In biological systems of tissue repair and growth, *tension* is a recurrent theme. *Tension balances* exist between the antagonistic growth factors, some of which promote tissue matrix production, while some promote matrix removal. Protein-digesting enzymes which degrade matrix components like collagen work *in tension* against protease inhibitors (e.g. TIMPs). Some cells specialize in matrix removal and are *in tension* with cells depositing matrix (e.g. bone osteoclasts and osteoblasts), but there is another, potent form of controlling tension in many of our tissues: the controlled balance of mechanical tension acting on the matrix-fabric of those tissues. How much better could our tissue engineering be if we understood how that tension control operated?

7 Other Ways to Grow Tissues?

7.1 General philosophies for repair, replacement and regeneration

As with so much of conventional tissue engineering, we tend to run helter-skelter past the basic concepts and potential clues which could be at the very centre of the squelchy problem. This is nowhere more likely than for the central question of '*How* do we intend to make tissues?' So, in the best traditions of extreme tissue engineering (i.e. doing it differently), this chapter will take a sedate walk around the question of whether there are any examples out there where we *already* 'make' tissues, particularly *in situ* (i.e. *in vivo*; see Text Box 7.1). Clearly, if we could identify any such examples *and understand how they work*, we would gain considerably from the clues and parallels they would provide (and perhaps also develop a healthy modesty).

As discussed in Chapter 1, the idea of replacement of tissues is not exactly special to TERM. Indeed, we are entering a crowded field where it is sometimes easy to miss the occupants because they are so familiar. There is considerable advantage, then, in carefully identifying and analyzing the available options and existing cues before committing to any general strategy for making tissues. In this case it is helpful to revisit (from Chapter 1) one of the most basic divisions in implant logic, i.e. to identify where we definitely *do not* want to go. This is the division between replacing

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

Text Box 7.1 Has someone out there already solved our problem?

When I was a young researcher, a pathologist-mentor passed on to me one of the great deflators of scientific pomposity: 'My boy, if you think you have a truly original idea, you probably just don't read German!' In fact, it can get worse. It might be that the answer is already out there, and *in English*, but you don't see it because it is in a dialect called 'Surgeon'. How many non-surgeons read surgical textbooks?

tissues with inanimate devices rather than living implants.

As discussed in Chapter 4, concepts based on the former, prosthetic type of replacement are, paradoxically, a more important influence on current tissue engineering than might be expected, given their position on the wrong side of this division. This is a consequence of their recent therapeutic success and the faulty aspiration for tissue engineering solutions to provide the same *immediate* benefits as a good prosthesis.

A characteristic feature of successful prosthetic implants is their almost immediate therapeutic benefit to the patient. In fact, as stated in Chapter 4, such implants never work better than on the day they are put in. The paradox here with tissue engineering is clear, since the whole aim of engineered tissues is that they take on a lifetime body function (i.e. they last as long as the surrounding tissues). The compromise we *must* accept is that engineered tissues are unlikely to be as functionally effective as prosthetic devices *in the first instance*.

Consequently, it should, perhaps, be a core principle of extreme tissue engineering that we avoid strategies based on the logic of making prosthetic implants. A more honest strategy-building logic would accept the downside compromise of delayed optimal function and focus on making the most of the strengths of living implants: their bio-activities (Text Box 7.2).

Although blurring across the prosthetics/living tissue boundary is common and, in some cases, useful, it can also have very troublesome consequences. However, as a boundary which is easy to identify, it is well worth either avoiding it or clearly marking its crossing. A more difficult distinction to make is that between *engineering tissues* (from the ground up), and the *biological expansion of existing tissues*. Engineering, or modification, of existing tissues is routinely performed by surgeons

Text Box 7.2 Contrasting 'wide' with 'narrow' approaches to problem solving

A useful analogy to illustrate the decision-making implications of opposing problem-solving strategies is that of two solutions for keeping warm in winter. People can either wear thicker clothes and run around more (i.e. self-only, inward facing strategies) or turn up their central heating and decorate their homes in rich warm colours (non-self, unfocused, outward facing strategies). The two classes of solution to the problem operate in completely different directions. Neither one is necessarily correct in all cases and sometimes combinations of the two may produce the best result. The only problem comes when we mix up the two logics and expect things to work, for example:

- I wear a thick sweater and expect this to make my party guests feel better.
- I paint the walls a warm colour and hope that the dog* is more comfortable.

* **Trivia note:** Dogs seem to be red/green colourblind, although they probably see brighter images than humans, with better night vision and detection of movement. So your dog probably would not see your lounge walls as a 'warm' colour even if he cared. '*The results of the colour vision tests are all consistent with the conclusion that dogs have dichromatic colour vision.*' Neitz, J., Geist, T. & Jacobs, G.H. (1989). Color vision in the dog. *Visual Neuroscience* **3**, 119–125.

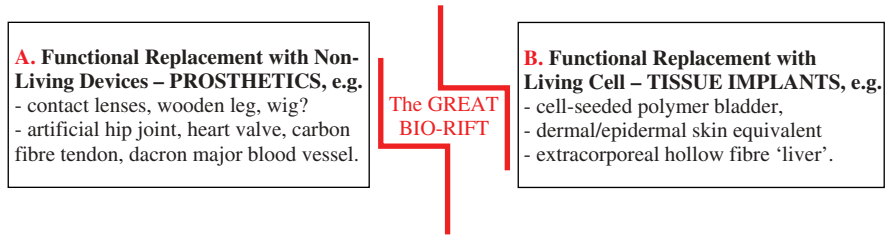


Figure 7.1 The basic non-living/living division ([A] and [B]) between tissue replacement strategies.

as they repair, reconstruct or remodel their patient's tissues. Wherever (or indeed, *if* ever) this interface is finally identified, it is clearly a good idea to learn what we can from surgical tissue modifying skills which have been developed over many decades. In particular, clues from this sector can teach us a great deal about the tissue-like processes and controls which we are keen to build on, as the benefits of the living/non-living compromise (Figure 7.1).

The purpose of this chapter, then, is to look more closely at one particular bio-process which is used, often incidentally, by reconstructive surgeons and repair biologists. The hope here is that we can copy or harness it. This is the process of **connective tissue 'growth'**, particularly where it occurs in adults. After all, many strategies implicitly accept that our engineered constructs will (magically) grow. This assumption is made on the simplistic basis that they are living and so will 'grow' up to the physical dimensions they reach naturally. For example, this is implicit in the concept of the *in vivo* bioreactor and extended to our *culture* bioreactors (see Chapter 8). Yet, without understanding what makes adult (post-embryonic) tissues grow and how that growth switches on and off, we really are going on very little else besides instinct. If we don't understand:

(i) what limits the size of natural tissues;
(ii) why they grow symmetrically (same left and right arm and index finger lengths); or
(iii) what makes wound tissues stop growing,

then what makes us so sure that our constructs will:

(i) grow at all;
(ii) stop growing, *ever*; or
(iii) not grow *smaller*?

7.1.1 What does reconstructive surgery have to teach us?

In its simplest form, surgical reconstruction uses the surgeon's skills and anatomical knowledge to recover part or all of the function of a tissue with the help of *natural tissue repair processes*. This involves repositioning some tissue-parts and joining others together, then encouraging the tissue repair process to 'paper over' the gaps that this leaves. For those from the non-bio tissue engineering tribes, the real extent of 'the gaps' can be illustrated by a plumbing analogy. Imagine that a plumber fitted up your new kitchen sink by pushing the pipes together and holding them together with a fine silk threads, delicately sewing a loose joint together. You might reasonably consider the inter-stitch gaps where water sprays out to be a terminal problem (particularly when the plumber's bill arrives), but this is exactly how a vascular surgeon would fit in a cardiac graft to your failing heart. And it works: the gaps fill!

We have naturally tended to focus so far on the rebuilding and repair of tissue or the movement of small living tissue spare parts from one body site to another. However, such reconstructions also rely on off-focus events which attract far less attention. These are the remodelling, reshaping and restructuring (indeed, growth or shrinkage) of tissues which *surround and support* the primary reconstruction. In fact, it is clear that the remodelling processes that we are so focused on during tissue repair are accompanied by a (much slower) remodelling of surrounding, but connected, body structures which were otherwise untouched.

The tensions across sutured skin injuries or around skin donor sites gradually decrease over

(a) (b) (c)

Figure 7.2 Two examples of skin growth in adults (a and b) and one in a child (c), where mechanical forces drive the tissue expansion – but how? In obesity, the retaining skin clearly grows (a), rather than stretches, under tensions from the accumulating underlying tissue. The same seems to be true in pregnancy (b), often at such a rate that scarring is common. Tissue expanders (c) are used by surgeons to 'create' new skin needed elsewhere on the body for graft-repairs. This is 'mechano-engineering' of skin *in situ*. If only we knew how it works! Credits: (a) © iStockphoto.com/Rob Friedman. (b) © iStockphoto.com/Steven Frame.

time, as surrounding skin 'remodels'. Comparable tensions are set up where cut nerves are repaired, for example following hand injuries, but these also seem to reduce in time where functional recovery is successful. Less helpful, but certainly instructive, the process can work against us. Facelifts inevitably sag again as months and years go by. More of a problem is the connective tissue contractures (shortening) which can occur in adhesion tissues around many surgical interventions. These are common, for example, between body wall layers (fascia) and internal organs, around abdominal hernia repairs or around injured tendons in the hand.

Such contracture is a process where (sometimes following repair, sometimes for reasons unknown) the affected or surrounding tissues become geometrically smaller – they 'contract'. This is a strong process, and any moveable structures which are attached get pulled together. If it is a finger, the digit gets stuck in a bent position; if it is around a section of gut wall or urethra, it can lead to dire and painful blockages.

Could we learn a thing or two, then, about how these shrinkage and sagging processes occur. Better yet, could we harness them, by looking more closely at what goes on during reconstructive surgery, to help us make and expand tissues in the lab? Alternatively, is it possible to persuade the body to make extra little bits of this-or-that tissue *in situ*, for use as grafts elsewhere in the body? This latter idea is basically the *in vivo* bioreactor strategy proposed by some tissue engineers. In reality, these techniques may already be in use by orthopaedic and plastic surgeons in the form of bone distraction (lengthening) and skin tissue expander procedures.

7.1.2 Clues from the natural growth of tissues

While there is a purist argument that tissue engineering is distinct from these surgical procedures, it is a pragmatic no-brainer that we must use whatever lessons they can teach us. Tissue engineering can be considered as aiming to grow *substantial amounts* of *new* functional tissue (Figure 7.2). The keywords in this phrase are underlined. First, there will be general agreement that we aim to engineer *substantial* pieces of tissue, in other words in the millimetre rather than in the micron scale (or below). This is important, as it distinguishes tissue engineering from basic repair biology, say at a wound margin, where tiny amounts of new tissue are formed by local cell action.

Second, there would be general agreement that in engineering of tissues, the aim is to work through processes which implicitly form *new* tissue. This point distinguishes tissue engineering from the successful areas of transplant biology/surgery, where any amount of modifications are developed to enhancement and improve the function of a previously used (i.e. not new) tissues and organs.

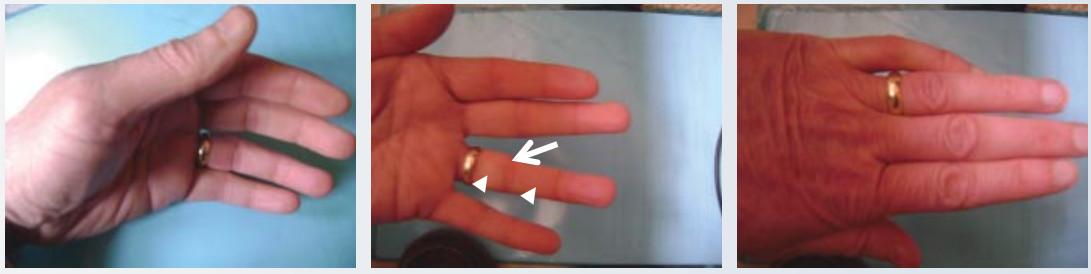
Text Box 7.3 Balanced tension in the skin of the hand

Take a look at the palm of your hand. The skin is folded over the simple hinge articulations at the front of your finger joints (Figure 7.3) but it is smooth and crease-free over the finger bones. Now turn over your hand, with your fingers pushed back and extended as far as they will go, and take a look at the back of your hand. The skin is now most likely wrinkled and in folds – clearly neither tight nor under tension. But you should now be able to feel the reason why.

This is definitely *not* a neutral, at-rest position for your hand. In this position, you have stretched or hyper-extended (hence the 'feeling' you have) the skin of your palm, so much so that the blood circulation changes (Wright *et al.*, 2006). In fact, if you find the resting or neutral position for your hand by letting the whole arm go limp, you will find that your fingers are slightly flexed into a partial 'claw'. In this position, the skin of both your palm and back of your hand should be in *balanced tension* – smooth except for over the joints. This is a visible manifestation of the effect, noted by many human anatomists, that once structures such as a major artery are dissected out of a limb, they seem too short to fit. In other words, *in situ*, they are constantly extended under a tension.

Reference:

Wright T. C., Green E., Phillips, J. B., Kostyuk, O. & Brown, R. A. (2006). Characterization of a 'blanch-blush' mechano-response in palmar skin. *Journal of Investigative Dermatology* **126**, 220–226.



(a) (b) (c)

Figure 7.3 Hands: (a) in neutral, relaxed position; (b) showing extended-stretched palm skin: between (arrow heads) and over the joints (arrow); and (c) extended, folded skin on the back of the hand.

Finally, this leads us to the 'grow' word. Of the three keywords, this one is perhaps where we may learn most and build better tissue engineering concepts. Understanding this would lead to the greatest advances, making the other two seem useful but technical caveats.

To conclude this section, then, we should definitely take on board the idea that THE key objective in tissue engineering is to grow tissue structure, with the emphasis on **grow**. Since we are all-too-familiar with the mechanisms by which man-made structures are made to 'grow' by engineering and fabrication processes, the next section will consider natural examples from which we might learn how natural tissues grow biologically.

7.2 What part of *grow* do we not understand?

'Grow' is an interesting word in its ambiguity and multiple meanings. In biology and medicine it tends to have a rather specific but also, to be honest, somewhat poorly understood series of meanings. In reality, it is more widely used to describe any form of geometric increase in size or proportion. For example, we like to think of children *growing* in height and salamanders *growing* new limbs after injury as rather special (i.e. biological). Yet the same word is used for both processes, even though they are clearly very different. Similarly, we are perfectly happy with the idea that the Eiffel Tower grew over a

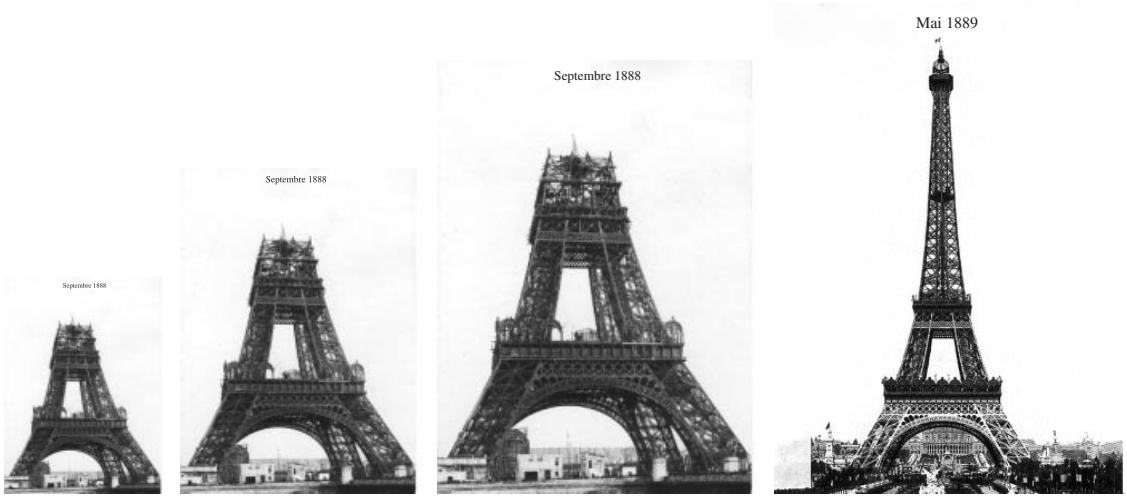


Figure 7.4 The Eiffel Tower grows in dimensions in images (a) to (c) – but this clearly is different to that growth which occurs between (b) and (d). Interestingly, (a) to (c) is analogous to soft tissue (interstitial) growth, but (b) to (d) better represents bone/hard tissue (appositional) growth. Equally interesting, (a) to (c) is a photo-trick which we cannot achieve by engineering.

period of months (Figure 7.4) or that the Himalayas grew by seismic activity (as do tsunami waves on a different timescale!). Intriguingly, we even consider that a tunnel (i.e. tubular void) *grows*, although in this case as a result of excavators *removing* material.

In other words, growth can be the appearance and extension of three-dimensional structure by almost any animate or inanimate means. As we shall see in the next chapter, the diversity of how we achieve growth may be at the very core of our thinking about how to engineer tissues. In particular, we shall wrestle with the tension between growth as a biological cell-driven process and growth of structure that we can achieve in the human world by routine engineering and fabrication.

First, though, let us make the distinction we have used before between cell-rich and matrix-rich tissues. In effect, most of embryology and early mammalian development can be seen as a series of cell rich tissue organisations, template formation and growth processes, under relatively tight gene control. Clearly, the major cell-rich organs (liver, kidney, lung, heart) continue to grow in size by the addition of more cell-rich tissue throughout childhood, halting mostly (though not completely) in adulthood.

The major matrix-rich connective tissues have, in the main, been laid down in their basic template shape and form by the time of birth. These templates grow in size during childhood and adolescence by addition of new matrix-rich tissue. In fact, the proportion of cells to matrix falls precipitously as they take on increasingly the mechanical support functions necessary for a growing, active body.

In a way, this shift, through childhood, is completely inevitable. On the one hand, cell-rich tissues inevitably have only limited mechanical strength. On the other hand, the rapidly increasing mass of a growing child demands increasingly strong, stiff structures for support of its shape and movements. Consequently, to achieve adult load-bearing function, such support-tissues must undergo a dramatic *reduction* in their content of cellular (weaker) material, if only to make way for the ever-increasing proportion of strong, load-bearing extracellular matrix (ECM) protein material.

The extent and pattern to which this happens differs between body tissues and their age, as each one

adapts to the load-carrying demands placed upon it. If we are interested in hunting clues, then, our question must be '**How does that happen naturally**?'

7.2.1 *Childhood growth of soft connective tissues: a good focus?*

Using the analysis above, we can see that human embryology and development represent the formation and patterning of **cell-rich** tissues. In this case we have a neat distinction, because the years between birth and adolescence, where childhood growth is greatest, is the period where the vast majority of connective (**matrix-rich**) tissues are laid down. Interestingly, while entire journals are dedicated to the study of development, far less is known about childhood tissue growth. Although this growth is 'only' simple geometric extension of the embryonic patterns, understanding the processes that control and drive it would be key to engineering *new* adult tissues. The section heading here specifically focuses on soft tissue growth, as we already have a good knowledge of how bone grows – and paradoxically, that is where we must start.

Long bones (i.e. most of the skeleton) grow during childhood through cell and matrix addition in small tissue areas known as physeal growth plates (Figure 7.5). Physeal growth plates are thin strips of specialized cartilage towards each end of each long bone. Such growth plates are directional bone-producing machines, comprising three basic parts in a sequence of three layers. The first (closest to the end of the bone) is a depot of uncommitted progenitor (stem) cells. The second is a layer of rapidly dividing chondrocytes, and under this is the third layer, where cells undergo rapid swelling. These swelling cells eventually die to be replaced by bone and vascular tissue.

In other words, this bone-making machine operates by briefly producing a swelling cartilage tissue, which is rapidly removed and replaced by hard bone.

The reason that the growth plate is important for us to consider here is that it is a mechanism to

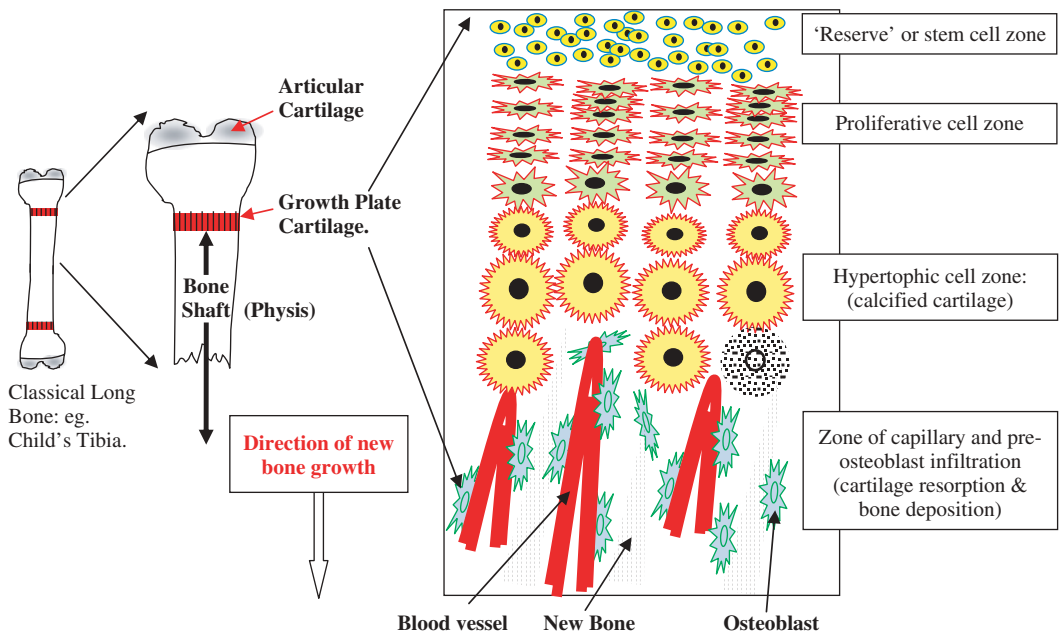


Figure 7.5 Diagram illustrating the main components of a long-bone growth plate – the 'motor' for soft tissue growth. Right hand detail shows stem cells (top) proliferating, then undergoing hypertrophy, death and remodelling by incoming vessels and osteoblasts. Expansion comes from the swelling hypertrophy. Notice that there is strong direction to the growth, downwards here, as columns of cells and, later, cartilage and bone matrix are deposited.

develop (grow) new tissue very rapidly *in a principal direction*. The rapid division of cells in the growth plate (second, proliferative zone) is under tight hormonal control by the body – in fact, by the pituitary gland. This means that throughout childhood, most of the growth plates undergo concerted spurts of action and inaction. These are seen as childhood growth bursts, with eventual cessation of growth and loss of the growth plates after puberty. These bursts of cell division, followed by cell swelling or hypertrophy (the third, hypertrophic zone, see Figure 7.5), inevitably generate compressive loads down onto the adjacent shaft of the bone. Since the bone shaft cannot compress significantly, the ends of the bone are progressively pushed apart and the bone-shaft elongates.

To summarize, this means that we can see long bone extension as a mechanically driven cyclical process. In each cycle, a tiny new layer of cartilage is swollen with water and is replaced by a new layer of bone matrix. The cycle repeats regularly, producing a form of ratchet-extension of the long bones. In rapidly growing vertebrates, this can generate 1–2 mm or more of new bone per day. Derivative versions of this process increase the dimensions (widths and diameters) of the articulating joints at the end of the bones, although of course at *much* slower rates.

But why the focus here on bone/hard tissue extension, when we were supposed to be considering *soft tissue* growth mechanisms? The answer is simple: 'mechanisms'. Almost all of our soft tissues (skin, tendons, vessels, nerves, etc.) are firmly attached to, or stretched over, our bony frame and around its articulations. Thus, the expansion/growth of that stiff frame inevitably becomes the *directional motor* and the *rate-limiting accelerator* which drives expansion of the soft bits that are attached.

Suddenly, it becomes a whole lot simpler to understand the apparent miracle of this biological control process, which leaves our body shapes so symmetrical, wrinkle-free and predictable. In effect, all of these diverse (soft) parts react to adjacent growth-plate driving motors, which are themselves under hormone, and so central, synchronized control.

Exercise Box 7.1

Exception: As always, exceptions are *so* informative. As a short test, list the accessible parts of your body that are *not* stretched over some part of your skeleton. This exercise does not need a formal solution – there are few such body parts. Interestingly, they tend to have much more size-shape variation between people, as well as asymmetry and size variability within the same individual, than those which are attached to bones. Equally interestingly, these also tend to be the parts which attract most angst and cruel attention during adolescence and in our later sex lives – but this is more to do with psychology than structure!

7.2.2 Mechanically induced 'growth' of tissues in children

But, what about *soft* connective tissues? After all, this is the subject of the present section. For soft tissues, there can be no single point of growth as there is in the growth plates of bones. Soft tissues grow at all points throughout their mass. This is called *interstitial* growth, as opposed to *appositional* growth, at the outer surfaces, in bones (illustrated in Figure 7.4). So what is it that controls and drives soft tissue growth? As we hinted at in the last section, the mechanism is apparently both simple and plausible. The directional motor for soft connective tissue growth can be thought of as growth plate expansion during long bone growth, centrally synchronized by pituitary and sex hormones.

The basic hypothesis which explains this linkage demands only that the cells within soft connective tissues can be stimulated to extend and grow their surrounding matrix when they are subjected to an appropriate tensile load. If we accept this working hypothesis for a moment, it is possible to understand how the concerted complexity of limb and trunk growth in childhood can occur. Extension of the spine and long bones through their growth plate machinery must inevitably place *directional* tensile loads onto all of the soft surrounding connective tissue that are attached. Our tensile load hypothesis then explains how all of these surrounding soft

tissues (skin, blood vessels, nerves, fascia, ligaments) grow in a progressive manner linked in space and time with growth of the skeleton.

This is a catch-up growth system in which the soft, elastic connective tissues are under a constant, background tension, sufficient to stimulate growth. This growth would not only follow, directly on bone-extension in time and rate, but also spatially, in shape. In fact, cells would then also respond to the principal directions, or *vectors*, of tensile force set up by the patterns of bone extension.

Not least, this would explain why children and young people, over progressive growth cycles, never seem to have unpleasantly wrinkly and then painfully tight-stretched skin, as a result of some independent or partially synchronous control of growth between the hard and the soft tissues.

7.2.3 *Mechanically induced 'growth' of* adult *tissue*

Clearly, such a **tension-driven** growth process would be an excellent source of clues and controls to mimic in our tissue engineering, if we understood it. On the other hand, all of this would be rather incidental to tissue engineers if it only worked for cells and tissues *in children*. Happily, the extreme tissue engineer can be encouraged by indications that the basic process of tension-driven soft tissue growth is present, and indeed is ready to go, in *adults*.

We are actually quite familiar with some forms of adult tension-driven growth of soft connective tissues. For example, lots of us know someone who is seriously overweight but, on occasions, diets back down to more reasonable proportions. Such obese-slimming cycles (Figure 7.2a) are not uncommon and, especially amongst younger individuals, they are accompanied by something which is remarkable for its absence (so easy to miss).

The abdominal circumference, notably the skin, might conservatively increase in length round the waist by 25–50 per cent in large numbers of people, particularly in the USA (Text Box 7.4). Yet these are substantially greater increases than could be expected if the skin were just stretching. This is particularly striking, as we do not see any of the expected signs of skin thinning, such as increased visibility of blood vessels, changes in tone or texture or tendencies to split, burst,

Text Box 7.4 Obesity and waist measurements

According to Ford *et al.* (2003) and data from 1999–2000, average US male waist measurements at that time were 97 cm (all ages and ethnic backgrounds). The 95 and 99 percentile figures were 126 and 143 cm, respectively. This suggests that even if an individual *started* at an average waist circumference and increased their girth until it reached a level of 1 in 20 of the population (the 95 percentile level), their skin and abdominal wall circumference would have increased by 25 per cent. Indeed, if they reached the heady heights of one out of 100 Americans, then their skin and tissue would have increased in length, in that plane, by 50 per cent.

These are two useful points for our ball-park extrapolation. First, 50 per cent linear increase is, at 1 in 100, not an uncommon level of expansion (albeit this is the USA). It implies that 1.5 million males would have this waist measurement, and that at least some of them were originally average girth. Second, try, if you can, to stretch your own waist skin by anything approaching 50 per cent in that plane. With a struggle, you may manage around 10 per cent extension. In fact, abdominal tissues are twice as stiff in this (the transverse), as opposed to the axial plane (Kureshi *et al.*, 2008). The take-away message, then is that millions of adults apparently manage to extend their skin and body walls by 3–4 times as much as might be reasonably expected by tissue stretching alone.

References

Ford, E.S., Mokdad, A. H. & Giles, W. H. (2003). Trends in Waist Circumference among U.S. Adults. *Obesity Research* **11**, 1223–1231.

Kureshi, A., Vaiude, P., Nazhat, S. N., Petrie, A. & Brown, R. A. (2008). Matrix mechanical properties of transversalis fascia in inguinal herniation as a model for tissue expansion. *Journal of Biomechanics* **41**, 3462–3468.

bruise or rupture[14]. Slimming is more variable, and perhaps age-dependent, but it is equally striking that these lucky individuals are happy to show off their new shape. This slimmer shape does not normally include hanging layers of loose skin, free to sag without its adipose support (except in some extreme examples). It certainly looks very much as if, in most cases, the excess bulk of skin has been removed (remodelled away), leaving the skin as smooth and tight as it was when it held an extra 10 kg of adipose. Although the common assumption might be that our skin *stretches and recoils* as we get fatter and slimmer, in fact we can now see that this stretch-and-recoil idea does not really stack up, either for weight gain or for weight loss.

A more commonplace, though equally dramatic, example occurs in pregnancy (Figure 7.2b). For its extent, rate and focal nature of skin extension, this must exceed that of almost all obese-slimming cycles. Of course, unlike obesity, it always occurs in a complete 'growth-shrink' cycle and, by definition, is restricted to adults. Again, the skin grows to accommodate the expanding abdomen and uterus and then resorbs back after the birth (Figure 7.2b). This can often be so close its original dimensions (again without excess tissue) that even self-conscious mothers are perfectly happy to play with their babies on the beach in last year's bikini. In this case, the tissue expansion/growth can be so rapid that there are residual signs of the process. These are the dreaded 'stretch marks', again indicating that the skin was not as stretchy as we might have thought and could not, in this case, grow fast enough. Stretch marks are scar-like tissue structures which seem to form as a result of minor local connective tissue injuries. In this case, they may form where local strains generated by the expanding foetus exceeds the material properties of the overlying dermis and its growth potential.

These, then, represent two of the natural examples of adult, tension-driven soft tissue growth.

7.2.4 Growth has a mirror image – 'ungrowth' or shrinkage-remodelling

Hopefully, most of you will already have spotted the extra enigma of both the pregnancy and the obesity-slimming cycle examples (do not worry if you missed this though, as it is so obvious and familiar as to be largely invisible). The extra enigma here is that these forms of tension-driven adult tissue growth are *reversible*. They can act in cycles. In the case of obesity, additional tension is generated by the localized deposition of adipose tissue. Obviously, in pregnancy the skin over the abdomen must accommodate rapid intra-uterine growth of the foetus. Both inevitably act as sources of progressive tensile loading on the surrounding soft tissues, which can be seen as comparable with that of extending bones in children*.

* Happily, *shrinkage* of children after they have grown is uncommon.

So, there seems to be a mirror image of tension-driven adult tissue growth, namely the loss of bulk tissue when the tensile load across it falls. This can be interpreted as meaning that the loss of basal tension in soft tissues *normally*[15] stimulates tissue-matrix contraction and resorption such that the tissue becomes smaller. Clearly such a geometric shrinkage (or tissue contraction) would also tend to restore the pre-existing tissue tension, so signalling the end of this shrinkage episode of the process. In other words, cellular activity seems to change tissue dimensions by deposition (or in this case *removal*) of bulk connective tissue to minimize changes in the basal tissue tension. This, in effect, is the description of a cell-based, tensional homeostasis system – implying that tissues work within (and maintain) an overall, background resting tension

[14]In fact, many patients with inguinal hernia ('ruptures') are not obese, and the body wall layer does *not* rupture (as in burst)!

[15]Tissue tension is so 'normal' that we are generally not aware of it until, for some reason, the tension is lost – see Figure 7.6.

Figure 7.6 Tissue tension is so 'taken for granted' that it only shows when it is not there, as in the skin of this oversized Shar Pei dog. © iStockphoto.com/Vitaly Titov.

(Figure 7.6). The presence of such a tension would explain why skin is taut, rather than wrinkly, following loss of body fat – and in pregnant mothers, following child-birth.

In everyday life, we take completely for granted this 'taking-out and adding-back' of material in the connective tissues and we barely notice it. In connective tissue and repair biology, we call this amazing and constant effect 'remodelling' and leave it at that. However, by any analysis, this is a specific, mechanically driven form of material restructuring, apparently controlled through a basal tissue tension. Doubtless we would give it a great deal more attention, and discussion, if some bright anatomist a century ago had named it '**ungrowth**'.

7.3 If growth and ungrowth maintain a tensional homeostasis, what are its controls?

At this point, we need to ask ourselves if is it likely that we are looking at two control processes held in approximate balance. One would involve a stimulus going to cells, perhaps circulating hormones to push connective tissue cells to make more matrix (so growth) every Tuesday, Wednesday and Saturday until the hormone is switched off. A completely separate stimulus, perhaps loss of background tensile load or a second hormone, might bring about tissue contraction or shrinkage at other times by matrix removal.

Such balanced systems, based on opposing mechanisms working in Yin-Yang fashion, are beloved of biologists. For example we think of the coagulation cascade balanced against the clot lysis (fibrinolytic) system of the blood. However, such antagonist systems have to have intimate and interlinked controls, and even then there are inevitably common pathologies where the balance fails, such as haemophilia on one hand and thrombosis/stroke on the other. Though it is always hard to rule out the existence of complex control systems, in this case the idea of a complicated two-process system, with interlocked controls, seems less attractive. For one thing, there are natural examples (Text Box 7.5) where one would expect a two-process system to produce consequences which we do not, in fact, see, but this cannot be conclusive either way.

The cell-mechanics theory, though, is more persuasive in this case, since it represents a single, unifying control mechanism for all tissue remodelling. After all, such a 'material tension' control system *is* available to cells anyway. So, while it is possible that an *additional* and complex biological control process has evolved *as well*, this simple mechanical system is available whether cells use it or not. It is based in fundamental mechanics: if tension in a material rises, insertion of more material (generating greater 'length') *must* reduce that tension. If tension falls, then removal of material bulk (shortened length) *must* increase load on its attachment points, increasing tension.

It is tempting, then, to expect that the 'KIS' principle (keep it simple) operates here. In effect, a simple control system is available anyway, based on the relationship between material geometry and tensions within its material components. So why would a more complex Yin-Yang system need to evolve? So the theory remains.

An important consequence of the material-mechanics control mechanism theory for tissue growth and remodelling is that a background tension needs to exist in soft tissues. Furthermore, the resident connective tissue cells would need to actively maintain that tension. That such a background tension exists in soft connective tissues, even at rest, is evident in our most visible of soft

Text Box 7.5 Author's reflection on the conundrum – can childhood growth reverse?

In old age, osteoporosis can cause loss of skeletal height/length, and wasting or loss of muscle-mass leaves many overlying tissues loose and sagging. The question is, does this represent an example of control failure or an uncoupling of the controls of tissue growth and shrinkage? Unfortunately, in this case the answer is tricky, as *all* cell processes slow down with age anyway, so they may just not keep up with external changes any more. Also, 'oldies' are inherently wrinkly anyway! So this test of ungrowth is ambiguous.

At the other end of the age scale, children with inherited bone growth disorders are left as very short adults, but their soft tissues are appropriately sized for their short stature. Their skin and vessels are suitably smooth and tight, like the rest of us. Similarly, where a bone growth plate is lost in childhood (due to a bone tumour) the bone cannot extend but the overlying skin, nerves and vessels stop growing also. These soft tissues do not grow into folds, even though the contra-lateral leg (bone and skin) continues to grow normally.

The Shar Pei dog skin (Figure 7.6) presents an interesting, if weird, exception. Our hypothesis (yet to be tested) must be that this strain of dog has an unfortunate (some consider cute) mutation of a gene which is key to the mechano-responsiveness of skin. Tissue engineers and repair biologists alike may have much to learn from this dog.

tissues – the skin. This is remarkably smooth and tensioned over the main bones, where it is under constant load. It only sags into folds over joints where regular variation of loading is unavoidable (see Text Box 7.3). As suggested in Text Box 7.5, the gradual failure of this remodelling control system with increasing age may only highlight its dependence on mechano-responsive cells. We can assume that the number and activity of these decline with increasing age, reducing tissue remodelling rates (adaptive growth and ungrowth) and leading to loss of tissue tension.

7.3.1 Tension-driven growth and tensional homeostasis – the cell's perspective?

Support for the idea that connective tissues grow in response to tensional forces acting on resident cells comes from the description of a fibroblast 'tensional homeostasis' system. As discussed previously, many cells – most commonly fibroblasts – are known to generate their own tensile forces on the collagen fibril network in which they are embedded. This is most easily seen where cells are cultured in native 3D collagen gels which are allowed to float freely in medium. Over the following hours and days, the resident cells attach to the collagen fibrils which make up the gel and apply their own tiny motor forces on the gel. As a result, the gel visibly shrinks in diameter and thickness.

In effect, in this system, we can 'see' the forces generated by resident cells as a change in gross dimension of the floating gel. The experimentalist measures the reduction in diameter ever 2–6 hours, as a percentage of starting diameter. Based on the 'simplest-theory-possible' principle, the observer then assumes that the change in shape reflects the sum of all the cell contraction forces generated independently and locally. Obviously, there are other, less simple possibilities. For example, not all of the cells in the gel need to have the same contraction abilities (the different-populations interpretation). There may be 'tough guys', or even 'thug-type' cell populations mixed in with the 'softies'. In modern mechano-biology, it seems all too likely that 'independent and local' is an assumption too far. Nevertheless, clusters of cells could act together, as a cooperative, coordinated by biochemical or mechanical signals.

Rather less obviously, we might question the assumption that cell contraction is isometric, with forces of the same magnitude applied in all direction, 360 degrees around the spherical cell. The isometric tension assumption arises because the actin-myosin (muscle-like) movements come from the cell cytoskeleton, and these can act in any and all axes. While it might be that cell-forces are isometric in the first instance, this would be a very special, extreme position which would be unlikely to persist

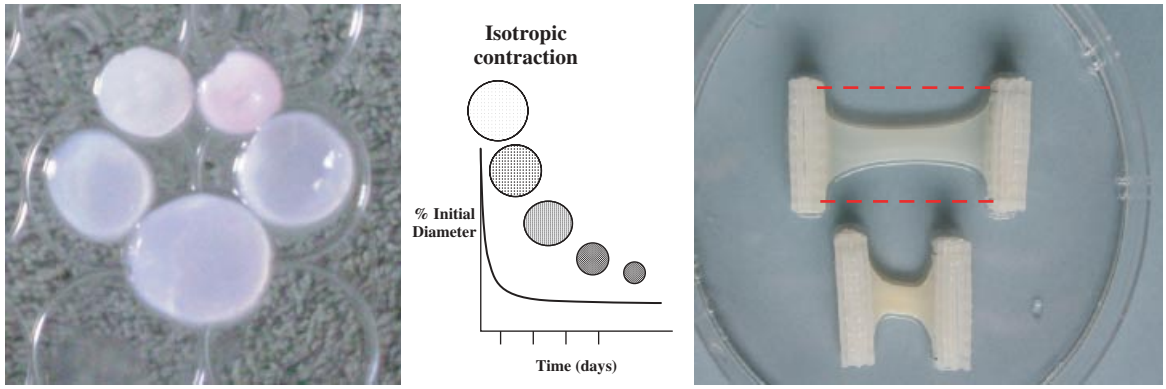


Figure 7.7 (a) Free-floating collagen gels plus fibroblasts contracting (left to right). (b) Graph indicating the contraction rate over time. (c) Same gels uniaxially tethered between porous bars. Top shows gel narrowing, perpendicular to the tethered axis and the original shape, as the dotted line. Lower gel was released just before photo was taken.

for long in any natural system. In particular, for the cytoskeleton to generate loads onto the extra cellular material, it must first *attach* to that material.

In practice, and especially for materials made of long thin, highly asymmetric fibrils, such as collagen, this step will inevitably end up producing very *an*isotropic forces as the fibril component reorganizes and realigns. In fact, the assumptions surrounding the fibroblasts-in-collagen contraction model were developed more than four decades ago and, as we shall see (Figure 7.7), are ripe for revisiting.

Quite by chance, this model is also remarkable in being an extremely low-force, low-resistance system. Forces generated by the cell cytoskeleton are very small, even when magnified by many cells – normally far too small to produce measurable gross-scale movements, least of all those we can measure with a cm ruler. However, the first requirement is that such 3D cell gels are free floating in their culture medium, so friction to movement is minimal. Secondly, the gels are nearly all water, with 0.2 per cent collagen fibres: hence 99.8 per cent water!

Consequently, when cell-cytoskeletal fibres attach to and pull on collagen fibrils through their integrin membrane receptors, there is almost no resistance to their movement. What is more, the gels are so hyper-hydrated that cells are able to contract them to 50–80 per cent of their initial size before the collagen stiffness increases to a level which stops further shrinkage (Figure 7.7).

The story of the collagen-gel-contraction model, with its indirect, visual read-out of contraction could have continued on much the same lines but for the development of direct, real-time measurement of force generation. This changed many of the basic assumptions of the system (mostly beyond the scope of this analysis). One example of this direct cell force measurement involved tethering rectangular gels at either end of porous bars. Sensitive strain-gauges could then monitor the contraction forces, as they were generated, in the single tethered axis (Figure 7.7c). At the same time simple uniaxial loads could be applied to the cell gel tissue through a computer-controlled motor. The precision and rate measurement of these systems, with the ability to monitor cell reactions to applied loads, has gradually changed how we understand these cell-matrix forces.

It turns out that forces generate by cells on the collagen network increase gradually, up to a relatively constant level, over 2–10 hours, depending on the cell type (Figure 7.8a,b). This relatively constant matrix tension is normally maintained by the resident cells over many hours or days (as long as

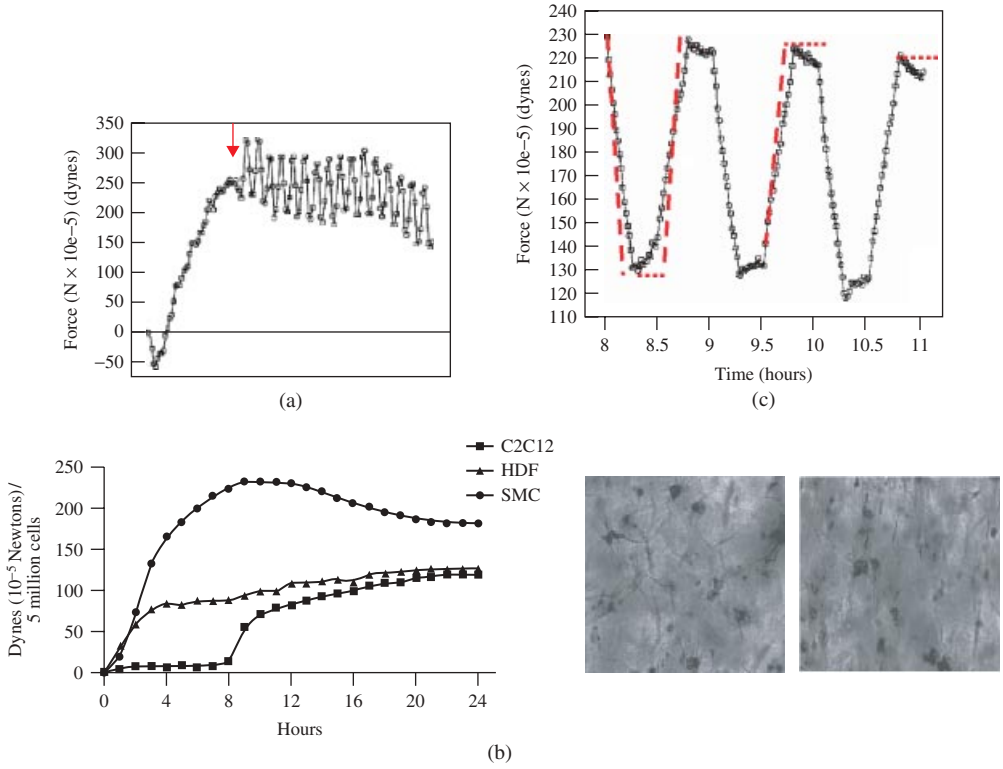


Figure 7.8 Tensional homeostasis graph. (a) Force generated by fibroblasts in collagen (24 hrs) rises to a maximum over ≈4 hours and remains relatively constant, maintaining at a characteristic 'equilibrium'. In this case, at the stage where cells began to maintain 'tensional homeostasis', a external cyclic load was applied via a motor (red arrow). Source: Brown, R.A., Pajapati, R. McGrouther, D.A., Yannas, I.V. & Eastwood, M. (1998). Tensional homeostasis in dermal fibroblasts: Mechanical Responses to mechanical loading in 3-dimensional substrates. *Journal of Cellular Physiology* **175**, 323–332. (b) Force-time responses (24 hrs) for three cell types: smooth muscle cells (greatest), dermal fibroblasts (same rate, lower max), myoblasts (delayed onset). Inset: myoblasts in multi-axial (left) and uniaxial loaded zones (right, aligned parallel to the load). (c) Detailed expansion of the four-hour loading cycle with the cell response to external load. Actual applied loads are shown by the dotted extension lines. Cells seem to work to maintain a constant endogenous tension, increasing and decreasing their loading in reaction to each part of the applied load cycle, so distorting the cell-free pattern.

is measured so far, or until another process intervenes).

Interestingly, one such 'other process' can be the application of an external load to the mini-tissue in the same plane. When this happens, there is almost immediately a mechanical reaction from the resident cells to reduce the external change. In other words, where a small additional load is added by the motor, there is a reduction in cell contraction until the baseline returns. Where the baseline tension is reduced by the system motor, there is a corresponding adjustment in endogenous contraction from the cells (Figure 7.8c).

This behaviour represents a tensional homeostasis at the cellular level. Cells are maintaining a constant tensile load against changes in external loads on their matrix. They seem to do this from inside the gel, by altering the cytoskeletal loads they generate in reaction to the changing external loads in an equal and opposite manner.

At first glance, this behaviour appears to be a futile reaction to the changes in the outside world/environment. As mentioned previously, the cell forces involved are absolutely *minute* (and cell numbers small), and this is particularly true in relation to the relatively huge external loads acting

on native skin and tendon tissues. However, tensional homeostasis behaviour is exactly what would be predicted from the concepts of mechanically reactive remodelling that we have been discussing. In other words, perhaps these fibroblasts are *not* involved in some heroic but futile tussle of the few and the weak against the irresistible. This small band of matrix material maintenance cells is not trying to correct short-term shape distortions of their parent tissue. Rather, this may represent the detectable signs of their constant vigil to maintain the material's properties and its 3D dimensions by ECM remodelling.

In fact, since these first observations, further evidence of mechanically reactive cell collagen remodelling has been reported. These cell responses seem to be part of the mysterious remodelling process we are so interested in – the same remodelling process by which tissues acquire and adapt their mechanical function (and 3D architecture) against the ravages of a changing external environment (including calorie-intake!). It is almost as if the fabric of our tissues were maintained by *a thousand blind but constant tailors*.

7.3.2 Mechanically reactive collagen remodelling – the 'constant tailor' theory

We may, then, have reached the point where it is possible to put some meaning and mechanism to a whole series of bio-observation and tissue behaviour anecdotes. It seems plausible, at the very least, to say that connective tissue cells constantly adapt their matrix stiffness by increasing or decreasing collagen deposition in response to changes in the basal matrix tension. If this is correct, it would resemble how a blind craftsman repairs or reshapes a piece of fabric, testing the strength by pulling across the materials, then adding stitches or patches until it can hold the tensions that are routinely applied. We can think of this as the '***constant tailor***' theory, helping to define the relationship between cells (e.g. fibroblasts) and the extracellular matrix which makes up the fabric material of our tissues. In this case, the tailors are our matrix-maintaining cells.

The idea here is that their maintenance programme is to constantly apply a small but steady tension on the fabric in which they live. Where the *x*, *y* or *z* planes of the material are stiff, the cell itself will be distorted. Where they are less stiff, the matrix will be pulled together and *it* will deform. Either way, the cell-tailor will receive an unmistakable message and presumably deposit or remove collagen matrix (a few stitches) in that location and plane.

The constant tailor theory presents us with an interesting consequence, as it makes it clear why the tailor-cell needs to maintain a tensional homeostasis. This is an essential component, in that it provides the constant baseline against which to measure material stiffness. In fact, it is far easier and much more accurate to monitor changes in dimensions or material properties where the material is under a constant, *known* load, rather than hanging loose.

This can be pictured in one of three forms: in one plane (1D) for a rope or chain, in two planes (2D) for a fabric and all thee planes (3D) for a bulk structure such as an office-block or a body-tissue (Figure 7.9). Without a background tension in the tethering rope between a kite and the flyer, information from the loose cords is ambiguous. Has the rope broken/stretched, is the kite being blown towards the flyer or is the kite falling? Indeed, could the flyer himself (i.e. the cell) be moving? If the kite is falling, for instance, a slack string provides absolutely zero information about direction or rate of fall. When the rope or fabric is tight we can quickly detect decreases or increases in background tension, but much of this information-stream is lost when the material or structure is loose.

There is a second (obvious) consequence of the constant tailor theory hinted at repeatedly throughout this section. This is that the *direction* or **plane** *matters*. As shown in Figure 7.9, this system can provide unambiguous information to the cell-tailor about which direction its material is more or less stiff. We must presume, at present, that this allows the cell to deposit or remove matrix material (e.g. collagen fibres) in a corresponding plane, to keep the tissue strongest in the direction it is loaded most.

Though there is still much to learn about the detail of this process, the importance of the main

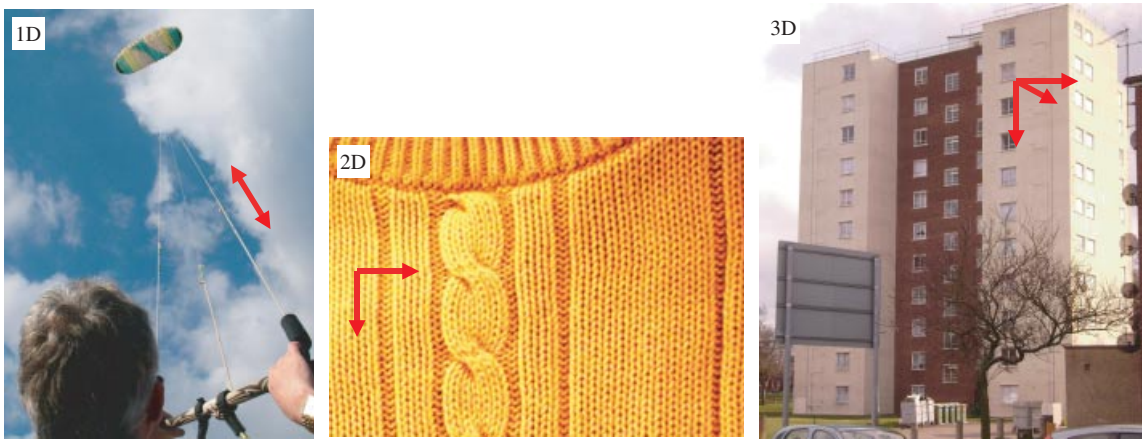


Figure 7.9 Illustrations of useful background tensions: (1D) in a kite or a boat-tethering rope; (2D) 2D planes in clothing fabric; (3D) a block of flats, where all three matter (arrows).

direction of loading, or primary force vector, cannot be overstated. The fact that many tissues are adapted to function under regular and repetitive load directions may be particularly important, as the constant tailor control process is particularly sensitive to changes in *force vector*. This leads on to the idea that the cells responsible for adaptive mechanical remodelling may respond most to local changes in force vector. This is the idea of 'OOPS', or 'Out Of Plane Stimulation', discussed in the appendix to this chapter.

7.4 Can we already generate tension-driven growth in *in vivo* tissue engineering?

It may be that we must endure some modest wobble of our tissue engineering self-esteem. To use a heroic image, we may have struggled to reach high into the foothills of the 'Sierra Tissue Engineering' only to find someone else's flag above us!

7.4.1 Mechanical loading of existing tissues

As we have seen earlier, plastic and reconstructive surgeons commonly use tissue expanders, inflated under patient's dermis, to 'grow' new skin, for grafting. This involves inserting a silicone bag under the skin and forcing in saline under pressure, with top-ups every few days over a period of weeks. The result is 'new' skin, which can be grafted elsewhere once the bag is deflated. In this case, it is clear that the tissue has grown under the applied load in dimensions and mass. Consider the potential for engineering of new tissues if the triggering process for such localized adult tissue growth could be understood *and applied* when and where we need it.

Orthopaedic surgeons have, for many years, been able to extend the long bones of patients with growth deficiencies by osteogenic distraction. This involves breaking the femur, tibia or humerus in question and allowing a soft fracture callus to form naturally. This is the normal bone tissue repair process, in which the callus eventually mineralizes, joining the opposing bone fracture ends. However, in osteogenic distraction, a strong frame is implanted around the fractured bone and this frame is expanded to pull against and stretch the fracture callus tissue. This stretching (i.e. the distraction) is carried out at a standardized rate, usually a few mm per day, in the axis of the bone. In effect, the soft fracture callus grows in length under (and in the plane of) this applied load.

The clever part here is that the rate of extension of the frame is designed to *just* match the rate at which the stretching fracture callus is mineralized and

turned to bone. As a result, distracted callus cannot fully mineralize as it would in a normal facture repair. However, neither can the soft callus get any longer. Rather, the new material becomes calcified as part of the patient's bone, which progressively elongates until the process is stopped and the frame removed.

A third surgical example, the stretched scar, is rather less a treatment than a form of damage limitation. In many skin incision wounds (i.e. surgical) it is necessary to suture the wound margins under a tension. Such tensions are normally an inevitable consequence of the patient's anatomy around or beneath the injury site. For example, tensions form across the abdominal wall, near to major arm or leg joints, or over the face under the effects of jaw movements. In such cases, the sutures carry most of these external loadings in order to *unload* the wound margins while new tissue and scar forms. Unfortunately, it is often necessary to remove these sutures at relatively early stages, which throws the load onto the new repair tissue in the same way that the distraction frame stretches the new fracture callus. The result is similar, producing growth of the scar tissue across and between the original wound margins, which produces a characteristic 'V' shaped stretched scar by lateral growth of new skin (scar).

7.5 Conclusions: what can we learn from engineered growth?

It is quite clear that these clinical examples can be regarded as forms of *in vivo* tissue engineering or, in some cases, *in vivo* bioreactors. It is even possible that some are already in use for questionable 'engineering' of skeletal geometry, for example to enhance sports performance such as finger action or jump height. Paradoxically, it is difficult to envisage how such processes, legitimate or nefarious, can progress far beyond their present empirical base without the sort of detailed understanding which is coming from new, *ex vivo* tissue engineering. However, in the best 'Looking-Glass' traditions, extreme tissue engineering can undoubtedly teach us much and throw up critical clues through careful analysis of its tissue-production success stories.

The big question now facing extreme tissue engineers in this field is how can we change the external loading demands on tissues (engineered or natural) to trick the resident cells into growing their extracellular matrix, when and where *we* need that growth?

Appendix to Chapter 7

Note: this appendix is designed to stimulate (where appropriate) an insecurity-driven desire to learn more about cell-matrix mechanics, rather than to answer all the questions you may have.

Forces on cells are a bit like bagpipes on people: position and direction are *critical*.

Those of you who have ever heard Scottish or Irish bagpipes being played live may appreciate this analogy very well. Heard in the open air, especially massed, or across distances over an open hillside, the bagpipes must produce one of the most haunting, enchanting and stirring sounds imaginable. However, experienced at close range in small rooms, especially with soft furnishings, they can represent a banned form of torture. So it must be when mechanical forces act on cells embedded within their 3D support material. How they affect cells

Text Box 7.6 Monolayer cell culture on plastic is not a control for mechanical 'stimulation'

Cells culture in monolayer on plastic is, in fact, a very special and extreme form of cell-mechanical loading because, when cells attach to plastic, they rapidly assemble a contractile (actin-myosin) cytoskeleton which generates very high intra- and inter-cellular loads on their trans-membrane attachment points. Since the plastic is so unnaturally stiff and completely symmetrical in its stiffness, in all three planes, it represents a cell environment which is both high-load and poorly, or potentially, non-physiological.

Text Box 7.7 Reflecting back on the value and vulnerability of the tribes of tissue engineering

As we saw in Chapter 1, the constituent tribes of tissue engineering are its unique and strongest feature, but they also bring special needs and vulnerabilities. One of these is the question of the shared, pseudo-shared and downright misinterpreted language we use. It is easier to see this effect where peoples of different cultures speak different languages, as in Figure 7.10. In this case, it is necessary to understand the French language to be struck by a cultural and philosophical difference concerning where and when it is appropriate to urinate in public. To other (more repressed?) cultures, it might seem surprisingly inappropriate *ever* to have to indicate where *public* urination is unacceptable (the assumption being 'nowhere, *ever*'). Having an industry which makes and sells the official signs is a further surprise!

The rich variety of approaches brought to tissue engineering by its tribes can be obscured by our *apparently* common scientific English. An example of this was experienced by the author. After talking for 15 minutes to an eminent, if patient, biological colleague about cell guidance and force directions, he finally tired and enquired how *gene* vectors could possibly come into the story . . .

Figure 7.10

depends on the materials they pass through and the direction they come from to reach those cells. To our colleagues from the physical science tribes of tissue engineering, this is completely obvious – knowledge of a force vector is at least as important as its magnitude or frequency. Within the biological and medical-related tribes, however, this is not so obvious and can be a real surprise (Text Boxes 7.6 and 7.7).

It is important to understand from the start that the nature of these '*load carrying demands*' is not at all simple and merits a detailed analysis. An idea common in the biological literature is that cells or tissues are either mechanically *stimulated* or not. But this does not come close to adequate. It leads to the disarming idea that if cells can be 'stimulated' by mechanical forces, we absolutely must have a 'control'. In familiar cell-molecular systems, a baseline or 'control' output comes from using a zero concentration of the compound in question. Unfortunately, this is not appropriate in the case of mechanical controls, mainly as it embarrassingly misunderstands the initial concept. *Mechanical load* is not a single, simple, dose-dependent form of 'stimulation' which can be switched on, or more particularly, *off*. Specifically, cells in monolayer culture on plastic are most definitely *not* a zero-force or baseline 'control' system, as sometimes thought.

In the first place, on this planet ($g = 1.0$), we can reliably assume that pretty well all tissues are constantly under some form of mechanical loading as a result of their mass. Thus, unless we are employing large amounts of expensive equipment or an experiment on the International Space Station, the very

idea of 'mechanically stimulated' (or unstimulated) is a mistake.

Interestingly, where cells respond to micro- or zero gravity experimentally, this may not be due to the direct effects of removing the cell *itself* from gravity. Given the tiny mass of a single cell, it may not be able to detect the effects of gravitational forces directly. However, pretty well all mammalian cells are part of, and firmly attached to, a great mass of other cells and extracellular matrix. Since such tissues have substantial mass, the resident cells will inevitably be deformed by the resultant compressive, shear and tensile forces. However, in isolated cell experimental terms, this is a very different *indirect* response.

Secondly, it is a dangerous simplification to look at the main form of loading on a tissue and conclude that such a tissue is simply compression-, tension- or shear-responsive. Although an artery wall is under strong shear loading at the blood/vessel interface, this will also generate directional (axial) tensions below the vessel surface, compression of the mid-layers, turning to circumferential tensions in the outer, elastic retaining wall layers. Similarly, compression on the articular cartilage of our joints (e.g. as we stand on our leg bones) generates tensile loads at both the cartilage margins and menisci, as well as shear at the articulating surfaces as the joints roll and slide. The reality is that combinations or *patterns* of loads act on different micro-sites and cell zones or layers, and that these patterns change during the period over which loads are applied (Text Box 7.8).

The detail of these patterns may be characteristic (potentially like a fingerprint), with one or two 'principal elements' dominating during any regularly performed movement. Different points in a tissue, then, are subjected to combinations of mechanical stress, in tension, compression or shear, with one of these predominating at any given site or time in the loading-relaxation cycle.

As we have seen, some of these loads can be described as 'static' or anatomical. These are tensions *built into* the physical dimensions of our stiff skeletons and less stiff but attached soft tissues. Other loads, commonly those generated by skeletal muscles, will have patterns which are complex and dynamic. These are generally very *repetitious* (e.g. cyclical) and *constant*, such as when we run. The cyclic repetition of this loading is again a consequence of the 'constant' anatomy around which the muscles act. This is because our anatomies (muscle position/bulk, joint locations and bone-length levers) tend to remain fairly constant (aside from injury and aging).

As a result, we can predict that cells within any of the main tissues will be subject to complex

[16] Actually, this still leaves us with a serious paradox – that the cells, in this case, also manufacture the ECM which they so constantly and diligently adapt.

Text Box 7.8 Paradox in the ranks of the cell-mechanic-biologists

As in previous chapters, we find so much to learn by tackling the big paradoxes head on. In cell mechanics (biology of cytomechanics) there have now been many, many works demonstrating that application of changing mechanical loads to cells in culture 'stimulates' them. The nature of the stimulation depends on what is being asked. The 'elephant in the room', though, is the question of 'how come all our bodies are not growing all the time, expanding with warts, tumours or just new mass in all sorts of directions?' If this were the case, then sports stars should be completely bizarre in physical form and hugely different between, say, swimmers and golfers.

Yet, when our cells are in our bodies (rather than naked on a culture dish), they become almost completely deaf to the vast majority of loads our tissues experience. The big difference is that cytomechanics is *not* the same as tissue mechanics. Cells in a physiological tissue are surrounded by a material (the extracellular matrix – ECM[16]), and this completely dominates how cells respond.

Exercise Box 7.2

Discuss the idea that 'zero mechanical loading is not really an option for physiological cell systems', including the use of zero gravity!

and highly dynamic **load patterns**. These are, in effect, localized 'load signatures' characterized by the main direction of loading (the principal force vectors) as well as their magnitude and frequency. Most importantly, however, these signatures will be remarkably constant for any given group of cells over considerable periods. This makes it possible for such cells to adapt the shape, composition and load-carrying characteristics of their extracellular matrix to resist each particular local load signature.

All we must propose, then to make such a system work, is that stromal cells are programmed to replace existing matrix with new materials of a type, density and orientation which minimizes their own (cytoskeletal) deformation during this routine (signature) pattern of loading. In proposing this, we are effectively describing a mechanism for what is generally assumed to be **mechano-dependent tissue remodelling**. In bone, this has been known for well over a century as Wolff's law.[17]

This suggests that connective tissue cells lay down an extracellular matrix which optimally stress-shields those same cells from the prevailing pattern of external loading. Any changes to that pattern of loading will tend to elicit a cellular response, which is to remodel that matrix material to again minimize the strain on local cells. In effect, it is a completely cyclic and interdependent relationship between connective tissue cells and the mechanical properties of the matrix material they lay down. The cells at the centre of this relationship (fibroblasts, chondrocytes, smooth muscle cells, etc.) are the 'constant tailors' of our earlier theory.

[17]Wolff's Law: the theory that healthy bone will adapt its composition and architecture to the loads it is placed under (Julius Wolff, surgeon, 1836–1902).

To conclude, if this analysis is correct, one potent way to influence the constant tailor's work-pattern will be to apply our new 'activating' or controlling forces along *vectors* which are outside the signature pattern. In other words, according to the 'OOPS' principle mentioned earlier (**O**ut **O**f **P**lane **S**timulation), loading tissues out of plane of the existing collagen fibre alignment is most likely to 'stimulate' cell adaptive mechano-remodelling.

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[Analysis of how mechanical forces, at the cell level, can and do affect tissue remodelling – including the 'oops' principle.]

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[Wrinkly (Shar Pei) dogs have 5 × normal levels of hyaluronan, with HAS2 gene over-expression: but why should a surplus of charged polysaccharide prevent Shar Pei fibroblasts maintaining a skin tension?]