–1778). Portrait in oils, by L Pasch after A Roslin, 1775 copied for Sir Joseph Banks. With kind permission of the Linnean Society of London.

course for the cycle is shrinking from centuries to decades.

Through tissue engineering, however, it can be argued that a completely new pattern of progression could be emerging. In their pivotal review of tissue engineering, Langer & Vacanti (1993)[5] outlined the aim of *building up* tissues and generic biological structures from their basic units, rather like engineering would be expected to fabricate a human-designed device. In this case, the basic building blocks consisted of suitable cells, 3D support materials and suitable growth control signals (mechanics, growth factors, nutrients). As we shall see later, having one of the basic building components as complex as 'a living cell' makes the assembly process rather more complex than one would normally choose for a fabrication process. Nevertheless, this is similar to the process that many engineers would immediately recognize as bottom-up logic.

Bottom-up approaches lead to intimate understandings of the operating mechanisms of the systems by virtue of their relative simplicity and the many iterative assembly cycles needed. These cycles are characteristic, first to make the system function, then function better, then faster, cleaner and cheaper, etc. In the automotive industry, developments have progressed for so long that much of what can be known about the basic process *is* known. Innovations now commonly come via other technologies which impact on the industry, such as computer-based engine management, aerodynamics, surface coatings or changing social pressures. In the case of assembling biological systems, the final target level is where it operates in exactly the same way that the original tissue does in nature.

In effect, almost 500 years post-Vesalius, we now have a pretty sophisticated idea of what any given tissue should look like and even how it should perform when intact. Tissue engineering effectively aims to make increasingly complex versions of simplified (reductionist) tissues, to assess how they perform and to keep reiterating the process to improve the functional result. In theory, we should know when our efforts are approaching functional and useful, as the tissue we fabricate will start to work more and more like the 'real thing'. Indeed, there is a case for the term 'biomimetic engineering' to cover this process.

The astute reader will see immediately that we are now, after almost half a millennium of tradition, peering the other way up the research avenue. This process promises to show us how biological mechanisms operate through progressively refining what we can *make to work*, rather than what we *think the parts should do*. Critically, each time we design and fabricate a tissue and it does *not* work, we can eliminate one more possible operating mechanism(s). Indeed, this view is already pointing the

[5]Langer, R. & Vacanti, J.P. (1993) Tissue engineering. *Science* **260**, 920–926.

way to completely new (often remarkably simple) understandings of how cells might assemble and refine tissue structures in nature. These will emerge periodically in later chapters.

However, perhaps the most persuasive glimpse of this mechanism inaction comes from the realization, through a number of reviews, that the engineering of tissues promises much more than the simple fabrication of body parts for clinical use. The driver of early stage clinical applications (initially accelerated by commercial forces) has tended to be premature and out of proportion. What we are seeing now is a whole segment of tissue engineering research dedicated to making biomimetic model tissues. These have a value in their own right as test platforms, 3D screening tools and diagnostic systems. But they are also the visible evidence that the 'make-assess-improve' iterative cycle is turning.

1.5 So, really, *is* there any difference between tissue engineering and regenerative medicine?

1.5.1 Questions never really asked: repair versus regeneration?

Many gardeners will have experienced that all too common, but slightly squirmy, moment when digging a flower bed – the 'earthworm incident'. They just get in the way. There you are: one worm, two halves. This may quickly be followed by reinterring the parts and a guilt-removing recollection that both (or was it just the head?) ends will regenerate into new animals. Have I done my bit for soil ecology, then? Rightly or wrongly, the idea that invertebrates can grow complete new, working parts even after complete and major losses seems relatively unsurprising.

Where this '*regeneration*' extends to fellow vertebrates, and in particular regeneration of amputated limbs in amphibians, such as newts and salamanders, it may seem a little more special. We can, after all, identify much more closely with the new limb and its movements – though not in any shape or form with the idea that this could happen to us. In fact, writers of comics and films frequently use this idea in their plots and story lines for superheroes who heal as fast as they are injured. But no matter how good the film graphics or comic storyline, this is still just fiction and a dream for we mammals. Indeed, the mammalian reality is that our tissues *repair*, and this repair process is a pale shadow if the ideal, which is *regeneration*.

Although not all amphibians or their wounds regenerate quite this well, some (Figure 1.10) will go on to form complete new limbs at the site of amputation. This occurs by a type of growth resembling that of embryonic limb formation and

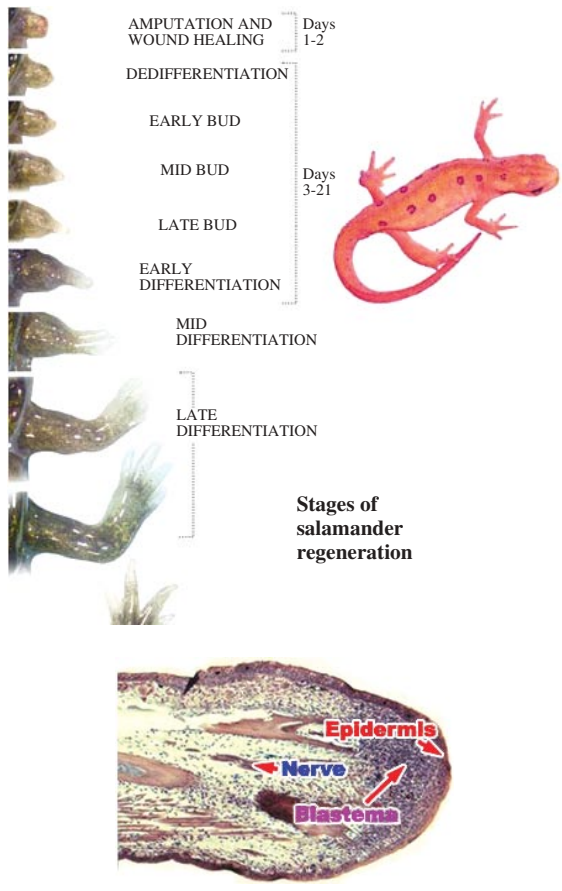


Figure 1.10 Serial images showing the regeneration of a salamander limb over 42 days. Inset: Histological section showing the growing point of the regenerating limb with its differentiated extensions. Including nerve and covering epidermis. From: Mescher, A.L. & Neff, A.W. (2004). Loss of Regenerative Capacity: A Trade-off for Immune Specificity? *Cellscience*. Reproduced by kind permission of A. L. Mescher.

some impression of it can be seen from the figure, in which the remaining stump of the lost limb starts to grow and extend. The growth and extension occurs predominantly at the extreme distal edge (i.e. most remote from the body). This growing strip, or edge, is a rather ill-defined (non-differentiate) cell mass which pushes away from the body wall. Behind it, component structures of the limb (blood vessels, nerve, bone etc) begin to form as cells progressively differentiate down each of the specific tissue lineages involved.

The same process of programmed differentiation, from generalist ('stem' or 'progenitor' cells) to specialist (tissue) cells, along a programmed series, is typical of limb development. This continues as the main bones of the limb grow, ending eventually with the formation of separate terminal digits (toes). Admittedly, the anatomy is simpler than it is in human limbs, but the regenerate has a new, normal skin, covering normal long and toe bones with tendons and joints, so they can bend and move.

Though less obvious, the appearance of another feature should be equally impressive (perhaps *magical* if we are considering how it might be engineered). This key step, so familiar to us that it can be almost invisible unless pointed out, is known as ***integration.*** After all, the new tissue could (in theory at least) have formed as an independent limb on the end of the stump with little connection to the body. However, this is not what happens; the limb bones and tendons organize and physically link to the rest of the salamander so that they move and function. This means that the new limb is not *just* physically joined to carry physical load, but is fed by nerves and blood vessels, which must grown *into* the new limb from the pre-existing body side of the stump (Text Box 1.3).

In addition, the size and geometry of the new limb are, mostly, similar to the original and they are a match to the size and needs of the animal. This dimensional (size) or spatial control is particularly intriguing and is poorly understood. By no means all of it can be explained by the idea that the cells 'know' (are genetically programmed) how to rebuild a salamander. One example of this is the enigma of the 'switch-off' control of new tissue formation, typical of repair, regeneration and normal tissue growth seen throughout the body, though (perhaps simplistically) not so for tumours. How does the new limb come to end up just the right size to match the others, and not three times or half the original length?

The process of limb regeneration in amphibia bears a strong resemblance to that of limb formation in the embryo. In other words, this form of natural 'tissue regeneration' is in the domain of developmental biology, which is significant, as we shall see later. The clue to this can be seen in Figure 1.10, and its inset showing a cross section through the regenerating limb-stump. At the growing tip is a plug of undifferentiated cells (stem cells), known in embryos as the blastema. These generate the forward outgrowth of tissue mass but, as this mass moves away (elongating the new leg), those cells behind start to differentiate into the structures we recognize as the layers and components of a new leg, skin, nerve, skeleton. Each gradually matures into their respective final parts of the leg, specialized to their individual functions.

So much, then, for our lower-vertebrate cousins. However, if we mere mammals are unfortunate enough to have a limb cut off in surgery or in an accident, it remains 'gone'. We, the victims, are left with the stump – that is, whatever (undamaged) tissue was left attached to the proximal, body side, of the injury site. Importantly, the stump will remain, non-sprouting, whatever we do and however long we try. Furthermore, the otherwise uninjured (distal) parts of the lost limb (e.g. fingers or toes) show absolutely no tendency to regenerate a new body (this one really *is* for the worms and sci-fi animators). Even though we are familiar, even resigned, to this and we take it for granted, our native abilities (relative to newts) are particularly modest and disappointing, because we do not even do particularly well with the stump end (or, to be more precise, the scar).

In the case of major limb loss such as this, human patients are understandably more concerned about the loss of the leg or arm function (or relieved to have survived at all). What sort of tissue reformation occurs at the stump is not, perhaps, the victim's main concern when you would prefer the stump not to end as it does at all. At least bleeding from

Text Box 1.3 Integration in tissue repair and regeneration: so familiar, it's almost invisible

Integration is a very small word for a critical part of both tissue repair and regeneration. It is the process by which any new tissue structures become attached and 'plumbed in' to the existing surrounding tissues. This ensures that the newly formed repaired or regenerate tissue is connected into the central systems of supply-and-control (i.e. is *systemically* linked).

The most basic of these linkages is the in-growth of new blood vessels (see (1) on Figure 1.11). This automatically brings 'connection to' (and control by) the host animal, in the shape of immune surveillance and inflammatory cells, hormones and growth factors, as well as coagulation, nutrients and oxygen. The in-growth of nerves (2), of course, brings its own control, where the nerve tips 'dock' with muscles or sensory endings. Finally, (3) a durable mechanical linkage, attaching the new tissue into the surrounding, parent tissues, is almost always essential. This is formed when connective tissue collagen fibres are woven, by fibroblasts, across the new-old join.

The importance of vascular integration is very widely appreciated (though mostly for nutrient and oxygen supply), but this is just the most colourful of the set. Without appropriate levels of integration in all three of these areas, the new tissue would either die or have very poor function. Indeed, the pattern of integration can affect the very nature of the new tissue; for example, in the cartilages, the entry/non-entry of vessels can determine when/where it is calcified (and forms bone), or where it forms articular or fibro-cartilages (as in the meniscus). Figure 1.11 summarizes these three elements. We can, then, consider there to be three distinct (but linked) integration processes:

1. Revascularization (normally angiogenesis, the in-growth of new vessels by sprouting of existing surrounding capillaries).
2. Re-innervation; outgrowth/sprouting of the injured surrounded nerve axons into the new tissues, establishing both sensory and motor controls to new tissues where appropriate.
3. Mechanical integration/attachment. This is probably the most fundamental, and so least noticed, element (i.e. the salamander's new limb does not drop off its stump when moved), and your repaired skin wound does not pop out to leave a hole when you wash (though, interestingly, the blood clot eventually does).

Rather like the work done by a team of skilled plumbers, electricians and plasterers who follow the builders of your new house or extension, these stages only become obvious where they *do not* work properly.

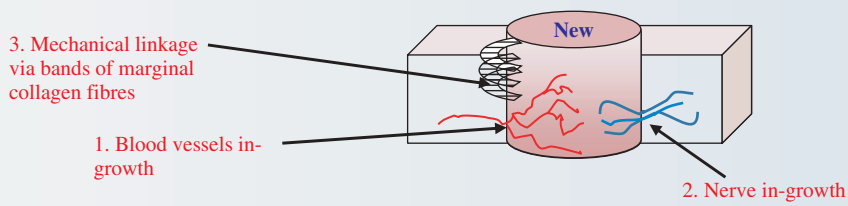


Figure 1.11 The three areas of integration.

the major arteries has been stopped in time and a covering of sorts has formed to keep out massive infections. Indeed, there is now a well accepted view that we mammals have evolved a system dedicated primarily to *survival*, and less to the quality of life afterwards.

However, some clues to the wider problem are perhaps evident when we hear of the 'phantom limb' effect (the failure of integration between injured nerves and the brain), necrosis or infection due to poor re-vascularization or skin contractures/deformation. These are features of a mammalian tissue *repair* process (i.e. not regeneration). In effect, evolution has favoured rapid, aggressive tissue in-filling, over the restoration of three-dimensional structure and spatial organisation which would restore function.

In short, higher vertebrates seem to have evolved to minimize the imminent and lethal dangers posed by rapid fluid loss out and equally rapid pathogen

access through the wound. The process of generating bulk in-fill (i.e. repair tissue and scarring), as fast as possible, seems to apply to most parts of the body, even where they do not border the outside world as the skin does. The process of bulk-dumping of poorly organized connective tissue into repair sites is what we call 'scarring'.

It is worth emphasizing, though, that scarring is the *generic* loss of normal tissue architecture and function. In other words, it is the default, and it occurs to lesser or greater extents at most injury sites (not just the skin). The dermal scar in Figure 1.12 is poorly functional. It has different (stiffer) mechanical properties than the surroundings and it affects facial movement, social interactions and appearance (commonly with psychological impact). Notice, though, how the new tissue stands up from the surrounding skin. In effect, it did NOT stop growing at an appropriate point (unlike the salamander limb) and is too big to accurately replace the injury site. Spatial control in repair tissues is poor.

The uncomfortable truth is that in higher vertebrates, scarring is the normal, default process in response to injury. This is a key concept for tissue engineers to recall and hold close, as it means that we live with the constant possibility that we are engineering scars!

Scarring, comparable with the type we are familiar with in skin, also occurs in blood vessels, tendons, heart and other muscles, and all major organs from lung and kidney to guts and urogenital tract. The fact that scarring/repair is the default mammalian response to injury is rather poorly appreciated, as we only refer to repair sites as 'scar' where they are a problem. When the scar is not a problem (a mixture of luck and insensitivity to loss of function), we are happy and we call it tissue repair.

What determines whether a post-injury repair tissue (for example in Figure 1.12) impacts significantly on function is generally down to luck, location and injury size (with a smattering of genetics). However, scarring is the source of an enormous variety of major and minor forms of human suffering, proportional to the perceived impact of the lost function. It is also important to recall that scarring/repair is the *normal* response.

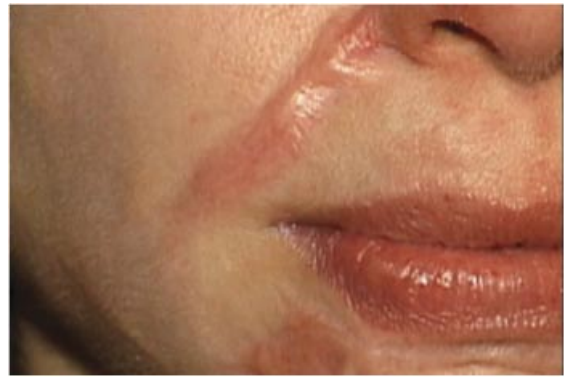


Figure 1.12 Image of a facial burn scar, long after injury. The new replacement tissue is the wrong size, geometry, colour and texture (material properties). Its function is seriously altered from the original. The fact that this *repair* tissue is on the face (and *after* surgical correction) helps us to understand the nature of the problem, but in fact this repair default occurs almost everywhere in the body. Reproduced with permission © R. K. Mishra.

There are, in fact, many abnormal or downright pathological forms of the process. The tip of this particular iceberg can be glimpsed in examples of pathological *dermal scarring* conditions such as hypertrophic and keloid scars. In these exaggerated repair tissues, the shape and material properties of the scars show signs of a failure to shut down at the appropriate time (in some cases ever), leading to oversized or physically deforming repair tissues. We are probably only now starting to understand how such faulty repair processing affects other internal tissues.

We have now identified the first plank in our understanding of how tissue engineering and regenerative medicine might be distinct – based on what each is trying to achieve (Text Box 1.4).

1.5.2 Understanding the full spectrum: tissue replacement, repair and regeneration

Is there *really* any difference between tissue engineering and regenerative medicine? The answer for most workers in the field is 'yes', though it is often less simple to explain why. In fact, both terms describe an aspiration which is as old as mankind – to restore previously lost function to body parts (whatever the cause). It is helpful to

Text Box 1.4 How tissue regeneration differs from repair

Tissue regeneration (whether in major part of a salamander or small layers of a human) is the replacement of lost or injured tissue structures by near-identical structures with the same function as the original.

Tissue repair (the default process in mammals) is the replacement of lost, damaged or injured tissues with an approximation to the original tissue (sometimes, but not always, of the same shape and dimensions), which may or may not substitute effectively for all of the original tissue functions.

Exercise: Write a short analysis of the difference (especially spatial organisation) between regeneration of the salamander limb in Figure 1.10 and adult mammalian skin in Figure 1.12. Suggest one or more environmental cues which might help to explain how the size and shapes of mechanical tissues such as these are controlled (or fail to be controlled).

Tip: *how is it that almost everyone's left leg grows to almost exactly the same length as their right, yet variation between even close relatives' leg lengths can be large?*

know, though, that there are probably only three broad approaches to this vision. These are:

1. to *replace* the tissue (with a device providing *some* function);
2. to get back *better* function by enhancing the natural *repair* process;
3. to *regenerate* functionally *perfect* matching tissue to that which is defective.

Modern bio-medicine, with its development of advanced artificial prosthetics and its ability to suppress rejection of transplants and to re-connect microvasculature, has made major progress with item number 1 above (replacement). From this standpoint, tissue engineering and regenerative medicine can be seen as different approaches towards the same goal (i.e. restoration of function) using very different techniques.

However, there is a vision among clinicians and researchers that we are moving (Figure 1.13) progressively along a left-to-right, past-to-future, time-line. This is moving away from the era of replacing defective body parts with metal/plastic implants or pieces of previously used tissues or organs. These are approaches which, paradoxically, have been and will continue to be enormously successful and important for real patient care well into the foreseeable future. This timeline ends at the point where we can achieve perfect restoration of function (i.e. in regenerative medicine). En route, it passes through tissue engineering, in which the aim is to develop ever better levels of engineered biomimetic repair.

According to this analysis, we now find ourselves at an intermediate stage (Figure 1.14), in which the dominant research questions and clinical objectives are designed to improve more on the natural *tissue repair* process than to achieve true *tissue regeneration*. In other words, tissue engineering (engineering enhanced repair) is characterized as using advanced bio-processing, monitoring and control technologies. This is currently a wide progression-front. Such technologies include those of biomaterials processing and tracking, bioreactor and monitoring/sensing systems, drug and growth factor-controlled release and biomimetic engineering of the extracellular matrix.

In contrast, regenerative medicine, with its more distant, elevated target, is based largely on new and evolving fundamental concepts of stem cell biology and cell plasticity. While some cell therapies and clinical applications are in clinical trial and evaluation, these are at early stages and tend to be characterized by a heavy reliance on the native behaviours of certain, selected cells. The cells in question can be adult, differentiated (such as cartilage chondocytes) or adult progenitor or stem cells (for example derived from bone marrow, corneal limbus or fat) or, more commonly, uncertain combinations of the two. Embryonic and reprogrammed stem cells are as yet on the horizon.

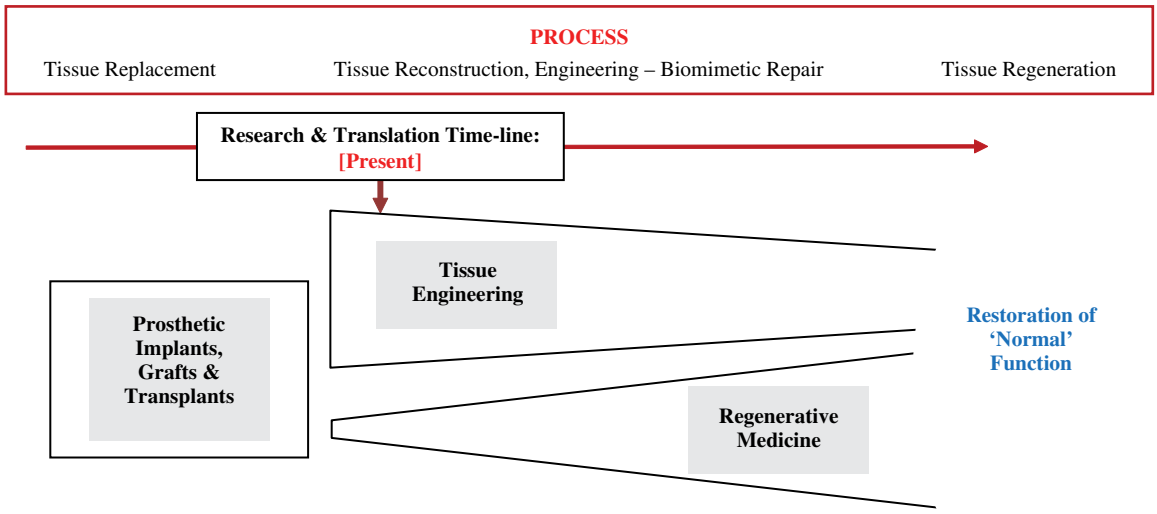


Figure 1.13 Diagram to illustrate how tissue engineering and regenerative medicine can be considered to relate to each other, based on the understanding of repair/regeneration biology. From this standpoint, they are moving towards the same goal (restoration of function) but are using different approaches. Importantly, they are running in parallel, though not necessarily at the same rate or using the same track or starting point. Unfortunately, to those outside the field, these two very distinct approaches *sound* the same, and they are often viewed as almost the same thing. The evolving approaches are indicated in the top box, while the general timeline of progression is indicated below it (left/past to right/future).

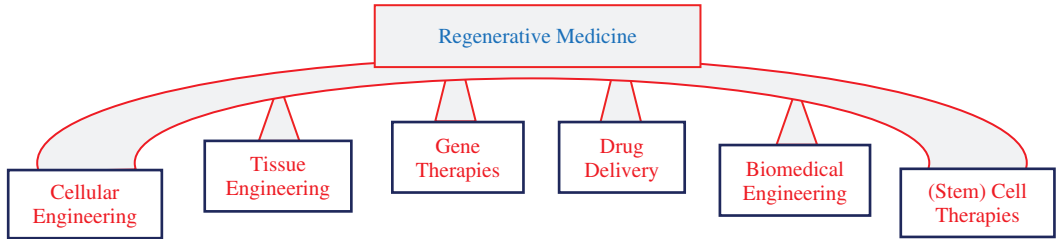


Figure 1.14 Diagram showing an alternative (aspirational) view of the position of regenerative medicine as an overarching, umbrella term. Here, it is supported by a clutch of related disciplines, using the idea that technologies which feed into the broad aspiration of regenerating perfect tissue function represent part of the same, target-defined structure. Such a technology-based structure, with regenerative medicine as its ultimate aim/aspiration, might also logically include developmental and tissue repair biology.

The opportunities for *engineering* what happens in such cell therapies are often fairly low, and there is frequently much less ability (even aspiration) to control what happens externally. This is an inevitable position, given our limited understanding of what these cells might be and how they might achieve the therapeutic ends we hope for. In the case of adult stem cells for therapy, the extent of this limited understanding is made clear by the complex and empirical immune-staining patterns that remain the only way to identify many populations of 'stem cells' and their early differentiation.

As a result, we can crudely distinguish between the two basic strategies (tissue engineering and regenerative medicine) in terms of how we approach them practically. Tissue engineering might be viewed as involving our best attempts to fabricate 3D biomimetic structures, i.e. tissues. It is generally

biomimetic of tissue *repair*, using any biological process available to achieve or improve on natural repair. Clearly, this means that tissue engineering is not limited to using or mimicking regeneration processes, or even to having regeneration as its goal, however welcome it is when it happens.

Cell therapies, aiming at achieving tissue regeneration and regenerative medicine therapies, largely *do* aspire to achieving tissue regeneration, frequently by trying to identify special cells which will mimic regeneration when implanted. However, these approaches presently depend heavily on identifying, tuning and isolating cells with such innate regenerative capacity and behaviour. Technological manipulations in this field are at an early stage and are largely limited to the selection of promising cell populations (ideally enriched in stem or progenitor cells) and encouraging some of these to differentiate towards cells needed in the target (in short, cell farming). Such culture or farming-like technologies are designed primarily to expand populations of desirable cells, to drive unsuitable cells in the required direction or to eliminate cells with irrelevant or unhelpful activities.

This is illustrated in Figure 1.13 as the expanding, diverging bar of regenerative medicine. This analysis suggests that tissue engineering tackles much higher technical and process control targets within a rather less ambitious overall vision (i.e. tissue repair rather than regeneration). In contrast, regenerative medicine aspires to a highly ambitious end vision but is forced to set itself relatively low, empirical targets, chiefly because of the currently modest technology base.

Attentive readers who identified with the earlier part of this chapter and its description of the tribal nature of TERM should now be expecting the inevitable 'alternative' or caveat. In this case, the alternative view of the tissue engineering and regenerative medicine relationship avoids reference to goals and relies on technological interdependencies.

Figure 1.14 illustrates this apparently regeneration-centric view, in which a number of existing research areas are considered to be supporting the overall, umbrella vision of regenerative medicine. In fact, it is possible to see this as a derivative form of the previous model, with the time dimension omitted. In this structure, regenerative medicine takes on an overarching position precisely because it is such a distant and high aspiration. In fact, regenerative medicine then becomes the vision or aspiration of those other (component) disciplines which do have sound, technically definable foundations (i.e. not requiring an open ended basic research commitment). Of course, where and/or if it turns out that clinically useful regeneration can be achieved *without* detailed technical understanding of how it occurs, then such empirical approaches will come to dominate.

So the answer to the question we started with, on the difference between tissue engineering and regenerative medicine, seems to come down to the *quality* of the function which it is hoped to restore. In previous decades (even centuries) we have concentrated on *replacing* tissues with the modest ambition of giving back some function (or for limited periods of time). In some areas, this approach (e.g. wooden legs to artificial hip replacements, eye-patches to contact lenses and corneal transplants) is perfectly adequate for the problem and, indeed, continues to improve the lot of patients.

We have also been trying to improve on natural tissue *repair* for centuries, though mainly by tackling its grossest, most acute failings of infection, haemorrhage and deformity (Figure 1.15).

Research into the problems of scarring, unstable repair tissue material or poor integration and organisation have only really become mainstream within the past 25–30 years. Simple patient survival is now not enough. We are now far more fussy about what is considered the 'functional' quality of

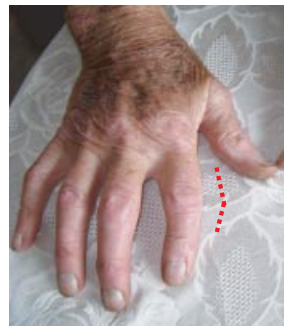
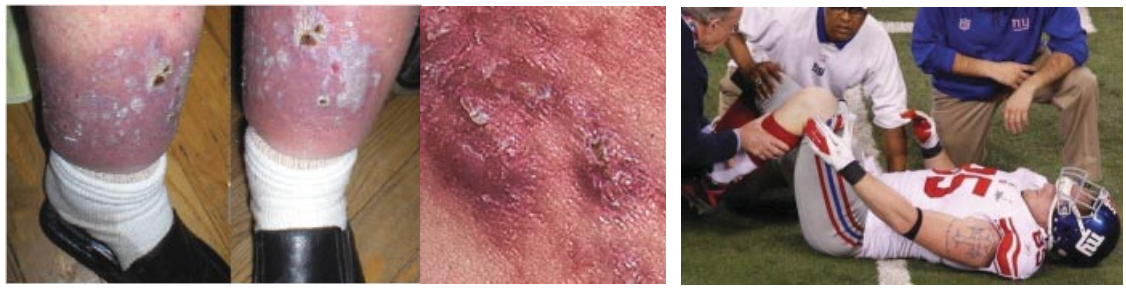


Figure 1.15 A bending finger, known as a fixed flexion deformity (in this case as a consequence of rheumatoid arthritis), can also follow from Dupuytren's disease or scarring after a tendon injury and adhesion.



(a) (b)

Figure 1.16 Modern society sets higher and higher demands on the traditional concept of restoring function. These have gone from just getting a wound to heal over (a) to current needs for it to 'look good' as well, or from previously getting back some walking/running function after a foot injury to now returning to first team performance (b). Photo in (b) © http://deadspin.com/nfl-roundtable/.

the repair tissue at the end of our treatments. These new aspirations to functional restoration are far higher than before and clearly are unlikely to be satisfied by the typical properties of either natural tissue repair or prosthetic implants (Figure 1.16).

Such solutions tend to leave:

- large red or inelastic scars;
- poor ranges of body motion;
- no feeling;
- poor circulation;
- limited implant survival expectation.

In fact, 'functional' has most recently migrated to include:

- convenience (too slow, too many stages – e.g. slow-healing or chronic wounds); and
- cosmetic/aesthetic (including concepts of *desirable* – rather than necessary – shape, colour or size).

With this has come an increasing understanding that many of these aims might be achieved by learning how to better enhance the native *repair* process. Examples have included:

- injections of growth factors (e.g. PDGF or TGFβ1);
- alteration of cell type or activity;
- manipulation of the mechanical forces acting; and
- fabrication of 3D physical repair-templates; or
- fabrication of graft tissues (ideally off-the-shelf).

Here we can recognize the emergence of tissue engineering approaches (though closely entwined with other therapeutic strands of repair biology). Only when the outcome of these treatments is sufficiently effective to produce a 'regenerate' (as good as the original), as opposed to a repair tissue (i.e. not bad but not perfect either), will we be over the border into the kingdom of regenerative medicine.

1.6 Conclusions

A great deal of this chapter has been spent dissecting the nature and aspirations of tissue engineering and regenerative medicine. If the student wishes to take this subject seriously and make any new contributions, it is important to have a grasp of these concepts and basic understandings. Initially, this can seem surprising, but on closer examination it is clear that we are not dealing with a conventional field of research. This makes it unusually important to understand how the 'cogs and pulleys' operate in this case and, more particularly, what makes them different.

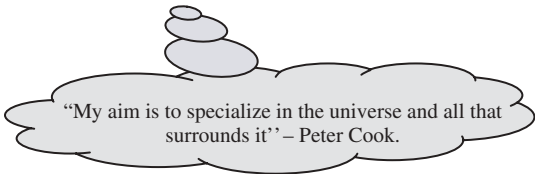
Critically, TERM has had a very different evolution to that followed in the past by other major initiatives. It was not born out of a revolution in technology, nor a breakthrough in scientific understanding (e.g. molecular biology to genomics/proteomics, or histology/optical microscopy to electron microscopy to magnetic resonance and computer tomography). Rather, it was generated from a fusion of technologies and concepts which were actually rather well known. It is this *fusion* aspect, drawing on three or four very different but major subject areas, which has moulded

tissue engineering. Where it can, this chapter has explored:

- how these ideas and technologies start to fit together (or where they have trouble fitting);
- what they really *can* do together; and (*more than ever before*)
- how the emphasis is on getting the most out of the crossovers and novelties which are generated every time an idea or technique migrates across a traditional discipline interface.

In chasing this particular rabbit to ground, we have tackled the question of which disciplines tissue engineers come from (and why it matters). We have looked back at our origins and the way that one's 'home discipline' might explain why definitions tend to be either bland or partisan. We have identified the defining factors embedded in the greater aspirations – the vision – of tissue engineering (namely whether we aim to replace, repair or regenerate a tissue). Finally, we have begun an initial sketch of how different approaches, understandings and requirements of the bio-science and physical science/engineering communities can generate their own form of scientific revolution. Indeed, we may now only just be starting to recognize the nature of this revolution.

As for defining the field, the message perhaps should be one of continuing to modify this, as the field continues to evolve, expand and subdivide (see Text Box 1.1). We, in fact, may just be too soon at the dance to know exactly what style the band will be playing. Critically, students of tissue engineering will always be a risk from Peter Cook's all too plausible warning* on the danger of weak self restraint in the face of the apparently endless diversity of options discussed here. As we have seen, this can be resisted by cold and critical analysis of what we propose to do and how we plan to reach our extreme tissue engineering goals.



1.7 Summarizing definitions

Definitions are, at least at some levels, an essential part of most intellectual activities. However, along with their obvious value can come many (well known) difficulties. This is especially true in the case of tissue engineering and regenerative medicine (TERM). Not least, the whole idea can be regarded as a fusion of already well-established concepts and fields of research. It might be, then, that we have the best result we can expect – a series of retrospective representations each looking at the topic from its own standpoint (e.g. biomaterials, surgery, cell and repair biology, bioengineering, etc.).

However balanced the author tries to be, it must come from one or other bias, because of our training. This is also true of the version you are reading now, though others may have more or less critical analysis. Listed here is a small collection of the more widely published efforts. Interestingly, while it is most common to use the entire string (TERM), published definitions generally focus separately on tissue engineering and regenerative medicine, usually without trying to explaining the difference. This may be that there is currently no clear idea of how and why they are different (so it is safer to lump the two together). Alternatively, it may be that the two terms are truly synonymous, covering essentially the same ground (with only subtle differences). This author's view is that they not only *are* quite distinct, but that it is important for our comprehension that we are clear about the differences. Below is a small collection of published definitions, for reference, starting with the pivotal 'Science' description/review of tissue engineering from Langer & Vacanti in 1993.

Annex 1 Other people's definitions of tissue engineering

Definitions are, at least on some levels, an essential part of most intellectual activities. However, along with this come many (well known) difficulties. In the case of tissue engineering and regenerative medicine (TERM) this is especially true, not least as the original idea might be said to be a fusion of already well established concepts, which almost immediately began to sprout retrospective definitions in every direction.

As a result of this it might be, then, that we have a series of perspectives on what the field really represents, each looking from the standpoint of one of the component traditional disciplines – exactly as I have done, now! This annex provides a small collection of the more widely published efforts, as an instruction of what might be and how the field has evolved. Interestingly, these pretty well all define either 'tissue engineering' or 'regenerative medicine', despite the fact that most writers will use the entire string (TERM). This may be that authors do not have a clear idea of the differences (so err on the safe side), or that they are truly synonymous, as sometimes claimed (almost certainly wrongly). Here we provide a small collection for reference. Since Langer and Vacanti are often (though far from universally) credited with coining the term, this starts with a definition of 'tissue engineering' from their widely cited *Science* review article of 1993:

> *'Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ.'*
>
> Langer, R. & Vacanti, J.P. (1993). Tissue engineering. *Science* **260**, 920–6.

> *'The term regenerative medicine is often used synonymously with tissue engineering, although those involved in regenerative medicine place more emphasis on the use of stem cells to produce tissues.'*
>
> Addendum from current entry (2009) in Wikipedia (i.e. popular definition).

Many authorities (particularly those dependent on US funding) might choose the NIH definition to be the most useful:

> *'Tissue engineering is an emerging multidisciplinary field involving biology, medicine and engineering that is likely to revolutionize the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining or enhancing tissue and organ function. In addition to having a therapeutic application, where the tissue is either grown in a patient or outside the patient and transplanted, tissue engineering can have diagnostic applications where the tissue is made* in vitro *and used for testing drug metabolism and uptake, toxicity and pathogenicity. The foundation of tissue engineering for either therapeutic or diagnostic applications is the ability to exploit living cells in a variety of ways. Tissue engineering research includes biomaterials, cells, biomolecules, engineering design aspects, biomechanics, informatics to support tissue engineering and stem cell research.'*
>
> NIH definition of tissue engineering.

A decade on from Langer and Vacanti, in 2004, amidst the many other 'perspectives' that had emerged came this example of tissue engineering from a biomaterials viewpoint:

> *'There is an inherent, virtuous logic to tissue engineering that sounds too good to be true. By my definition, tissue engineering is the persuasion of the body to heal itself, achieved by the delivery to the appropriate site of cells, biomolecules, and supporting structures. It specifically involves the regeneration of new tissue to replace that which has become diseased or injured, the significance of which is that we, as adult humans, do not normally possess this ability. We may repair ourselves under some very limited circumstances (for example, bone fractures and injured skin may undergo repair) but, even when this does occur, this usually involves nonspecific reparative tissue (i.e. scar tissue) rather than the regeneration of the specific functional tissue that has been affected.'*
>
> Williams, D.F. (2004). Benefit and risk in tissue engineering. *Materials Today* **7**, 24–29.

Annex 2 Other people's definitions of regenerative medicine

The current NIH working definition states:

'Regenerative medicine/tissue engineering is a rapidly growing multidisciplinary field involving the life, physical and engineering sciences that seeks to develop functional cell, tissue and organ substitutes to repair, replace or enhance biological function that has been lost due to congenital abnormalities, injury, disease or aging. It includes both the regeneration of tissues in vitro *for subsequent implantation* in vivo *as well as regeneration directly* in vivo. *In addition to having a therapeutic application, tissue engineering can have a diagnostic application where the engineered tissue is used as a biosensor. Engineered tissues can also be used for the development of drugs, including screening for novel drug candidates, identifying novel genes as drug targets and testing for drug metabolism, uptake and toxicity.'*

This can be qualified by the addendum from the Pittsburgh Tissue Engineering Initiative:

'A distinguishing characteristic of regenerative medicine is that it has the potential to cure disease through repair or replacement of damaged or failing tissues.'

The NIH Facts sheet goes on to state:

'Regenerative medicine is the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage or congenital defects. This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal themselves. Regenerative medicine also empowers scientists to grow tissues and organs in the laboratory and safely implant them when the body cannot heal itself. Importantly, regenerative medicine has the potential to solve the problem of the shortage of organs available for donation compared to the number of patients that require life-saving organ transplantation.'

Though it is perhaps unusual for a definition to include the 'potential' or 'promise' of its subject matter rather than what they actually are now, this aspirational quality may, itself, be a defining characteristic of the new field.

After almost a decade of use a new definition, based on its brevity, comes from the journal *Regenerative Medicine*:

'Regenerative Medicine replaces or regenerates human cells, tissue or organs, to restore or establish normal function.'

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Further reading

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   [A good start to the origins of general tissue engineering from a combined biomaterials and surgical perspective.]
2. van Blitterswijk, C. A., Moroni, L., Rouwkema, J., Siddappa, R. & Sohier, J. (2008). Tissue Engineering – an Introduction. In: van Blitterswijk, C. A. (ed.) *Tissue Engineering*, pp. 14–36. Academic Press, London.
   [Modern introduction to basics, with biomaterial leanings.]
3. Clark, R. A. F. (1996). *The Molecular and Cellular Biology of Wound Repair*, pp. 611. Plenum Press, New York.
   [Classic view of the basic teachings of the wound repair biology tribe, excellent analysis – still widely referenced.]
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5. Stocum, D. L. (2006). *Regenerative Biology and Medicine*, pp. 464. Academic Press, Burlington, MA.
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   [The classic review which acted as the starting-pistol for the international expansion of effort to engineer tissues.]