Studies in Multidisciplinarity, Volume 3
Editors: Ray Paton† and Laura McNamara

25 Tissue engineering: the multidisciplinary epitome of hope and despair

David F. Williams

UK Centre for Tissue Engineering, Division of Clinical Engineering, The University of Liverpool, Liverpool, UK

1. TECHNOLOGICAL PROGRESS IN MEDICINE

During any of the years of the last decade, a contribution to the multidisciplinary approach to the theory of regenerative medicine, a subject that we shall see subsumes tissue engineering, could well have been written with immensely different perspectives, emphases, and prognoses depending on the precise time of writing. This may not sound too surprising, since many emerging sciences, including medical sciences, flow through troubled times, often with alarming perturbations, before they emerge with hypotheses which are intact or refined, and practical applications ensured. Neither the pharmaceutical and biotechnology industries experienced smooth passage towards billion-dollar revenues, nor did the theories of anatomical or functional imaging emerge unchanged on their way to clinical reality. The question arises as to whether regenerative medicine is any different. Will each of the depths of despair to which the subject has descended at times, prove to be a nadir out of which only success can emerge? Or is it a subject so fatally flawed by a misappropriation of medical principles and commercial hype that it can only serve to deceive and ultimately fail? At the time of writing, there is no clear answer to this, and the possibility of abject failure is there for all to see. This chapter attempts to provide different perspectives on this subject and place the well-founded hopes and despairs of clinical and commercial reality on a balanced scientific foundation.

The practice of medicine is built on progress, and often that is technological progress. It is rare for technology not to be synonymous with progress, and even rarer for technological progress to go into reverse,

although the recent cessation of supersonic civil air travel shows that this is not impossible. This chapter explores the reasons why regenerative medicine, and tissue engineering in particular, finds itself in this position at this moment.

2. THE RECONSTRUCTION OF THE HUMAN BODY

Let us first discuss the nature of regenerative medicine and tissue engineering, two terms that were not in our dictionaries twenty years ago. The human body is susceptible to a wide variety of diseases and traumatic events, hence the very existence of medicine. Diseases are conventionally addressed through a small number of medical paradigms, including the pharmaceutical approach, either palliative or curative; the destructive approach, for example radiological or surgical; the nursing palliative approach, and the psychotherapeutical approach. Until very recently the concept of treating disease through a regenerative paradigm was not really considered either sensible or feasible, since it is counter-intuitive, and essentially counter-Darwinian. The natural lifespan of the human, edging up from the biblical three score years and ten to the late seventies and early eighties for males and females, respectively, in western civilisations at the end of the twentieth century, and extending upto 100 years in favoured places, we are led to believe, is predicated on apoptosis and senescence; in other words, our bodies are designed and indeed programmed to get weaker and fade away. If it were not the case that humans died of old age through these phenomena – in the event that either disease or trauma did not get the better of them – the scenario of a world increasingly populated by ill-tempered nonagenarians and upwards would be very disconcerting. The concept of a medical technology that allowed us to obviate natural apoptosis through the simple ability to regenerate any tissue or organ that got into trouble through the interference of micro-organisms or physical trauma does not make much sense, either philosophically, psychologically, economically, or emotionally.

This statement is based on the unwarranted, but entirely logical assumption that whilst it is conceivable that it may become a practical proposition to effect the regeneration of a specific tissue or organ that has become diseased (for theoretical exemplar purposes, the regeneration of the kidney in renal failure, the bladder in urinary tract dysfunction, the myocardium in heart failure, and even the retina in macular degeneration), it will be impossible to simultaneously address all the features of multiple organ failure that usually brings an end to life in the above nineties, or even to equally address the multiple and independent aetiologies of complex

organ failure that lead to degeneration of the nervous system (e.g. Alzheimer's disease) or of the musculoskeletal system (e.g. multiple sclerosis, osteoporosis, etc.)

At this point we should address the different technologies for reconstruction of the human body and the profound conceptual differences between some of them. Until recently, there have been three major procedures for reconstructing or replacing tissues or organs (or their functions) that have become diseased or damaged. The first is the physical replacement of the affected tissue by an implantable device. This involves either the excision of the tissue and replacing it with a synthetic substitute, or the augmentation of the tissue without physical replacement. Although, as explained later, some of these devices give very good performance, by definition they are limited in the functions they can replace since synthetic materials can only be expected to have physical or mechanical functionality, and generally cannot achieve active biological functionality. We, therefore, see the major successes with implantable devices in sectors, such as total joint replacement (with purely stress transfer capability), prosthetic heart valves (mechanical control of fluid flow), intraocular lenses (light transmission), breast implants (simply filling space and generating volume), and cochlear implants (sound conduction), with not an active biological function in sight.

Equally importantly, there is a limit to the length of time that these devices can perform within the human body. The tissues of the body are not only aggressive to foreign materials but also very sensitive to their presence. This is an immensely important point as far as implantable medical devices are concerned since it represents the absolute limit of their performance, and perversely it is to the patient's advantage that this is so. It is necessary here to recall that evolution has provided the human body with a very sophisticated immune system that has memory and aggressiveness. This system has been designed teleologically to respond to the invasion by foreign objects through a cascade process of significant power. Once the body recognises a foreign object, through a variety of processes, it mounts an aggressive humoral and cellular response designed to trap and destroy the object. Without this process, humans could not survive. It was, however, designed for the specific threat from natural invading objects, and in particular micro-organisms such as bacteria, using an antibody-based defence against the antigens of these predator organisms. But we do encounter problems with long-term implantable devices and the materials of their construction, which may, one might say ''inadvertently'' trigger one of the incipient defence mechanisms and suffer from one or more destructive processes. Bearing in mind that these defensive agents, which operate within a powerful oxygenated electrolyte,

include a myriad of enzymes, lipids, free radicals, and other oxidative species, it is not surprising that virtually no materials come through the implantation experience unscathed (Williams, 2003).

The story does not stop there, however, and it is necessary to consider the consequences of *in vivo* degradation of these biomaterials. The immune response referred earlier includes elements of a cellular reaction to invading agents. This involves cells of the inflammatory system, including phagocytic cells such as macrophages and giant cells, and highly specific cells of the immune system such as lymphocytes. These respond to both physical and chemical signals such that when a biomaterial suffers a degradation process mediated by the biological environment, this process itself stimulates the cells in that environment to become even more aggressive, establishing an autocatalytic process that inevitably results in accelerated destruction of both the material and tissue. This is the essential reason why biomaterials and implantable medical devices have to have a finite *in vivo* lifetime.

The second process for resolving organ or tissue failure, which we shall deal with only briefly, is that of extracorporeal devices. These involve procedures where body fluids, specifically blood, are taken out of the body on a transient basis, where they are subjected to one of a series of processes that the organs of the body are no longer able to supply. The best example is the ''artificial kidney'', in which, during end-stage renal failure, blood is regularly removed from the body and passed through an extracorporeal device in order to remove toxic metabolites. Other examples address deficiencies of heart, liver, and lungs, usually on a temporary acute basis. These devices may provide functionality in order to save lives but are no substitute for long-term effective replacement of organ function (Iwata and Ueda, 2004).

Since, whether by *in vivo* or *ex vivo* performance, classical engineering solutions have very limited functionality when it comes to replacing physiological processes, we have to consider the only logical alternative and this is through the replacement of organs with organs and tissues with tissues. The third process for reconstructing the body is, therefore, by organ or tissue transplantation. With whole organs, this inevitably involves the use of donor organs derived from another human, usually recently deceased or occasionally donated by a live relative. Under certain conditions, this could also involve grafting from one part of the body to another, the significance of which is seen later. Several major scientific hurdles have had to be overcome with organ transplantation, primarily associated with the immune system, which is exquisitely designed to reject any such large mass of tissue derived from anyone other than a very closely matched donor. It has, of course, proved possible, through major developments in the technology of microsurgery, intensive care medicine and anaesthesia, and

immunosuppression, to overcome the many challenges of organ transplantation, but the ever-present issues of donor supply and cost, together with the uncertain cultural and ethical difficulties in many countries, limit the extent to which this component of medical technology can assist in the global provision for the reconstruction of the human body (Goudarzi and Bonvino, 2003). This leads very conveniently into the new modality of tissue regeneration.

3. THE NATURE OF REGENERATIVE MEDICINE AND TISSUE ENGINEERING

Regenerative medicine involves any therapy that aims to induce the regeneration of tissues or organs following disease or injury, or in the presence of birth or developmental deformities. It may be achieved through cell therapy or tissue engineering, either of which may be assisted by concurrent gene transfer or pharmaceutical intervention, or by gene therapy alone. Gene therapy itself involves the insertion of specific forms of DNA into the cells of a host in order to correct a genetic error or alter a particular host characteristic. Cell therapy involves the administration of a group of cells to a patient to replace or augment a deficient cell population; for example, implanting a volume of dopamine producing cells derived from an embryo into a Parkinson's patient. Tissue engineering is a little different in detail but has the same objective.

The real problem with the treatment of any degenerative disease in humans, or indeed in any higher mammal, is that we have largely lost the inherent ability to regenerate tissues once they have been destroyed or damaged. Lower organisms still have this ability, but higher mammals have sacrificed this capability whilst concentrating on improving mental functionality. Humans normally deal with injury by the generation of non-functional, non-specific scar tissue, which is usually fibrous or fibrocartilagenous in nature. Thus, if we damage muscle by a major incision, the wound can be closed, but largely through the generation of fibrous scar tissue rather than the regeneration of functional muscle. More importantly, if we damage nerve tissue by a major incision or just mechanical damage, it may be eventually healed, but again by scar rather than functional nerve tissue; and since fibrous scar does not conduct nerve impulses, this is a rather useless process and leads, at the minimum to loss of sensation, or parasthesia, to, at the other extreme, paraplegia or quadriplegia. In between these extremes we have the widespread "irreversible" conditions that affect the nervous system such as the neurogenic bladder, leading to incontinence; the damaged intervertebral disc, leading to chronic back pain

and worse; and age-related conditions of the optic nerve leading to blindness, all of which demonstrate the importance of developing strategies that can, at least temporarily, reverse this evolutionary process and restore some ability to regenerate functional tissues in adult humans.

We have some clues to show how this might be achieved through few examples where some degree of regenerative capability has been retained by tissues in higher mammals. It is interesting to note that evolution has been rather precise here, since this involves two tissues that are easily compromised through daily activity and where an inability to heal functionally would certainly compromise both the quality and quantity of life in the vast majority of people.

These two tissues are skin and bone. If the skin is breached, a common, indeed possibly daily occurrence in active infants, eager sportsmen, and careless adults, it is absolutely necessary that it is repaired quickly and with good functionality in order to restore the barrier properties and thus keep out the ubiquitous bacteria, otherwise we would be destroyed by infection very easily. Thus skin has an effective, although limited, ability for regeneration; in other words, damaged skin can be replaced by new skin (Yannas, 1998). It is important to note here that this ability is indeed limited, and does not extend to major skin injuries, such as extensive burns or those which are caused by deficiencies of the underlying blood supply, as with diabetic foot ulcers or pressure bed sores. It will be dealt with in detail later.

In the case of bone, a fracture, although not quite so common, also frequently affects the same population at risk. If bone fractures did not heal, then mobility would be significantly compromised, again a debilitating condition which would have been fatal in the early days of homo sapiens' evolution. Bones actually heal better than skin provided that the fragments are kept in close proximity, hence the auxiliary use of plaster casts and, if really necessary, internal fracture plates, wires, and screws (Davies, 2000). This is necessary since bone is one of the two tissues whose mechanical characteristics are essential for mobility and, being a mineralised tissue, its repair with scar or poorly mineralised tissue would not be effective. Interestingly, the other major mineralised tissue is found in the teeth, and evolution determined that it was not worth retaining a comparable regenerative function in the dentition, at least in the human.

Virtually all the other tissues of the body have far less capacity to regenerate in a comprehensive manner and damage is repaired, if at all, by non-specialised scar tissue as noted above. The processes of tissue engineering have the simple objective of stimulating the body to produce new functional, specialised tissue on demand when the treatment of disease or trauma requires it. This of course is not a trivial point, and the hopes

and despair indicated in the title of this chapter represent the enormous potential that tissue engineering has to offer and the extreme difficulty that is being experienced in translating this potential into clinical and commercial reality.

It should be noted in passing that the term ''tissue engineering'' does cause some confusion since it appears to suggest that new tissue is ''manufactured'' under classical engineering conditions, rather like a hip replacement or mechanical heart valve is produced in a factory out of synthetic materials. Although there may be some mass production of tissue-engineered components such that this proposition is not so implausible, this is not the reason why the term ''engineering'' is used. Instead, engineering is used with its original meaning associated with creation. Just as the engineer, as an artisan, creates objects, so tissue engineering creates new tissue. My conceptual definition of tissue engineering is ''the persuasion of the body to heal itself through the delivery to the appropriate site of cells, molecules and /or supporting structures'' (Willams, 1999). We shall see later just how these components interact and how this form of engineering compares to classical engineering within medical technology. For now, let us take a few examples of current (i.e. the beginning of 2005) thinking on this broad subject of the reconstruction of the human body to examine the extent of the dilemma facing us, with the choice between, on the one hand, replacement with medical devices and, on the other hand, regenerative medicine with tissue engineering products and processes.

4. THE HEART: CURRENT REPLACEMENT THERAPIES

The heart is literally and technologically the best place to start with, and it is an organ that is quintessentially multi- and inter-disciplinary. It is an organ that is remarkable both in its simplicity and functionality. It is a muscular pump that controls the flow of blood through the circulatory systems. The scientific bases for this action have their origins both in electrophysiology and fluid mechanics and the durability is associated with a combination of fatigue-resistant myocardium and a non-thrombogenic endothelial lining. Interdisciplinarity exudes from cardiology. We have, however, several problems to face in the long-term management of cardiac function, relating to the immediacy of death should something catastrophic happen to the heart (which tends not to happen with other organs and tissues), or to the slow prolonged and expensive death when we abuse our bodies and place too much strain on this organ (which does happen with other organs, such as the liver, lungs, and kidneys). We must also consider the socio-economic and ethical issues arising when non-life-threatening

parts of the body give up but the heart refuses to do so, with neurodegenerative diseases, such as Alzheimer's and Parkinson's coming to mind.

4.1. Arrhythmias

Medical technology has little to offer in the case of the catastrophic entry of a bullet or knife into the heart and so we will concentrate on the chronic conditions. Several diseases and conditions can affect the functionality of the heart and over the past 50 years some extremely successful artefacts of technology have saved millions of lives. Disturbances to the electrical conduction system, for example, give rise to a series of arrhythmias, such as tachycardia or brachycardia, and rhythm management through implantable cardiac pacemakers and defibrillators is an essential front-line approach to the treatment of these conditions (Bourke and Healey, 2002). The provision of a hermetically sealed unit of power supply, sensing system, and pulse generator, that can be programmed externally, which is placed subcutaneously in the shoulder region, and communicates with an electrode placed within the heart, is very sophisticated and can give more than ten years of uninterrupted and unnoticed service to the patient. This remarkably improves the quality of life but does not necessarily extend quantity of life. Moreover, in health economic terms this is very cost effective. Although the capital costs of the device are not trivial, the recurrent savings of a maintenance-free therapy coupled with a return-to-work outcome are significant. Whilst it is easy to see that the provision of pacemakers is highly beneficial to all concerned when judged by any medical, social, or economic parameter, a fascinating ethical and technological issue arises when a patient fitted with a pacemaker dies from an unrelated cause within a few years. It is quite common practice to explant the pacemaker after death and, after re-sterilisation, re-use it in another patient. As with many issues in medical ethics, there is no right or wrong solution to the dilemma posed by this possibility.

4.2. Heart valves

Let us turn now to the valves of the heart. These four valves, all different from each other, perform with tremendous efficiency in controlling blood flow between the chambers of the heart and the major vessels of the circulatory system. They are, however, subject to disease, resulting in stenosis or incompetence, both of which result in lower effective cardiac output and higher energy losses, which are serious issues reducing quality of life. It has been possible in the past forty years to replace diseased

valves with synthetic structures or some form of tissue valve (Moffat-Bruce and Jamieson, 2004). A typical mechanical prosthetic heart valve has a series of leaflets that are retained within a ring through a hinge mechanism, this ring being sewn into the heart muscle in place of the defective valve. The movement of these leaflets controls the flow of blood in diastole and systole. Current designs give very good haemodynamic performance that results in excellent clinical functionality in most patients, this being a widely used, cost-effective treatment (Milano et al., 2001). There is just one problem; the valve replacement itself is inherently thrombogenic, the combination of altered fluid mechanics and intrinsically foreign materials providing a tendency for blood to clot on the valve, a process that could cause the valve to malfunction or result in the release of a potentially fatal embolus. It means that all such patients are at serious risk of death from this consequence, the principal risk management tool being a daily dose of systemic anticoagulant. There is no way to deal with this problem and the added complications of compliance with this regime and achieving the optimal level of anticoagulation that provides protection against a thromboembolic event without undue risk of spontaneous bleeding, turn an exceptionally good mechanical solution to disease into a high-risk therapy.

It should be said here that the mechanical valves are normally very robust and do give good reliability from most classical engineering perspectives. This is just as well, for if a valve does suffer structural failure, the results are often fatal. Bearing in mind the fact that a valve operates at a frequency of around 1 Hz, the fatigue performance becomes quite critical, at 40 million cycles per year. Considering even further that environmental factors will influence the material behaviour, i.e. the performance becomes one of not just fatigue but of corrosion fatigue, or more precisely biological corrosion fatigue, the interdisciplinary nature of this subject becomes even more obvious. There have been some significant examples of heart valves that have had a higher than acceptable rate of mechanical failure. In one situation, some 80,000 valves had been implanted in patients before this became known, resulting in one of the most serious dilemmas in the management of risk (Actis Dato et al., 1999). The solution to the resulting problem required the input of statisticians, ethicists, politicians, regulators, and lawyers as well as a variety of engineers, scientists, and clinicians, who collectively had to weigh up the risks of re-operation (with a moderately high statistical chance of mortality or significant morbidity) and compare that to the risk of valve failure, which depended on a plethora of manufacturing, personal, and clinical factors. We shall bear this dilemma in mind when considering the risks of tissue engineering later.

The main alternative to the purely mechanical heart valve is the bioprosthetic valve (Vesely, 2003). These are manufactured from animal tissues

and can be either derived from an animal heart valve with appropriate chemical treatment or fabricated from some suitable tissue into the shape of the required valve. Cow was the best source for the latter alternative, but it has become less popular after the bovine spongiform encephalopathy crisis, and most procedures have involved porcine-derived valves. These are treated in some way in order to remove infectivity and antigenicity (Zilla et al., 2004). For many years both these objectives were managed through the use of a glutaraldehyde fixation process. The results can be very successful but the valves do eventually denature and/or calcify. The susceptibility to these biological processes varies with the individual. The paediatric case is particularly difficult since valves tend to calcify much faster in young people, and of course, a non-living valve does not grow with the child.

There are a few other alternatives which we shall not deal with here but the main choices are obvious. Manufactured replacements for heart valves give good performance in many cases, but there continue to be risks, higher in some patients than others. The profound question for tissue engineering is whether there is any possibility that a tissue-engineered heart valve could do any better. As the title says, is this an avenue of hope or despair?

4.3. Coronary artery disease

A somewhat different story can be told with the coronary arteries. It is widely recognised that coronary artery disease is one of the major life-threatening diseases in developed countries, being caused by narrowing down of the lumen of these small vessels as atherosclerotic plaque forms on and within the endothelium. Although we can replace or bypass major blood vessels such as the aorta and femoral artery with synthetic structures, it is far more difficult to do so with small diameter vessels, and the 3–4 mm internal diameter of the coronary arteries has so far defeated the biomaterials scientist. The traditional surgical route to the alleviation of this condition has been the bypass graft (coronary artery bypass grafting, CABG for short, wonderfully spoken of as ''cabbage'') in which a natural blood vessel, such as the saphenous vein or the internal mammary artery, is transposed from one part of the body to the heart. This is classical heroic surgery, the chest opened up by cutting through the ribs in order to gain access to the heart, stopping the heart beat by means of cardioplegic arrest, re-directing the blood to an external oxygenator in cardiopulmonary bypass, harvesting the saphenous vein by means of an incision from groin to ankle, cutting up this vein into small pieces and,

with exquisite skill, re-implanting these little pieces into the heart of the patient. The advances in medical technology in recent years have been directed towards less invasive and less damaging procedures, a feature that has become more important as patients requiring CABG are now older and have an increasingly higher incidence of co-morbid illnesses and greater seriousness of the coronary artery disease (Niccoli et al., 2001).

Two aspects of these technological developments may be mentioned. The first concerns the trend to carry out coronary bypass grafting without stopping the heart, with the so-called off-pump or OPCAB, also referred to as beating heart surgery. The main advantage here is the reduction in the morbidity associated with cardiopulmonary bypass, especially the systemic inflammatory response that arises from the contact between the blood and the surfaces of the bypass machine, and the reduced risk of the release of emboli, particularly debris from platelets, fibrin, fat, and red cells, which become trapped in the capillaries of organs such as the kidneys and the brain. All these sequellae that are consequent on the interactions between blood and foreign materials and devices form part of the broad subject of biocompatibility, a highly multidisciplinary component of biomaterials science that ultimately determines the performance of these devices. It will be dealt with below. As discussed by Murphy et al. (2004) several clinical trials are showing that OPCAB provides better short-term outcomes for patients than the conventional surgery, although the evidence for better long-term results is not yet overwhelming.

The second development that has affected cardiac surgery in recent years has been that of angioplasty and stenting. Many of the CABG procedures are carried out in situations of advanced coronary artery disease where there is close to complete blockage of the vessel. In many situations, and especially where the presence of atherosclerotic plaque can be diagnosed at an earlier stage, an alternative therapeutic process may be used, in which the plaque is removed, ablated, or compressed such that the lumen of the affected vessel is widened, rather than the vessel being replaced. This is the technique of angioplasty, in which typically the affected area is treated with an expandable balloon that is deployed from a catheter fed into the vessel from an inter-arterial approach following a minimally invasive insertion into the femoral artery in the groin. This can be highly effective in opening up the vessel, and it should be noted that the discipline which delivers this therapy is now radiology and not surgery (Arjomand et al., 2003).

The main problem with this technique is that the result is not permanent and in many patients, the endothelium reacts to the physical insult of the angioplasty procedure by slowly thickening and re-blocking the vessels,

with clinically serious restenosis occurring perhaps one year later. The solution to this problem has involved a small mechanical device known as a stent, which is an expandable tube that is left behind in the vessel at the site of the original lesion (Dundar et al., 2004). This physically holds the vessel open after the angioplasty, and is normally delivered to the site through the same balloon catheter. This provides a very interesting scenario in medical technology, since the technique of angioplasty is very effective in improving both the quality and quantity of life, but in delivering this benefit it actually predisposes the tissues to worse damage a little later, such that a mechanical device has to be used to constrain the endothelial response to the trauma. Not surprisingly, the endothelium does not really take too well to this insult either, and many stents become encased with further hyperplastic tissue, so-called in-stent restenosis, within a year or so The solution to this problem has been to try to minimise this hyperplastic response and proliferation of the endothelium, either through the technique of brachytherapy which involves the localised delivery of radiation, or more usually through the localised release of an anti-proliferative drug from a coating on the stent (Van der Hoeven et al., 2005). This latter technique looks very promising although it is again following this paradigm of trying to cover up the damage of the stent by an even more powerful compensatory mechanism and the eventual response of the endothelium when either all the drug has gone or its long-term effects negated, is not known. This story of the intravascular stent epitomises the dilemma often seen with invasive medical technology and provides a driving force for the development of tissue engineering.

4.4. Heart failure

Having dealt with the three major structural parts of the heart that can go wrong, we finally turn our attention to the whole organ and the possibility of heart failure itself (Hellerman et al., 2002). This is associated with the incapacity of the heart to pump the blood, and may itself follow on from a heart attack in which a significant part of the myocardium is damaged, apparently irreversibly, following an interruption to the supply of oxygenated blood from one or more of the coronary arteries (a myocardial infarction). For patients with the symptoms of congestive heart failure, there are some pharmaceutical options but death has been the usual outcome unless the patient was fortunate to be a transplant recipient. Medical technology has, in the last thirty or more years, seen attempts to replace the heart with a mechanical pumping device, the artificial heart programme being one of the more expensive and,

until recently, least successful bioengineering endeavours (Jauhar, 2004). Mechanical replacements for heart function have been highly damaging to the blood and have only been considered as temporary devices to allow a patient to remain alive until a transplant become available, the so-called bridge-to-transplant. Recently there have been some significant developments which are worth describing briefly. It has always been accepted that it may not be necessary to replace the heart's pumping function fully since it is usually the left ventricle that has the highest workload and is thus in need of support. Thus, the LVAD, the left ventricular assist device, has received as much attention as the total artificial heart and some highly successful developments in the engineering of some of these devices has now led to some unexpected clinical outcomes. A major clinical trial has shown that patients fitted with an assist device faired better than patients maintained on a conventional drug regime for up to two years (Dembitsky et al., 2004). Moreover, there is evidence that the hitherto considered irreversible nature of the myocardial damage can in fact be reversible with some recovery of the tissue and restoration of function during the period when the left ventricle is being assisted by the device (Dutka and Camici, 2003). It should be borne in mind that this is not an inexpensive treatment, with devices costing $70,000 and the hospital costs in the region of $200,000, one small fact that presages the debate that is central to the future of tissue engineering alongside medical technology.

4.5. The heart: the potential for regenerative medicine technologies

It has to be said that, in parallel with pharmaceutical approaches, medical device technologies (Zilla et al., 2004), have provided very powerful methods to alleviate the debilitating conditions associated with either the congenital malformation or deterioration of the heart and its constituent components. As good as they are, however, all have limitations in that they are not providing the optimal long-term solutions, for there is almost always a price to pay for the intervention. There are some classical studies of actuarial survival data for patients who have been the recipients of medical device technology which demonstrate that they do not compare favourably with the general population. This may appear self-evident since the recipient of a medical device does not correspond to an average person, but the analysis is worth pursuing. It is easy to determine the survival statistics for a person aged 60 who has no obvious medical condition. If you take a cohort that have untreated valvular disease at this age and plot their survival statistics, it will not be surprising

to see that the curve towards expiry dips rather sharply. The curve for an individual who is diagnosed with valve disease at the age of 60 and who is implanted immediately with a prosthetic heart valve is somewhere in between. In other words, the patient is better off but is not, statistically or functionally, in as good a state of health as the person who does not have heart valve disease.

If there is, therefore, an inherent deficiency in the medical technology approach to the conditions of the heart, the question arises as to whether there is any other concept or technology that could do any better. The tissue engineering solution is to replace like with like, tissue with tissue, rather than tissue with synthetic device. Thus we can see the logic of replacing a diseased valve with one that is regenerated by the patient, of replacing a coronary artery with a new artery grown by the patient, of repopulating the myocardium with new patient-derived functional cells, and of even persuading the patient to grow a new heart. Having stated earlier that we do not innately have this ability to grow new tissues, we have to determine under what conditions we can be persuaded to do so. But first, we should briefly examine what other tissues and organs can be considered as targets for this type of therapy.

5. THE TARGETS FOR TISSUE ENGINEERING

Having used the heart as an example of the potential for tissue engineering, it is worth considering the extent of the conditions that could be addressed by this approach. We shall deal with this briefly under the headings of the skin, the musculoskeletal system, the nervous system, the sensory organs, and the main organs involved in metabolic functions, although it should be borne in mind that this is not an exhaustive list.

5.1. The skin

There are two main conditions that involve the skin where there are significant unmet clinical needs and where tissue engineering is already having some impact. The first concerns burn wounds and the second is associated with chronic ulceration.

Burns are classified by degree and severity. A third degree, or full-thickness, burn destroys the entire depth of the skin, including the epidermis, dermis, and some subcutaneous tissue, and is normally treated by a skin graft, using an autograft derived from the patient, skin from a human cadaver or, rarely, a porcine xenograft. When a third-degree burn covers more than 20% of the body surface area, there are serious

problems with the otherwise preferred autograft and although cadaveric skin is effective, it usually results in a poor cosmetic outcome, largely associated with the extensive contraction that takes place. There is considerable scope for a tissue engineering solution to those patients with extensive third-degree burn (Kopp et al., 2004).

One of the most important types of chronic ulcer is the diabetic foot ulcer. These ulcers have a multifactorial aetiology, with peripheral arterial occlusive disease, very common in diabetics, being amongst the most significant. Many diabetics eventually develop foot ulcers, and 25% of all admissions to hospital of diabetics are related to this condition. The wounds become the principal portal of entry for infection and the poor vascularity means that they are very difficult to heal. Treatment is largely confined to debridement and local hygiene, but many eventually lead to amputation. The treatment of the diabetic foot ulcer has been one of the major early targets of skin tissue engineering, where clearly the intention should be to regenerate not only the skin but also the underlying vascular tissue (Mansbridge et al., 1999).

5.2. The musculoskeletal system

It will be recalled that, along with skin, the other major tissue that has retained some regenerative function is bone, and indeed bone that has been traumatised quite readily repairs itself through the regeneration of new bone. There are, however, many conditions in which the generation of new bone would be very beneficial but where this does not occur. Even more importantly, the other major tissues of the musculoskeletal system, including cartilage, tendon, ligament, and muscle, all have very limited capacity for regeneration.

In some of these situations, the current standard of care treatment involves replacement of the affected tissues by implantable medical devices and it is important in the overall consideration of the role of tissue engineering to determine the relative cost – benefit factors. For example, osteoarthritis is one of the major diseases affecting the joints within this musculoskeletal system. In the past forty years we have had total joint replacement prostheses available for the replacement of the arthritic hip, knee, ankle, shoulder, and elbow and, they are very successful. The data now shows that we can expect an overall 90% success rate in 10 years for both hips and knees, which is quite remarkable (National Institute of Clinical Excellence, 2000). This fact alone presents a very interesting dilemma for tissue engineering. On the one hand, we can see that this benchmark will be very difficult to match with any equivalent

tissue-engineered product; indeed it is quite difficult to imagine, with current thinking about tissue engineering processes, a system that allows us to regenerate a whole hip joint, involving the highly complex shapes of bone and cartilage, both of which display considerable heterogeneity and anisotropy, and which can be incorporated into the patient's skeleton. Should we therefore even try to go down this route? On the other hand, 90% success in 10 years is not perfect, and in the vast majority of patients, the joint will have to have deteriorated very considerably, with major deformity, lack of mobility, and pain before the arthroplasty is performed. The answer to this dilemma is far from clear, but some strategy for early, and minimal, intervention by tissue engineering approaches, possibly to be repeated at later stages, is an attractive option to some.

At this stage there has been some success with cartilage tissue engineering, but it is mostly in the area of trauma that it is being applied. Conceptually it is far easier to apply a regenerative therapy to small lesions on cartilage surfaces, for example arising in the knee following sports injuries, than to whole diseased joints. There are alternate clinical techniques that address cartilage injuries and it remains to be seen whether tissue engineering can provide any significant improvement. One factor is potentially very important here, and that is the question of time. Cartilage injuries, especially in the knee, are often sustained in sportsmen, and it is the time to recovery which usually determines the preferred option as far as treatment is concerned. The significance of this will be seen below.

A few other areas deserve passing comment. In the spine, the intervertebral disc is a source of weakness in many people and the treatment of a herniated disc is neither easy nor always successful. There are in theory two ways to solve a disc problem, one being to repair, regenerate, or replace the disc, the second being to obviate the problem by fusing together the two vertebrae either side of the affected disc. The disc itself is a rather complex structure consisting of a centrally located viscous gel (the nucleus pulposus) that is surrounded by a collagen annulus (the annulus pulposus) and it has been impossible so far to produce a satisfactory synthetic alternative (De Kleuver et al., 2003). There is, therefore, a major need for some regenerative technique to be able to restore a functioning intervertebral disc. The spinal fusion may involve mechanical components, such as screws and cages, which assist in holding vertebrae together whilst fusion takes place, assisted by bone grafts, usually autograft bone, but also possibly banked allograft bone. Again, it is easy to see why there is much interest in a tissue engineering solution to this problem (Gruber et al., 2004).

Finally, we have to consider the issues associated with reconstruction of parts of the body following resection of cancer. This is particularly

relevant to the head and neck where serious functional and cosmetic deficiencies arise after major resection, for example of either jaw. Treatment is often difficult because of the compromised healing associated with the radiotherapy that is given to patients and also due to the need to recreate specific geometrical forms of the face. Much success has been achieved recently with computer-assisted design and manufacturing of facial reconstruction devices (Hassfeld and Muhling, 2001), often using MRI-or CAT-acquired data, in highly multidisciplinary team efforts, but this does not necessarily get around some of the major biological factors that control the incorporation of constructs into the delicate features of the face, and once again we find that the tissue engineering solution of regenerating the complex structure and architecture of this part of the anatomy is very appealing.

5.3. The nervous system

It has also been noted earlier that nerve tissue has considerable difficulty in regeneration. There are several different aspects to consider, starting with injuries to peripheral nerves. Peripheral nerve injuries are very common and result in significant disabilities in hundreds of thousands of individuals per year (Belkas et al., 2004). When a nerve is cut, and with only a small gap opening up between severed ends, the axons distal to the injury degenerate and are destroyed, whilst Schwann cells proliferate within the endoneurial tubes. Nerve fibres proximal to the injury, sprout new axons which progress in a distal fashion. Through a variety of signalling methods, some of these axons extend and can reach the distal tubes, although there will be considerable mismatching of axons to tubes, and much reduced function results. For larger gaps, where spontaneous axonal growth is not possible, nerve autografting has been the gold standard method of treatment for gaps greater than 5 mm (Mackinnon and Nakao, 1997). These work because they contain Schwann cells and endoneurial tubes which provide the neurotrophic factors and adhesion molecules for the regeneration of axons. However, they have many defects, especially donor site morbidity and sub-optimal regeneration. One of the more important developments in this area has involved the use of nerve guides which are sutured between proximal and distal nerve stumps, these being made out of either inert synthetic materials, such as silicones, degradable synthetic polymers, natural biopolymers, such as laminin or collagen or, fabricated from blood vessels (Tina Yu and Shoichet, 2005). Again, a great deal more can be envisaged in this situation if either more sophisticated signals could be supplied to the

regenerating axons, or if additional cellular populations could be used to enhance the regeneration.

The spinal cord represents an even more difficult case. Injury to the cord usually involves the impingement of bone fragments on the nerve. This may not initially look severe, at least histologically, but progressive necrosis occurs leading to paralysis in many cases, depending on the kinetic energy transferred to the cord during the injury. There have been some attempts to attenuate these secondary effects through pharmacological interventions (e.g. with methylprednisolone) in so-called neuroprotection, but so far these appear to offer little benefit but at high risk. The unfortunate fact is that the process is effectively irreversible and axonal regeneration in the spine does not take place. It might seem as if this was an area that would defeat any tissue engineering/regenerative medicine strategy. However, it does seem that mature neurons in the spinal cord do have the ability to regrow axons, but there are several inhibitors of the process that prevent this from occurring in practice (Kwon et al., 2004). This gives us a hint that through manipulation of these inhibitors and other growth promoting molecules some form of regeneration could occur. Whether this would be achieved by direct pharmaceutical intervention, cell therapy, gene therapy, or tissue engineering remains to be seen.

Fortunately, the number of patients requiring spinal cord regeneration is rather low. Of far greater significance numerically are the patients who suffer age-related neurological degeneration. There are several examples of this type of process, including Alzheimer's and Parkinson's diseases. It is far from clear whether tissue engineering will ever be applicable in these situations but the challenge is very obvious. Take Parkinson's as an example. This is associated with degeneration of the basal ganglia in the brain and dopamine deficiency, leading to symptoms, such as rigidity, bradykinesia, and cognitive dysfunction. The classical pharmacological treatment involving the administration of leva-dopa is well known to have serious limitations and other methods of treatment have been long sought after. It is interesting once again to see two alternative concepts here, one that attempts to address the symptoms through an implantable device and one utilising a cell-based therapy. The former approach involves the implantation of a pacemaker-like device that is able to deliver electrical signals to the brain to counter the activity that is responsible for the tremor and rigidity (Mayumi et al., 2005). The cell-based therapy involves the transplantation of embryonic ventral mesencephalic tissue directly into the brain of the patient (Borlongan and Sanberg, 2002). Both the procedures are expensive and the latter is not unnaturally associated with severe ethical and logistics constraints. Both can be

successful, but both have failures. A cell therapy approach that does not involve embryonic tissue, or a direct tissue engineering approach that induces regeneration of the basal ganglia, are obvious targets for the future.

5.4. The sensory organs

Following on from a discussion of nerve tissue, we should address very briefly the situation with sensory organs, and we can use the eye as an example. The eye suffers from a number of conditions, two major generic issues being the changes to the tissues that process the light on its way to the retina and changes to the retina and optic nerve themselves.

Mechanical trauma to the retina, leading to detachment, can be treated by a number of techniques that involve a mechanical reattachment, but it is the functional impairment caused by the death of specific neural cell populations, including retinitis pigmentosa and age-related macular degeneration, that are the most troublesome conditions for which virtually no effective treatment exists. One of the more encouraging developments here is the possibility of transplanting neural stem cells into the retina to either prevent further degeneration or actually replace those that have irreversibly deteriorated. It has proved possible under experimental conditions to graft such cells into the retina (Chacko et al., 2003), but the efficiency is rather low and it remains to be seen whether some supporting structures will be required for this type of therapy. With respect to the light pathway, there are very good techniques already available for replacement by medical devices of the simple function of the lens, especially the intraocular lens for the treatment of cataracts (Lloyd et al., 2001) and tissue engineering may not have much to offer. However, on the surface of the eye, especially the superficial layers of the conjunctiva and cornea, there are several disorders characterised by the loss of the epithelium, which give severe cosmetic and functional loss and which are difficult to treat in any way other than by regenerating this epithelium. The ability to prepare a sheet of epithelium is crucial here, and although this is not a trivial problem, there have been some early successes (Scuderi et al., 2002).

The extreme loss of quality of life associated with the gradual or sudden loss of sensory function, and the frequently encountered situation where there is no current cure, and for which there is therefore a profound unmet clinical need, provide clear evidence for the urgency of making progress with these technologies of regenerative medicine.

5.5. Major organs

It is appropriate to finish this section with a mention of the major organs and the potential role of regenerative medicine in the treatment of major deficiencies in them. We have already discussed the heart and, in passing, the central nervous system, and so we should now concentrate on the liver, kidney, lungs, and pancreas. The fact that there is no mention here of the viscera does not imply that these tissues are unimportant, just that space and time preclude a comprehensive approach to all tissues and organs.

There is one fundamental question that underpins the approach to whole organ regenerative medicine and that is whether the goal is the regeneration of the organ physically or whether it is the function that is required irrespective of the presence of a recognisable organ. There can be no doubt that the ultimate goal for the majority of those working in the field is the regeneration of a fully implantable whole organ (Shieh and Vacanti, 2005), but there are many major difficulties to surmount and much of the progress to date has been related to the restoration of appropriate function. In the field of hepatic liver engineering (Kulig and Vacanti, 2004), for example, we do not expect to see patient-derived liver-shaped objects ready for implantation in the near future, but instead we are witnessing both the development of external bioreactors that perform the function of the liver transiently, much as the short-term left ventricular assist device (LVAD) supports the heart (in fact such devices are called Liver Assist Devices, LAD), and of procedures to use direct cellular injection, in this case of hepatocytes, into a vascular bed (with microcarriers and scaffolds as appropriate), where they are able to sustain the metabolic activity of that organ. Similarly there has recently been significant progress in the implantation into patients with Type 1 diabetes, of islets of Langerhans, derived from cadaveric donors. These cells are placed into the vessels that lead to the liver, where they become embedded in this organ and function in the production of insulin. A similar story may be told with the kidney, where there is little chance of tissue engineering a full organ at this stage, but various cell and tissue engineering approaches are being adopted as adjuncts to transplantation and dialysis regimes (Hammerman, 2003).

6. THE CENTRAL TISSUE ENGINEERING PARADIGM

If the concept of tissue engineering is ''the persuasion of the body to heal itself through the delivery to the appropriate site of cells, biomolecules

and/or supporting structures'', we must now consider how this concept can be translated into practice for the growth or regeneration of those tissues and organs mentioned above. It should be appreciated that there will be no single method applicable to all the situations and the techniques of this persuasion may vary considerably. However, we can identify a basic tissue engineering paradigm, one that incorporates the essential mechanisms, and use this as a basis for identifying individual strategies. This paradigm again embodies multidisciplinarity since it depends on so many areas that are usually considered quite disparate. These include, but are not limited to, cell and molecular biology, genetics and pharmacology, materials and surface science, biorheology and structural mechanics, and biomanufacturing and bioprocessing.

The central tissue engineering paradigm is this: We first decide which cell type (or types) is/are necessary to generate the required tissue and determine where best these are derived. We then obtain a sample of such cells (cell sourcing). Almost always these will be in relatively small numbers and we have to expand this number in culture to a required level (cell expansion). This is not a trivial process and the conditions of the cell culture have to be controlled vary carefully, ensuring that they maintain their phenotype during the expansion process. In order for these cells to express the tissue we are seeking, we now have to manipulate them by providing them with the appropriate environment and signals (cell manipulation). The basis of the ''appropriate environment'' is usually some material in the form of a scaffold or matrix. A scaffold is a microporous structure that will allow the cells to invade the pores or spaces and express the extracellular matrix within these spaces in order to constitute the new tissue. A matrix is usually a gel which supports the individual cells and provides a suitable viscous medium for them to function. In both the cases, the scaffold or matrix is designed to be biodegradable so that it is resorbed at the same rate at which new tissue is formed.

Simply placing cells within these scaffold or matrix configurations is not sufficient to persuade them to express the required tissue; they need signals or cues to persuade them to do so, and here we should recall that these cells, when present in adult mammals, do not normally have this capacity. The signalling is usually provided in one of the two ways. The first is molecular signalling, where highly specific molecules may be infused into the culture medium to trigger the cells into certain activity. Typically, and perhaps not surprisingly, these will be growth factors or any of the biomolecules, such as cytokines, that, under different circumstances, have the ability to control cell behaviour. This may also involve transfection of the cells with certain genes that optimise the

specific function that is required. The second type of signalling process is that of mechanical signalling, or mechanotransduction. It is well known that many physiological processes require mechanical forces for their stimulation or maintenance, for example, the induction of osteoporosis in individuals who have limited mobility. The same is true in many situations where cells are cultured *ex vivo*, and the stimulation of such cells by either the shear stresses encountered when the fluid culture medium is agitated or when structural stresses are transmitted to the cells *via* a scaffold or matrix, may be of considerable benefit to the functional performance of those cells. This normally takes place in a bioreactor.

The anticipated result of the signalling of these cells over a period of time is the expression of the required tissue. Within this central tissue engineering paradigm, we find that the clearest manifestation of a tissue engineering product is the so-called "construct" that is removed from the *ex vivo* bioreactor, ready to be placed into the patient, or host. This could be a piece of skin intended to be placed on wound from burns or chronic ulcer, a heart valve, an intervertebral disc, or a whole bladder. We come now to what is possibly the trickiest part, the implantation of this construct into the host. The response from the host could be quite variable, bearing in mind the previous discussion about the exquisite nature of the body's defence mechanism against most invading objects. Apart from the factors related to the clinical technique, there are several issues which control the way in which this incorporation takes place. These are related to those aspects of the host response we wish to minimise, usually including inflammation and the immune response, and those aspects we may wish to promote, including the innervation of the implanted construct and, where necessary, its vascularisation.

We shall now discuss each of these phases of the central tissue engineering process to see how the individual scientific disciplines contribute to the system as a whole.

6.1. Cell sources

Cells are clearly the main active component of a tissue engineering process, but the question immediately arises as to what cell to use for any particular application. It may be self evident that we should use those cells that are the natural synthesisers of the tissue we are interested in; thus we should use chondrocytes for cartilage, osteoblasts for bone, keratinocytes for skin, cardiomyocytes for the myocardium, and so on. But are these sufficient, and from where do we get them?

Considering for a moment the case where the required tissue can be derived from a single-cell type, we find that there are two fundamental, sequential choices here. The first choice is whether we wish to start with fully differentiated cells; i.e. cells which already have the required phenotype, such as the chondrocytes, osteoblasts, keratinocytes, and cardiomyocytes mentioned above; or whether we wish to start with some precursor to the required cell that will differentiate into the desired phenotype under the conditions that we impose. This implies that we start with stem cells or progenitor cells and persuade them to differentiate into the required chondrocytes, osteoblasts, keratinocytes, or cardiomyocytes. If we have made this decision, then we have to discuss where these cells originate. In other words, who or what is the donor? We have three broad choices, although with sub-sets of decisions in each case.

To many people the preferred choice of donor is the patient himself. Such cells are classified as autologous cells, and the products will be described as autologous tissue engineering products. For the fully differentiated cells, it implies that some biopsy of the relevant tissue is taken and then the relevant cells extracted, or separated, from the tissue mass (Jorgensen et al ., 2004). Autologous chondrocytes can be obtained from the patient's cartilage, osteoblasts from his bone, and so on. There is one obvious advantage of this concept, and that is the cells will present no immunological challenge to the patient since they are self-derived. There are also some obvious disadvantages, mainly concerning the need for extensive cell separation techniques and the time it takes for the process of cell expansion following the derivation process. One could also imagine the logistic issues concerned with the transport of cells between the patient and an appropriate cell expansion laboratory and back. The stem or progenitor approach may also be considered. Since the majority of patients will be mature, we can consider these as adult stem cells and there are several sources. Many tissues contain stem or progenitor cells, although in varying concentrations. The bone marrow is the obvious source and has become a very important source of the cells used in tissue engineering and cell therapy (Ballas et al., 2002). There is an obvious disadvantage concerning the invasiveness of the procedure to acquire the bone marrow, and we now know that adult stem cells can be derived from many types of tissue. In particular, stem cells can be derived from whole blood (Roufosse et al., 2004), obtained by simple venesection, or from adipose tissue, obtained as a by-product of liposuction (Gimble and Guilak, 2003). Recently it has been found that extracted teeth can provide a relatively rich source of stem cells (Miura et al., 2003). There is also one interesting source here that has a somewhat different objective, and that is cord blood retained at the time of birth and stored frozen until

required in later life for some regenerative process of the individual (Rogers and Casper, 2004).

The second type of source is another human donor, yielding allogeneic cells (Boyce, 1998). In theory there is no limit to the origin of such cells, but in practice these are cells obtained from donors and manipulated on a commercial basis, possibly by cell or tissue banks. The donors could be deceased, or possibly living where the ''donation'' is a by-product of a routine unrelated procedure, such as male circumcision. Several allogeneic products incorporate foreskin-derived fibroblasts obtained in this way. Allogeneic stem cells move us into a quite different arena since these are synonymous with embryonic stem cells, derived from a discarded embryo in IVF treatment (Hirai, 2002).

The third type of source, in principle, is a xenogeneic source, i.e. an animal. This is not really considered as an option at present because of the issues of infectivity (Martin et al., 1998).

It has been assumed in the above discussion that the required tissue can be generated by manipulation of single-cell type. Obviously in practice this will be an exceptional situation since most tissues are multicomponent. An artery may require smooth muscle cells, fibroblasts, and endothelial cells, for example. This obviously increases the complexity of the sourcing and manipulation processes since each cell will require its own optimal culture conditions, and they may compete with each other under the culture conditions (Bhandari et al., 2001).

6.2. Cell sorting and expansion

It will be obvious from the above section that the specific cells required for a tissue engineering process will not usually be found alone, but in combination with several others within a harvested sample of tissue or blood. It is therefore necessary to sort the available cells and extract those that are required from those that are not. There are two major techniques in use at the moment, magnetic cell sorting and flow cytometry (De Rosa et al., 2003). Neither has been specifically developed for tissue engineering, but their development for diagnostic and therapeutic purposes has proved valuable in this area. In magnetic cell sorting, magnetic microparticles, of which there are several types, are conjugated with antibodies targeted to the required cell, and passed through a separation column within a strong magnetic field. In flow cytometry, or fluorescence activated cell sorting, the required cells are tagged with a suitable fluorochrome and, in suspension, are passed through the path of one or more laser beams. Both the scattered light and fluorescence

are detected by photomultipliers which produce electric pulses according to the received signal and which directs individual cells into discrete paths. This can result in the sorting of cells at rates of several thousand per second and the separation of very small number of rare cells with a high degree of precision.

The output of cell sorting is often a very small volume of the required cells. While bone marrow may yield a large number of cells in total, the various lineages of these haematopoietic cells will be present in varying numbers. Even more important, when blood – either cord or adult – is used as the source of stem cells, the number will be extremely low and procedures are necessary for their expansion *ex vivo*. In the central tissue engineering paradigm, this process occurs after cell sourcing and sorting, but before the cells are seeded onto scaffolds and provided with the signals for tissue expression, although it should be recognised that there can be variations on this theme. One of the main issues here is that cell expansion is possible under predefined conditions with most cells, but this normally takes time so that, for example, the process of tissue engineering with autologous cells can be quite protracted. Adequate numbers may not be achieved for several weeks for both differentiated and mesenchymal stem cells. The conditions obviously control the efficiency of the process.

If we take haematopoietic cells as an example, there are several different culture systems that could be used. The first involves the use, in a flask, of a layer of marrow-derived stromal cells on which the haematopoietic cells loosely attach, the contact between the two cell layers, and the addition of appropriate nutrients, favouring the expansion of the cells, depending on temperature and other factors. The stromal cells in this example are referred to as feeder cells and the maintenance of this process of haematopoiesis is critically dependent on this layer (Han et al., 2004). This has become an important point in tissue engineering processes in general, for feeder layers are often required in other expansion systems and most often it is murine fibroblasts that are used, a point of some significance with respect to infectivity risks, as we shall see later. The second process is rather similar but involves the addition of cytokines to the culture, which enhance the efficiency up to a point, molecules such as the interleukins IL-1 and IL-11 and thrombopoietin being particularly effective. It should be noted that cytokines are usually very expensive. The third process is that of membrane-separated co-culture system in which, in order to avoid the issues of using murine cells in contact with the human haematopoietic cells, these cells are separated by a porous membrane. Indirect communication by soluble cytokines and direct contact between cells through the porosity by membrane-bound

cytokines provides the stimulus for proliferation. The fourth process involves culturing within a three-dimensional environment in which the cells are supported by, for example, collagen microspheres or some synthetic scaffold, with either or both feeder cells and cytokines.

It is not only the time it takes for expansion that controls the efficiency of this process. Amongst the other factors that are potentially of great significance are the difficulty of maintaining the cell phenotype during the process and the need to avoid chromosomal changes when the cells are replicating. With many cell types there are significant changes to telomere structure during expansion, especially telomere length, such that the cells are effectively extensively aged, with marked decrease in efficiency. Chondrocytes are amongst those cells affected in this way contributing to the difficulty in the *ex vivo* expansion of chondrocytes and the production of cartilage (Parsch et al., 2004)

6.3. Scaffolds and matrices

Most cells naturally require contact with discrete structures in order to function and, under normal physiological conditions, this contact is achieved *via* other cells or their specific extracellular matrix. It is not surprising, therefore, that in the rather unnatural situation where we take cells and try to persuade them to express new tissue in situations where they would not normally do so, their immediate structural environment is of immense importance. In practice, the cells that are used in tissue engineering are cultured within either a scaffold or matrix. Perhaps the matrix is more natural since this is essentially a gel-like structure in which the cells are suspended, somewhat like a viscous extracellular matrix. A scaffold has a greater physical identity, usually consisting of a material that has been fabricated in such a way as to have a three-dimensional architecture. At present, there are no specific rules or specifications for either the materials or designs of scaffolds, but a number of principles are emerging.

One of the more important differentiating characteristics for scaffolds and matrices concerns the degradation profile (Gunatillake and Adhikari, 2003). The concept on which the tissue engineering process is based assumes that the supporting structure will degrade as new tissue is generated. The rate of degradation and the characteristics of degradation products are therefore of crucial importance. In almost all the situations, the physical requirements of the scaffolds imply that it should be polymeric, although some procedures in bone tissue engineering involve degradable calcium phosphate ceramics (Arinzeh et al., 2005).

There are some serious limitations to the choice of degradable polymers since the breakdown products of many synthetic polymers will be toxic. We have two broad choices, first to use synthetic polymers with non-toxic breakdown products and second to use naturally occurring biopolymers. With the former category, there has so far been little innovation since it has been widely assumed that regulatory approval will be easier to obtain for a material that has existing approval for a degradable implantable medical device, and hence the now classical degradable polyesters, such as polyglycolic acid, polylactic acid, their copolymers, polycaprolactone, and polyhydroxybutyrate are all being used in tissue engineering processes (Cooper et al., 2005). This is unfortunate since the functional requirements are somewhat different. This is an example where multidisciplinarity breaks down under the imposition of regulatory processes. The natural biopolymers make more sense. These are either protein/peptide or polysaccharide based, with collagen being the best example in the former group and alginates, chitosan, and hyaluronic acid derivatives among the latter (Leach et al., 2003). These may be derived from tissues or produced by recombinant techniques and have the potential advantage of possessing specific biological activity which can positively benefit cellular function, rather than the synthetic polyesters which can be pro-inflammatory, with the potential to adversely impact the tissue regeneration process.

The manufacturing technology is also important, bearing in mind that a three-dimensional porous structure of complex architecture is required, although again it has to be stressed that detailed specifications have not been determined. The critical parameters are likely to be the geometry of the pores and pore interconnections, with a degree of heterogeneity and anisotropy depending on the precise application (Malda et al., 2005). The traditional route with the degradable polymers has been through solvent casting and leaching (Hou et al., 2003); i.e. mixing the polymer with a suitable water soluble component such as salt, dissolving the polymer in a suitable solvent, casting it into the required shape and leaching out the soluble component. Although satisfactory in many situations, more sophistication is likely to be required and technologies such as supercritical fluid processing (Quirk et al., 2004), electrospinning (Yang et al., 2005) and various rapid prototyping techniques including selected laser sintering and three-dimensional printing are now in use (Cooke et al., 2003).

It should also be mentioned that tissue-derived structures may have a role as scaffolds, with chemically treated small intestine mucosa being among the more prominent (Badylak, 2004). Finally, it should be noted that in some situations a two-dimensional construct may be more relevant,

especially with respect to the delivery of sheets of cells to the affected site. For example, sheets of cardiomyocytes could be delivered to the myocardium, and sheets of epithelium to the cornea. This is one area where there has been considerable innovation through the introduction of environmentally sensitive polymers, which can undergo a hydrophilic–hydrophobic transition by a minor adjustment to the pH or temperature which allows sheets of cells to adhere to and proliferate on, a substrate but then to be released onto the desired site when the conditions change (Yamata and Okano, 2004). This has been referred to as cell sheet engineering.

6.4. Molecular signals

Assuming that we now have the required number of appropriate cell type(s) and also a suitable scaffold material, we have to address the issues of signalling processes that will persuade the former to express the right type of tissue when they are seeded onto or into the latter, and when any construct is placed in the host. Leaving aside direct cell–cell signalling for now, we can divide these signalling processes into two main varieties, mechanical cues discussed in the following section, and molecular signals, discussed briefly here. It should not be surprising that molecular signals play a large role in tissue regeneration since they are immensely important in tissue development in the first place. Most important of all are the growth factors and their analogues, which can be utilised in soluble form or bound to a supporting structure. It is clearly important for the growth factors to be delivered to the appropriate site in a timely manner with the optimal dose. In the central tissue engineering paradigm where cells are being persuaded to expand, differentiate, and express the relevant tissue *ex vivo*, the growth factors may be added to the culture medium, bearing in mind the requirements of co-culture in most realistic systems. The growth factors are expensive and usually unstable, and since there is usually a need for growth factors to be present when a construct is incorporated into a patient, a great deal of attention has been paid to the immobilisation of these proteins onto scaffolds, either for them to exert their activity when surface bound or when slowly released into the medium or tissue. Amongst the factors that have been handled this way are the bone morphogenetic proteins (BMPs), epidermal growth factor (EGF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) (Boontheekul and Mooney, 2003). The full potential for the delivery of molecular signals in tissue engineering is discussed later.

6.5. Bioreactors and mechanotransduction

For several decades, it has been appreciated that cell cultures are often more successful when the fluid medium is agitated in some way, and the advent of commercial processes involving cultured cells in large quantities, for example with the production of vaccines, led to the development of systems designed to optimise this agitation with respect to the specific cell types and desired outcomes in question. These systems, in which cells interact with their nutrient media, possibly in the presence of some supporting structure, are referred to as bioreactors. This term appears to imply a sophisticated piece of equipment, but it need not necessarily be so. Indeed many bioreactors are still based on a simple stirred tank concept, in which a closed cylinder system contains the liquid medium, the cells and a fixed concentration of gas and where a rotating impeller stirs the contents. Other types are based on hollow fibre principles, rotating concentric cylinders, and so on. As scale-up of these systems has become very important in commercial tissue engineering, so the input from chemical engineering, biorheology, and bioprocessing has been quite profound. The critical aspects are based around the transfer of critical shear stresses to cells in a suitably large reactor, whilst maintaining optimal mass transport with respect to the nutrients, oxygen, and so on. If the stresses are too low, there is no significant degree of mechanotransduction, whilst it is necessary to avoid, uniformly throughout the system, excessively high shear stresses that damage the cells or alter their function, bearing in mind that different cells have different susceptibilities in this respect. Again, it is easy to visualise the additional problems here with co-cultures.

Central to the functioning of a bioreactor are the optimisation of mass transport in the culture medium and within the cellular mass that is producing the required tissue and the delivery of mechanical signals to the cells within the system. Mechanotransduction is the process by which cells sense and respond to mechanical systems (Ingber, 2003). In normal tissues, the forces that are sustained by the body are transmitted through the tissues and across the interface between a cell and the extracellular matrix, i.e *via* the cell membrane. Mechanosensory organs can recognise and respond to the physical stimuli. Cells adhere to their extracellular matrix through the binding of specific cell surface receptors, in particular the dimeric transmembrane protein receptors known as integrins. The external part of these receptors bind to specific protein sequences such as the RGD sequence while the intracellular domains bind with actin-associated domains of the cytoskeleton, thereby forming a kind of bridge, through which stresses can be transmitted. The human body becomes susceptible to many diseases when this interface ceases to function, and

by the same reasoning, it is vital that those cells responsible for the generation of new tissue are able to sense mechanical forces. Both the design of the scaffold and of the bioreactor play significant roles here. The combination of the analysis of mechanical stresses at the nanometre scale and the molecular biology of the cell membrane/extracellular matrix/cytoskeleton reinforces the multidisciplinarity of this part of tissue engineering.

6.6. Incorporation into the host

Within this central tissue engineering paradigm, we now arrive at the final stage, which is the incorporation of some construct into the host. (this point will be reinforced at the very end of this chapter.) This paradigm is rather flexible and the state of this construct at the time of implantation can range from a collection of cells within the confines of a degrading scaffold and some extracellular matrix already formed, to a recognisable volume of tissue, the scaffold already having disappeared and functional tissue already being present. Whatever the status of the construct, it has to be fully and functionally incorporated into the host, and in doing so, there are some events to avoid and some to encourage.

High on the list for avoidance are excessive inflammation, infectivity derived from a contaminated cell source or process, an immune response and tumour formation. Inflammation could be associated with the response to the degradation of a polymeric scaffold. Infectivity is a major and definitive concern for xenogeneic-derived cell sources, and of some concern for allogeneic-derived material, while the extensive time it takes for autologous cells to be manipulated does tend to invite the possibility of microbial contamination. It is also of some concern that antibiotics used during the process may form a residuum in the construct, leading to idiosyncratic responses in some individuals. An immune response is also a significant concern (Harlan et al., 2002) when allogeneic sources are used, although it has to be said that the extent of the danger is far from clear. It has already been noted that embryonic cells may be associated with teratogenic effects. It has not escaped attention that the ability to switch on certain regenerative processes carries with it an inherent danger that the processes may not be capable of switching off, the result being the uncontrolled proliferation of tissue. The magnitude of this risk is not yet clear.

Equally important are those mechanisms that ensure effective incorporation. There are many facets of this subject, most of which are tissue specific. One of the most important, which we can use as an example, is

the need for vascularised tissue growth within and adjacent to the construct. This has been the subject of intense research effort, much of which is related to the molecular signalling discussed briefly above since it involves the delivery to the site of the growth factors that are responsible for angiogenesis (Zisch et al., 2003). Two of the most significant angiogenic proteins are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). There is evidence that it will be necessary to give quite prolonged delivery of these factors to the site of tissue regeneration if effective revascularisation is to be achieved, but the dosage has to be well controlled since excessive amounts, especially of VEGF, have undesirable consequences. It should also be noted that soluble angiogenic factors are not the only possible modes of stimulation of vascularisation, and that the scaffold materials themselves may act as morphogenetic guides to the type of tissue that is required (Hubbell, 2003).

We should also recognise that the delivery of any signalling molecule is not without risk, and, whether it is cytokines or growth factors that are involved, the delivery profile is very hard to optimise, with only small windows of opportunity between ineffectiveness and toxicity. For this reason, there has been much attention given to the possibility of overcoming these difficulties by delivering these agents not as recombinant proteins but as plasmid genes (Bonadio, 2000). Plasmid DNA may possess a stable chemistry that is compatible with drug delivery systems and which could be delivered to the site of tissue incorporation.

7. THE STATUS OF TISSUE ENGINEERING PRODUCTS AND PROCESSES

The above sections have demonstrated the enormous potential of tissue engineering and explained some of the multidisciplinary science that underpins this potential. It is probably already clear that the potential is not yet being realised. In this final section, we address some of the societal issues that envelope this new technology, demonstrating even more profound interdisciplinarity as we discuss regulatory, ethical, and commercial facets. We then conclude with an assessment of risks and benefits and speculate whether hope or despair will prevail.

7.1. The regulatory environment

In most regions of the world, all commercial products used in health care require some form of regulatory approval. This implies that products

have to be assessed by the appropriate regulatory body with respect to a variety of factors related to quality, efficacy, and safety. As yet there is no global position on this and each country or region has its own procedures. Of most importance, the Food and Drug Administration (FDA) control these regulations in the USA, and the European Union controls the process within the member states of the EU through a variety of centralised and regional procedures. Although there are some similarities, there are profound differences between these two regimes. This adds to the complexity and costs of industry, who would much prefer to have just one regulatory barrier to overcome.

Procedures in regenerative medicine, and tissue engineering in particular, provide additional difficulties. Traditionally, in both jurisdictions, drugs and medical devices have been treated quite differently, and indeed in Europe, the procedures are so different that they are controlled by different legal instruments and administered by different institutions. The problems with legally binding distinctions between these two types of product started to emerge when products that contained both drug and device components (antibiotic releasing bone cements, anticoagulated catheters, thrombin containing haemostatic agents, bone morphogenetic protein – collagen composites for bone repair, etc.) were presented to the regulators. The USA has responded by creating new offices and new procedures within the FDA, but Europe takes a much more opaque position in which each case is effectively considered on its own merits.

The issues are complex but of considerable importance. It will be obvious that tissue engineering displays characteristics of medical devices, through the central position of the hardware of scaffolds and bioreactors. As we have seen, however, there are usually significant contributions from pharmaceuticals, or at least biologically active molecules such as growth factors and cytokines, not to mention antibiotics to counter the possibility of infection during the extended culture period and a variety of nutrient and preservatives. At the very least, there should be some element of device regulation and some of pharmaceutical regulation. But even more significantly, the really central characters are the cells. There are those who argue that autologous cells should not be regulated since they start off in one person and end up back in the same person after having helped the regeneration of new tissue. The argument has some basis since there are no legally binding regulations controlling the grafting of tissue from one part of the body to another (e.g. skin or bone grafts). On the other hand, such grafts are not manipulated in the same way as these cells, and regulatory bodies are still discussing how they can differentiate between these situations by defining what is meant by substantial manipulation. It should also be borne in mind that full organ

transplantation is not regulated in the same way, ostensibly because it does not involve a commercial process, and is governed by the licences of the medical staff.

Whatever be the situation with autologous cells, that with allogeneic cells may be quite different. As we shall see in the final section, many commercial tissue engineering products do involve allogeneic sources. It is usually assumed that they pose greater risks to the recipients: if there is any problem with the process, such as contamination, then large number of patients can be affected rather than the single patient treated, by definition, with autologously derived products.

The regulatory position is still uncertain and evolving, and it is difficult to make any rational scientific comments about these positions until they are resolved. It is clear, however, that both continued uncertainty and regulatory procedures that deny the scientific basis of risk assessment and management in this area are bound to adversely impact on the practical development of these technologies. A discussion of these risk factors was published by the European Commission in 2001 (Opinion of the Scientific Committee on Medical Products and Medical Devices, 2001).

7.2. The ethical environment

There are several highly significant ethical issues that confront tissue engineering and the other components of regenerative medicine, and although there have been some attempts to discuss some of these issues, the debate remains at an early stage (Hennon, 2003; Nordgren, 2005). Some have argued that these issues have held up the progress of these therapies, although this is not really the case, since there are many other factors that have been responsible for the delays in achieving clinical progress. Nevertheless we may consider these issues as on-going and potential barriers to success.

The nature of ethical considerations depends on whether the tissue engineering process is autologous or allogeneic. It could well be argued that autologous tissue engineering is free from any ethical consideration since the patient's own cells are being used to help his or her tissues to regenerate; indeed, it is hard to think of any therapy with fewer ethical demands. It could be, and indeed has been, argued that autologous tissue engineering processes are likely to be very expensive in view of the dedicated facilities needed to sustain the culture of an individual's cells over a prolonged period of time so that this constitutes an ethical problem over the restriction of the therapy to the richest in society, but this is not

a sustainable argument when considering the range of other expensive medical therapies available for these diseases.

Perhaps of greater logic, at least to some individuals, is the claim that to persuade the body to regenerate itself under conditions where it would not normally do so constitutes deviant behaviour, and it is true that the concept of ''growing a new heart'' is anathema to some individuals. Again, much of modern medicine is concerned with the techniques to circumvent the vagaries of nature and autologous tissue engineering would appear to be no different. In this respect there can only be one type of major ethical factor and that is when genetic manipulations are used to enhance or optimise the process of autologous tissue engineering.

On the other hand, we have to accept that far more serious ethical issues arise with allogeneic tissue engineering processes. These issues arise from the basic fact that the cells do not, indeed never have, belonged to the patient: Is it ethical to use someone else's cells or tissues to assist in that patient's cure? There is one generic issue here and two very specific ones. From the generic point of view, just as with live organ transplantation, there cannot be any significant objection to a donation of cells from one individual to another if either that person is alive and makes the donation willingly or made it known that they would grant permission for donation after their death. The two specific cases are more difficult. One is concerned with embryonic (or foetal) stem cells and the second with cell lines derived from unknowing individuals. Since ethics is concerned with philosophical issues in which there is no clearly perceived right or wrong, it is difficult to take a dogmatic and entirely rational view of the arguments for and against embryonic stem cells. Nevertheless, it is impossible to ignore the fact that the argument to suppress the use of, or indeed the research into, embryonic stem cells, ignores the full picture. Therefore, to deny the use of embryonic stem cells on the grounds that some unwanted embryos produced during IVF (which would otherwise be disposed off) would have to be destroyed during the acquisition of the cells, ignores the probability that many individuals would be denied the life-saving or life-enhancing benefits of stem-cell-based therapies that would emerge from this research.

The second specific case is just as difficult, but not often discussed. Allogeneic cells are used in a number of commercial tissue engineering products and the question has to be asked as to the origin of these cells. As noted above, there can be no ethical issues when the donors consent that they are, in fact, donors. It is far from clear that the donors of cells, that have become commercial cell lines used in this type of therapy, have agreed to do so, or even known that they had done so

(Thasler et al., 2003). Again, it could be argued that the tissues that are used for the acquisition of cells, for example the foreskin removed during circumcision, would be thrown away anyway, but questions have to be faced where commercial entities make profits out of tissues that were unknowingly donated.

7.3. The commercial environment

Health care is financed by many different mechanisms throughout the world, with immense differences in the ability to pay for any advanced therapies. The various mechanisms range from publicly funded systems (for example, the NHS in the UK) to private insurance, with the vast majority of countries operating a mixed healthcare economy. Whatever the system, unless health care is entirely personally financed, there will always be a problem that payers may be reluctant to fund treatments that are experimental or unproven. Moreover, there is often a reluctance to pay for expensive treatments when much less expensive treatments exist, even if they provide inferior benefits (Williams, 2003). The commercial environment for tissue engineering products and processes, therefore, is based on the costs of development and production and on the willingness of the health care system to pay for these services.

In relation to the central tissue engineering paradigm, we cannot ignore the fact that much of the underlying science and technology is very new and has required a considerable level of investment to pursue the necessary development to make tissue engineering applicable at a clinical level. This in itself is not insurmountable, since many other developments in medical and pharmaceutical technology have been in the same position and succeeded. The real questions arise when the cost basis for production is assessed alongside the revenue basis for providing the tissue engineering service. It should not be surprising to note that a major factor in the cost basis is determined by the nature of the cells used and the length of time for, and complexity of, the cell manipulation phases. On the one hand, we have the possibility of using allogeneic cell sources and commercial scaffolds or matrices where there is actually an off-the-shelf product that can be used directly on patients. Allogeneic chondrocytes within a polymer scaffold could be used as the basis for cartilage tissue engineering and used directly to treat cartilaginous lesions of the knee. It is likely that such products could be provided at a reasonable cost in view of the potentially large volumes of ''mass-production'' involved. On the other hand, we can imagine the real costs of autologous tissue engineering, where a physician

has to undertake an invasive procedure on a patient, send the acquired tissue to a laboratory where it is processed in order to derive and manipulate the required cells and grow the construct in a bioreactor, before sending it back to the physician who has to implant it in the patient. This is likely to be extremely expensive.

At this stage, no universally applicable business model has been identified for tissue engineering and there is no agreed basis for decisions concerning health insurance reimbursement schemes. As noted below, this uncertainty has been blamed for the difficulties faced by the industry. At present we can see a divergence of views and practices, dependent upon whether tissue engineering is seen as a product-based industry or a service industry. In the former case, the allogeneic products could be supplied to health-care facilities, similar to drugs and devices. In the latter case, we could imagine the commercial tissue engineering service operating within the health-care facility, deriving cells from patients and using commercially sourced scaffolds, matrices, growth factors, and other consumables in order to provide a custom-based service.

7.4. Commercial and clinically viable tissue engineering products

It is well known in the tissue engineering sector that, after a great deal of promise and enthusiasm in the early 1990's, it has been difficult to translate the concepts and paradigms of tissue engineering into commercially viable products or processes and into clinically successful procedures. The difficult commercial basis has been discussed recently on several occasions (for example, Lysaght et al., 1998; Lysaght and Reyes, 2001; Pangarkar and Hutmacher, 2003; Lysaght and Hazelhurst, 2004) and these reviews give details of the commercial institutions that have suffered considerable losses and closures over this period. Although several companies are still involved in tissue engineering, and there are signs that there are improvements in their viability, it is still difficult to see the small innovative companies on which medical technologies have previously depended upon, really making a significant contribution without early stage revenue streams. It is also interesting to note that those areas of tissue engineering initially considered to be the major markets have not fulfilled this potential in the clinical sense, and neither skin nor cartilage tissue engineering have made a significant impact clinically. On the other hand, we are witnessing some very important developments at the "higher end" of the clinical spectrum, in, for example, cardiology (Matsumura, 2003), the genito-urinary tract (Atala, 2004) and the gastro-intestinal tract (Grikscheit, 2004) which give some indications that major clinical utility from tissue engineering is possible.

8. CONCLUSIONS: HOPE OR DESPAIR

The title of this chapter alluded that tissue engineering was either an emerging medical technology full of potential and needing a little more time; or was an unworkable concept doomed to failure. Several points have emerged.

First, the processes of tissue regeneration in adult humans are biologically feasible but the conditions under which we can switch on (and off) the relevant mechanisms are not trivial. It should be of no surprise that the basic knowledge that allows us to achieve this is not fully developed. This is not a major concern and we should note that previous radical innovations in health care have taken just as long to develop. The fact that the science base is so multidisciplinary should be no deterrent to progress here, but it should be recognised that the increased complexity provided by this juxtaposition of contrasting disciplines does imply the need for greater resources.

Second, the development of tissue engineering as a viable industry has to take into account the infrastructure surrounding the new concepts that are involved, ranging from political to legal, to ethical, to reimbursement. There will be a number of critical pragmatic factors that control success. One of the most significant is the situation of unmet clinical need. It is highly unlikely that tissue engineering will succeed, at least initially, if it concentrates on areas that are already well served, with both clinical success and acceptable costs, by alternative therapies. Tissue engineering tooth enamel, for example, does not seem to be a sensible place to start. However, those major conditions that destroy life or the quality of life, including the neurodegenerative processes and major organ failure, where there are no acceptable and widely available alternatives, should be the prime targets for tissue engineering.

The logistics and costs of tissue engineering are obviously of paramount importance and, as noted above, decisions have to be made about whether the commercial drivers are products or processes and the nature of the optimal business model. At the moment, and with the current models, we are finding that the development of tissue engineering products requires investments that approach the astronomical amounts normally associated with the pharmaceutical industry, but with financial returns more equivalent to those of the lower cost based medical device industry. This gap is unsustainable. The best hope here most probably rests with a model in which tissue engineering services are provided by commercial operators working within a health-care environment, with appropriate accreditation concerning quality systems and manufacturing practices, and using approved and qualified materials

and consumables supplied by other vendors, such as scaffolds and biochemicals.

It may well be that the tissue engineering paradigm that forms the basis of this chapter needs reassessment. Indeed it is probably the separation of the tissue engineering process into discrete phases that is at the heart of the problems that this technology has faced. Central to this is the role or place of the bioreactor, represented here as the location where the cells express the required tissue under the influence of all the appropriate signals, with the implicit assumption that this location is *ex vivo*, within a closed, engineered structure. It could be argued that this is an unnecessary component and that the best bioreactor is the human body itself. We have already inferred that the signalling processes are required in both the *ex vivo* expansion/proliferation phase and the *in vivo* incorporation phase, and it might be more logical to consider how these phases could be integrated. It is time that systems engineering principles took on a greater role within tissue engineering, and then the process would lean far more towards hope than despair.

REFERENCES

Actis Dato, G.M., Centofanti, P., Actis Dato, A., Aidela, E., Punta, G., Poletti, G., di Summa, M., 1999. Bjork Shiley convexo-concave valve: is a prophylactic replacement justified. J. Cardiovasc. Surg. 40, 343–346.

Arinzeh, T.L., Tran, T., Mcalary, J., Daculsi, G., 2005. A comparative study of biphasic calcium phosphate ceramics for human mesenchymal stem-cell induced bone formation. Biomater. 26, 3613–3638.

Arjomand, H., Turi, Z.G., McCormick, D., Goldberg, S., 2003. Percutaneous coronary intervention: historical perspectives, current status and future directions. Am. Heart. J. 146, 787–796.

Atala, A., 2004. Tissue engineering for the replacement of organ function in the genitourinary system. Am. J. Transplant. 4, 58–73.

Badylak, S., 2004. Xenogeneic extracellular matrix as a scaffold for tissue reconstruction. Transplant. Immunol. 12, 367–377.

Ballas, C.B., Zielske, S.P., Gerson, S.L., 2002. Adult bone marrow stem cells for cell and gene therapies. J. Cell. Biochem. 85, 20–28.

Belkas, J.S., Shoichet, M.S., Midha, R., 2004. Axonal guidance channels in peripheral nerve regeneration. Oper. Tech. Orthop. 14, 190–198.

Bhandari, R.N.B., Riccalton, L.A., Lewis, A.L., Fry, J.R., Hammond, J.A., Tendlet, S.J.B., et al., 2001. Liver tissue engineering; a role for co-culture systems in modifying hepatocyte function and viability. Tissue. Eng. 7, 345–357.

Bonadio, J., 2000. Tissue engineering via local gene delivery. J. Mol. Med. 78, 303–311.

Boontheekul, T., Mooney, D.J., 2003. Protein – based signalling systems in tissue engineering. Curr. Opin. Biotechnol. 14, 559–565.

Borlongan, C.V., Sanberg, P.R., 2002. Neural transplantation for treatment of Parkinson's disease. Drug. Discov. Today 7, 674–682.

Bourke, M.E., Healey, J.S., 2002. Pacemakers; recent directions and developments. Curr. Opin. Anesthesiol. 15, 681–686.

Boyce, S.T., 1998. Skins substitutes from cultured cells and collagen3-GAG polymers. Med. Biol. Eng. Comput. 36, 791–800.

Chacko, D.M., Das, A.V., Zhao, X., James, J., Bhattacharya, S., Ahmad, I., 2003. Transplantation of ocular stem cells; the role of injury in incorporation and differentiation of grafted cells in the retina. Vision. Res. 43, 937–946.

Cooke, M.N., Fisher, J.P., Dean, D., Rimnac, C., Mikos, A.G., 2003. Use of stereolithography to manufacture critical sised 3D biodegradable scaffolds for bone implants. J. Biomed. Mater. Res. 64B, 65–69.

Cooper, J.A., Lu, H.H., Ko, F.K., Freeman, J.W., Laurencin, C.T., 2005. Fiber based tissue engineering scaffold for ligament replacement. Biomater. 26, 1523–1532.

Davies, J.E. (Ed.), 2000. Bone Engineering. Em Squared Inc, Toronto.

De Kleuver, M., Oner, F.C., Jacobs, W.C.H., 2003. Total disc replacement for chronic low back pain; background and a systematic review of the literature. Eur. Spine. J. 12, 108–116.

De Rosa, S.C., Brenchley, J.M., Roederer, M., 2003. Beyond six colors; a new era in flow cytometry. Nat. Med. 9, 112–117.

Dembitsky, W.P., Tector, A.J., Park, S., Moskowitz, A.J., Gelijns, A.C., Ronan, N.S., et al., 2004. Left ventricular assist device performance with long term circulatory support; lessons from the REMATCH trial. Ann. Thorac. Surg. 78, 2123–2130.

Dundar, Y., Hill, R.A., Bakhai, A., Dickson, R., Walley, R., 2004. Angioplasty and stents in coronary artery disease; a systematic review and meta-analysis. Scand. Cardiovas. J. 38, 200–210.

Dutka, D., Camici, P.G., 2003. Hibernation and congestive heart failure. Heart. Fail. Rev. 8, 167–173.

European Commission. State-of-the-Art concerning tissue engineering. Opinion of the Scientific Committee on Medicinal Products and Medical Devices, 2001. http://www.europa.eu.int/comm/food/fs/sc/scmp/out37-en.pdf.

Gimble, J., Guilak, F., 2003. Adipose-derived adult stem cells. Cytotherapy. 5, 362–369.

Goudarzi, B.M., Bonvino, S., 2003. Critical care issues in lung and heart transplantation. Crit. Care. Clin. 19, 209–231.

Grikscheit, T.C., Siddique, A., Ochoa, E.R., Srinivasan, A., Alsberg, E., Vacanti, J.P., 2004. Tissue-engineered small intestine improves recovery after massive small bowel resection. Ann. Surg. 240, 748–754.

Gruber, H.E., Leslie, K., Ingram, J., Norton, H.J., Hanley, E.N., 2004. Cell based tissue engineering for the intervertebral disc. Spine. J. 4, 44–55.

Gunatillake, P., Adhikari, R., 2003. Biodegradable synthetic polymers for tissue engineering. Eur. Cell. Mater. J. 5, 1–16.

Hammerman, M.R., 2003. Tissue engineering the kidney. Kidney. Int. 63, 1195–1204.

Han, J.-T., Chen, B., Liu, S.-J., 2004. A new tissue engineering technique: culture and pre-treatment conditions of feeder layer cells of embryonic stem cells. Chin. J. Clin. Rehabil. 8, 4900–4901.

Harlan, D.M., Karp, C.L., Matzinger, P., Munn, D.H., Ransohoff, R.M., Metzger, D.W., 2002. Immunological concerns with bioengineering approaches. Ann. N. Y. Acad. Sci. 961, 323–330.

Hassfeld, S., Muhling, J., 2001. Computer assisted oral and maxillofacial surgery – a review and assessment of technology. Int. J. Oral. Maxillofac. Surg. 30, 2–13.

Hellerman, J.P., Jacobsen, S.J., Gersh, B.J., Rodeheffer, R.J., Reeder, G.S., Roger, V.J., 2002. Heart failure after myocardial infarction. Am. J. Med. 113, 324–330.

Henon, P.R., 2003. Human embryonic or adult stem cells; an overview on ethics and perspectives for tissue engineering. Adv. Exp. Med. Biol. 534, 27–45.

Hirai, H., 2002. Stem cells and regenerative medicine. Hum. Cell. 15, 190–198.

Hou, Q., Grijpma, D., Feijen, J., 2003. Porous polymeric structures for tissue engineering prepared by a coagulation, compression moulding and salt leaching technique. Biomater. 24, 1937–1947.

Hubbell, J.A., 2003. Materials as morphogenetic guides in tissue engineering. Curr. Opin. Biotechnol. 14, 551–558.

Ingber, D.E., 2003. Mechanobiology and diseases of mechanotransduction. Ann. Med. 35, 564–577.

Iwata, H., Ueda, Y., 2004. Pharmacokinetic considerations in development of a bioartifical liver. Clin. Pharmacokinet. 43, 211–225.

Jauhar, S., 2004. The artificial heart. N. Engl. J. Med. 350, 542–544.

Jorgensen, C., Gordeladze, J., Noel, D., 2004. Tissue engineering through autologous mesenchymal stem cells. Curr. Opin. Biotechnol. 15, 406–410.

Kopp, J., Jeschke, M.G., Bach, A.D., Kneser, U., Horch, R.E., 2004. Applied tissue engineering in the closure of severe burns and chronic wounds using cultured human autologous keratinocytes in a natural fibrin matrix. Cell. Tissue. Banking. 5, 89–96.

Kulig, K.M., Vacanti, J.P., 2004. Hepatic tissue engineering. Transplant. Immunol. 12, 303–310.

Kwon, B.K., Dvorak, M.F., Fisher, C.G., Tetzlaff, W., 2004. Spinal cord injury regenerative strategies and obstacles. Curr. Opin. Orthop. 15, 196–201.

Leach, J.B., Bivens, K.A., Patrick, C.W., Schmidt, C.E., 2003. Photocrosslinked hyaluronic acid hydrogels; natural biodegradable tissue engineering scaffolds. Biotechnol. Bioeng. 82, 578–589.

Lloyd, A.W., Faragher, R.G.A., Denyer, S.P., 2001. Ocular biomaterials and implants. Biomater. 22, 769–785.

Lysaght, M., Hazelhurst, A.L., 2004. Tissue engineering; the end of the beginning. Tissue. Eng. 10, 309–320.

Lysaght, M., Reyes, J., 2001. The growth of tissue engineering. Tissue. Eng. 7, 485–493.

Lysaght, M., Nguy, N.A., Sullivan, K., 1998. An economic survey of the emerging tissue engineering industry. Tissue. Eng. 4, 231–238.

Mackinnon, S.E., Nakao, Y., 1997. Repair and reconstruction of peripheral nerve injuries. J. Orthop. Sci. 2, 357–365.

Malda, J., Woodfield, T.B.F., van der Vloodt, F., Wilson, C., Martens, D.E., Tramper, et al., 2005. The effect of PEGT/PBT scaffold architecture on the composition of tissue engineered cartilage. Biomater. 26, 63–72.

Mansbridge, J.N., Liu, K., Pinney, E., Patch, R., Ratcliff, A., Naughton, G.K., 1999. Growth factors secreted by fibroblasts; role in healing diabetic foot ulcers. Diabetes. Obes. Metab. 1, 265–271.

Martin, U., Kiessig, Y., Blusch, J.H., Haverich, A., Von der Helm, K., Herden, T., et al., 1998. Expression of pig endogenous retrovirus by primary porcine endothelial cells and infection of human cells. Lancet. 352, 692–694.

Matsumura, G., Miyagawa-Tomita, S., Shin'oka, T., Ikada, Y., Kurosawa, H., 2003. First evidence that bone marrow cells contribute to the construction of tissue-engineered vascular autografts in vivo. Circ. 108, 1729–1735.

Mayumi, K., Murata, J., Uesugi, H., Kikuchi, S., Saito, H., Tashiro, et al., 2005. Two year follow up of chronic stimulation of the posterior subthalmic white matter for tremor-dominant Parkinson's Disease. Neurosurg. 56, 281–289.

Milano, A.D., Blanzola, C., Mecozzi, G., D'Alfonso, A., de Carlo, M., Nardi, C., Bortolotti, U., 2001. Hemodynamic performance of stented and stentless aortic bioprosthesis. Ann. Thorac. Surg. 72, 33–38.

Miura, M., Gronthos, S., Zhao, M., Lu, B., Fisher, L.W., Robey, P.G., Shi, S., 2003. SHED: stem cells derived from human exfoliated deciduous teeth. Proceeding of the National Academy of Sciences of the USA. 100, 5807–5812.

Moffat-Bruce, S.D., Jamieson, W.R.E., 2004. Long term performance of prostheses in mitral valve replacement. J. Cardiovasc. Surg. 45, 427–447.

Murphy, G.J., Ascione, R., Angelini, G.D., 2004. Coronary artery bypass grafting on the beating heart: surgical revascularisation for the next decade. Eur. Heart. J. 25, 2077–2985.

National Institute of Clinical Excellence (UK). 2000/2003a Guidance on Artificial Hip Joints, Issued 31 March 2000. http://www.nice.org.uk.

Niccoli, G., Iacoviello, L., Cianflone, D., Crea, F., 2001. Coronary risk factors; new perspectives. Int. J. Epidemiol. 30 (Suppl 1), S41–S47.

Nordgren, A., 2005. Moral imagination in tissue engineering research on animal models. Biomater. 25, 1723–1734.

Pangarkar, N., Hutmacher, D.W., 2003. Invention and business performance in the tissue engineering industry. Tissue. Eng. 9, 1313–1322.

Parsch, D., Fellenberg, J., Brummendorf, T.H., Eschlbeck, A.-M, Richter, W., 2004. Telomere length and telomerase activity during expansion and differentiation of human mesenchymal stem cells and chondrocytes. J. Mol. Med. 82, 49–55.

Quirk, R.A., France, R.M., Shakesheff, K.M., Howdle, S.M., 2004. Supercritical fluid technologies and tissue engineering scaffolds. Curr. Opin. Solid. State. Mater. Sci. 8, 313–321.

Rogers, I., Casper, R.F., 2004. Umbilical cord blood stem cells. Best Pract. Res. Clin. Obstet. Gynaecol. 18, 893–908.

Roufosse, C.A., Direkze, N.C., Otto, W.R., Wright, N.A., 2004. Circulating mesenchymal stem cells. Int. J. Biochem. Cell. Biol. 36, 585–597.

Scuderi, N., Alfano, C., Paolini, G., Marchese, C., Scuderi, G., 2002. Transplantation of autologous cultivated conjunctival epithelium for the restoration of defects in the ocular surface. Scand. J. Plast. Reconstr. Surg. Hand. Surg. 36, 340–348.

Shieh, S.-J., Vacanti, J.P., 2005. State-of-the-art tissue engineering; from tissue engineering to organ building. Surg. 137, 1–7.

Thasler, W.E., Weiss, T.S., Schillhorn, K., Stoll, P.-T., Irrgang, B., Jauch, K.-W., 2003. Charitable state-controlled foundation human tissue and cell research; ethic and legal aspects in the supply of surgically removed human tissue for research in the academic and commercial sector in Germany. Cell. Tissue. Banking, 4, 49–56.

Tina Yu, T., Shoichet, M.S., 2005. Guided cell adhesion and outgrowth in peptide – modified channels for neural tissue engineering. Biomater. 26, 1507–1514.

Van der Hoeven, B.L., Pires, N.M.M., Warda, H.M., Oemrawsingh, P.V., Van Vlijmen, B.J.M., WQuan, P.H.A., et al., 2005. Drug eluting stents; results, promises and problems. Int. J. Cardiol. 99, 9–17.

Vesely, I., 2003. The evolution of bioprosthetic heart valve design and its impact on durability. Cardiovasc. Pathol. 12, 277–286.

Williams, D.F., 2003. Reconstructing the body; costs, prices and risks. The Geneva Papers on Risk and Insurance. 28, 331–339.

Williams, D.F., 2003. The inert-bioactivity conundrum. In: Ellingsen, J. (Ed.), The Implant-Tissue Interface, CRC Press, Boca Raton, pp. 407–430.

Williams, D.F., 1999. The Williams Dictionary of Biomaterials, Liverpool University Press, Liverpool, p. 318.

Yamata, M., Okano, T., 2004. Cell sheet engineering. Mater. Today 7, 42–47.

Yang, F., Murugan, R., Wang, S., Ramakrishna, S., 2005. Electrospinning of nano/micro-scale poly(L-lactic acid) aligned fibres and their potential in neural tissue engineering. Biomater. 26, 2603–2610.

Yannas, I.V., 1998. Studies on the biological activity of the dermal regeneration scaffold. Wound Repair Regen. 6, 518–523.

Zilla, P., Human, P., Bezuidenhout, D., 2004. Bioprosthetic heart valves; the need for a quantum leap. Biotechnol. Appl. Biochem. 40, 57–66.

Zisch, A.H., Lutolf, M.P., Hubbell, J.A., 2003. Biopolymeric delivery matrices for angiogenic growth factors. Cardiovasc. Pathol. 12, 295–310.