Tissue Regeneration

Biological Theory, Modeling and Applications

Shay Fisher

Volume I

Tissue Regeneration: Biological Theory, Modeling and Applications

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Preface

The main aim of this book is to educate learners and enhance their research focus by presenting diverse topics covering this vast field. This is an advanced book which compiles significant studies by distinguished experts in the area of analysis. This book addresses successive solutions to the challenges arising in the area of application, along with it; the book provides scope for future developments.

In a majority of incidents involving damage or injuries, the healing process involves formation of a scar, which is like a patch, reinstating structural integrity of the damaged tissue without reviving physiological functions. A far better option for a patient affected by tissue damage would be to replace the damaged tissue with something functionally similar. Increasing amount of study and research is being undertaken across the world in search for such a technology. This book provides a timely overview on crucial topics in tissue regeneration, in a well-researched manner, written by experts from around the globe. It facilitates a better understanding of normal healing and the methods of applying stem cells. This book will be a great source of reference for study and work undertaken by students, experts and medical professionals.

It was a great honour to edit this book, though there were challenges, as it involved a lot of communication and networking between me and the editorial team. However, the end result was this all-inclusive book covering diverse themes in the field.

Finally, it is important to acknowledge the efforts of the contributors for their excellent chapters, through which a wide variety of issues have been addressed. I would also like to thank my colleagues for their valuable feedback during the making of this book.

Editor

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Introductory Chapter

Tissue Regeneration – A Clinical Science Whose Time Has Come

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UK

1. Introduction

Tissue engineering is the application of knowledge gained in the study of basic developmental cell biology to the construction and repair of human bodies.

The surgically-focused side of the field has a long history, resting mainly on experience with wound healing and *ad-hoc* attempts to improve it. A well-known and long-standing example is modulation of bone healing by the application of physical force that gives rise to the image of a patient in traction, so common on humorous 'get well soon' cards.

The more cell biological side of the field is younger because its development had to await the gaining of significant amounts of basic knowledge in molecular cell biology, a field that is only a few decades old. The coming together of cell biology and experimental surgery to drive forward the development of tissue engineering is therefore a relatively recent phenomenon and only in this century has tissue engineering really taken off as a major area of research (Fig 1).

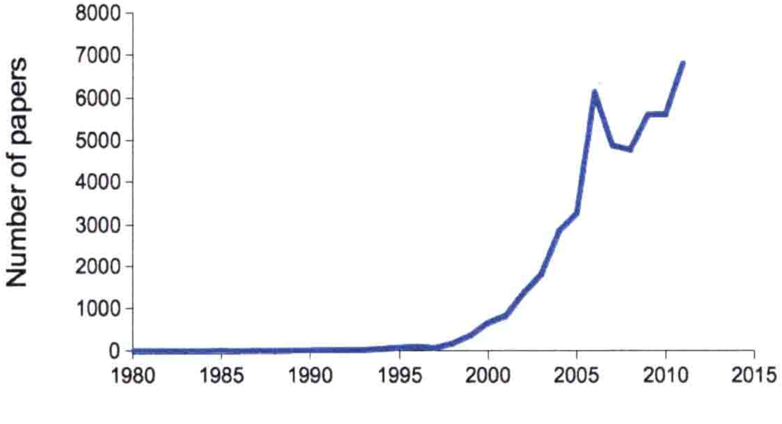

Fig. 1. Rapid growth of tissue engineering as a 21st century discipline. The graph shows the number of publications returned by a Pubmed search for ' *"tissue engineering"* <year>'.

Unlike many other young sciences, tissue engineering is growing very much as a global enterprise, perhaps because of the ubiquity of surgery and therefore the visibility of obvious need. It is noticeable, for example, that the contribution of China to research into tissue engineering is currently approximately equal to that of the European Union (judged by numbers of publications on a simple search: Figure 2).

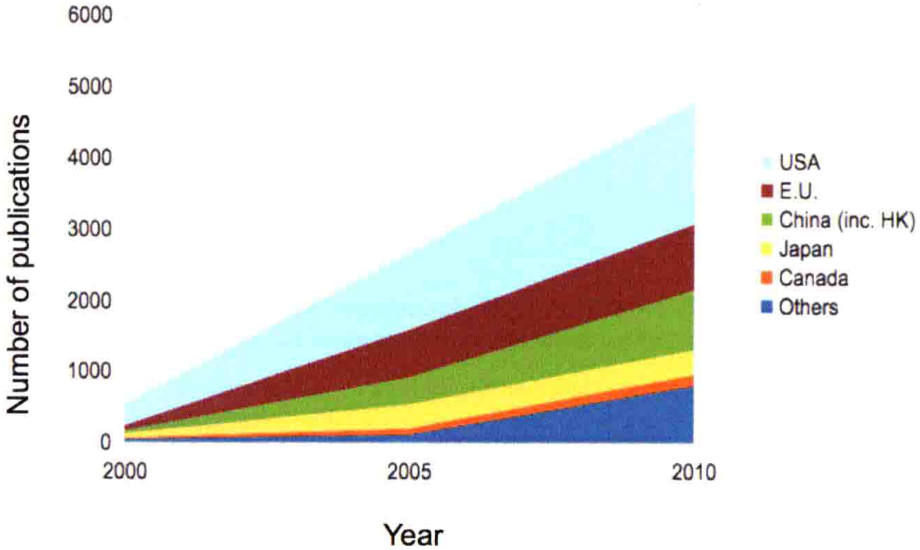

Fig. 2. Growth of tissue engineering output by country. This graph was produced by searching PubMed for '<year> *"Tissue Engineering" xxxx*', where <year> was '2000', '2005' or '2010' and 'xxxx' was the name of a country, or a Boolean expression combining, with a logical OR, a list of countries such as constituents of the European Union.

This global spread of research effort stands in marked contrast to the pattern seen in other new fields such as synthetic biology, which has grown more-or-less in parallel to that of tissue engineering and which is again very much of the twenty-first century. A comparison of pie charts of the national origins of papers in the two young sciences shows the difference immediately, about a third of research in tissue engineering coming from outside the USA and the European Union while only around fifteen percent does so in synthetic biology. The active engagement of so many countries and cultures in problems and applications of tissue regeneration ought to be a great strength for the field, encouraging the development of techniques suited to a wide range of problems and also to a wide range of health care economies.

Wherever it is done, research into tissue regeneration can be divided into three complementary sub-fields, and this book is organized around them. First, there is research that aims to understand and manipulate the endogenous healing processes in human tissues. This is the oldest part of the field. Second, there is the application of stem cell science to the regeneration of tissues (or to their *de novo* generation). Third, there is the construction of engineered scaffolds to guide normal healing processes and the behaviour of stem cells either in culture or *in vivo*. These different aspects of tissue regeneration link and overlap but, for convenience of organization, they will be considered in different sections of this book.

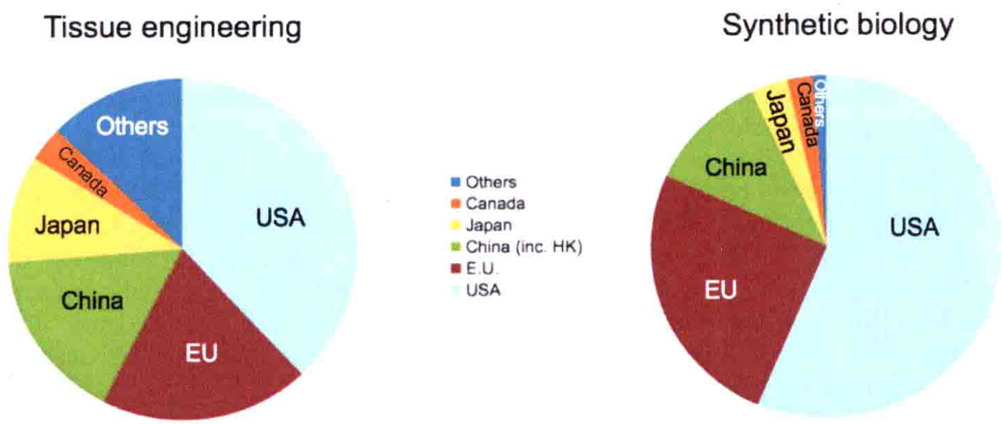

Fig. 3. Research in the young field of tissue engineering is a more global enterprise than research in synthetic biology, a field chosen for comparison because it is about the same age, is taking off about as quickly and is also an application of molecular cell biological knowledge. As with figures 1 and 2, data plotted were obtained by conducting PubMed searches for the name of the field AND the name of the country, or a Boolean expression combining several countries as appropriate.

2. Understanding and manipulating endogenous healing

There are two broad methods for assisting endogenous healing; physical and biological. Physical forces are relevant to healing because many cell types sense and respond to mechanical forces using sensory systems in their cell-cell junctions, cell-matrix junctions and cytoskeleton. The sensing systems, though currently the subject of intense research, remain poorly understood (Katoh et al. 2008). Their detection of appropriate mechanical forces can nevertheless be critically important and the development of many cells and tissues, including heart muscles (Vandenburgh et al. 1996), heart valves (Jacot et al. 2010), and blood vessels (Poelmann et al. 2008), is modulated or determined by mechanical force.

Three chapters in this book illustrate how purely physical interventions can be used to assist healing. Monici and Cialdai (chapter 1) set the scene by reviewing in detail the ways in which living cells sense and respond to physical forces. These include mechanical and gravitational forces (which are, in terms of how they interact with living things, effectively mechanical) and also electric fields, which modulate various types of cell signalling and can be used to stimulate cell motility. Electrical forces are relatively easy to apply from outside the body and therefore offer exciting possibilities for safe, long-term treatment to encourage the regeneration of damaged nerves amongst other things (Hamid & Hayek 2008). High-frequency electromagnetic radiation, in the form of microwaves, infra-red and light, has a long history of medical use (Kenkre et al. 1996, McCulley & Petroll 2008, Gravas et al. 2003, Owens et al. 2003, Simon et al. 2005, Goldberg & Sand 2000). Chapter 1 presents examples of laser light being used to promote regeneration of muscle and bone. In Chapter 2, Li illustrates how a very specific type of mechanical force, pressure waves from ultrasound, can be used to promote the repair of damaged nerves. Again, this technology holds particular promise because it can be applied from outside the body.

One of the major problems faced by surgeons and their patients is the replacement of bones that have to be removed because they harbour neoplasia. Amputation of a limb is clearly a major loss for a patient, so surgeons attempt to conserve whenever possible. If a large section of bone has to be removed, there is a problem finding something to replace it. The ideal replacement would be the excised bone itself, freed from all traces of the tumour. Crude attempts to kill tumour cells, by autoclaving or by severe chemical treatment, do result in a sterile bone but destroy so many of the normal guidance cues for cells that the bone is not properly integrated into the body and maintained when it is put back into the patient. In chapter 3, Diehl and colleagues describe an adaptation of a technique for sterilization that uses high hydrostatic pressures. This kills any cells in the bone but seems to leave intact the guidance cues necessary for the bone's efficient recolonization. It may therefore allow tumour-infested bones to be removed, sterilized safely and replaced, with the aim of leaving the patient with a normal limb.

Biochemical manipulation of cell behaviour is as ancient as medicine itself and the herbal remedies of the first physicians worked - when they worked at all - in this way. An improved understanding of the natural communications during healing and regeneration is allowing researchers to develop biochemical techniques for encouraging regeneration and discouraging damaging scar formation. An approach that is simple in principle even if not straightforward in practice, is to alter wound chemistry to make naturally-produced growth factors more effective. One way of doing this is to alter the extent to which wound matrix sequesters signalling molecules that would, if able to interact with cells, promote healing. In Chapter 4, van Neck and colleagues take this approach and discuss the use of synthetic, glycanase-resistant heparan sulphate mimics in mobilizing endogenous, regeneration-promoting signalling molecules. The chapter presents interesting data that suggest a beneficially-altered balance in favour of repair and against scar formation. Healthy tissue needs a good blood supply and, in chapter 5, de Mendonça describes how angiogenesis is controlled and draws attention to potential targets that would allow the process to be manipulated pharmacologically. Platelets are small cell fragments that travel in blood and that play a critical role in limiting the immediate consequences of wounding by formating a clot when a blood vessel is damaged. As well as performing this mechanical function, platelets are a rich source of growth factors and cytokines. In chapter 6, Ohkohichi and colleagues present data that argues that platelets can significantly promote the regeneration of damaged liver and possibly promote the survival of small grafts as well.

A well-known, and frustrating, fact about natural regeneration is that so-called 'lower' animals are a lot better at it than are humans. Have humans completely lost an ancient regenerative response still present in fish and amphibians, or is the difference a matter of degree, humans still retaining elements of the repair pathway but not activating it strongly enough? The difference is important, because the second of these hypotheses allows for the possibility of developing a clinical intervention that builds on an existing pathway but pushes it harder to give humans an amphibian-like power of rebuilding. Eleonora Grigoryan, in chapter 7, analyses the early cellular events that follow damage to the retina in amphibians and mammals. Although the final outcome of the type of damage is very different in these different organisms (amphibians making a new retina, mammals failing to do so), the early responses of the cells are the same but the conversion of non-neuronal cells to neurons and glia follows them only in amphibia. It is still not clear why the eventual fates of the cells diverge so much between species, but the work described in the chapter narrows down the difference to a specific stage in the time-course of the response and should help researchers to discover exactly why the cells of the different organisms end up behaving so differently from the same initial pathway.

Compared to the research in stem cell biology that is catching so much public attention, research on simple physical and biochemical methods to promote regeneration may strike some readers as pedestrian. From the point of view of the patient, though, the important requirement is not that a researcher keeps publishing in the trendiest stem cell journals, but rather that they invent something that actually works. With the honourable exception of bone marrow transplantation, most stem cell treatments are very new and most clinical trials have been small and have not been running long. It is therefore too early to say, in most cases, whether stem cells will prove as effective as is hoped (Trounson et al. 2011). If they are not, it may well be that, in the short-to-medium term at least, real patients will benefit more, and in larger numbers, from the development of 'conventional' physical and chemical based therapies.

3. Application of stem cells

Stem cells are cells that can maintain their own population and give rise to daughters that are committed to differentiate. Depending on where they come from, they can have an ability to differentiate into any cell type of the body (for example, embryonic stem cells) or only a restricted range of cell types (for example, haematopoietic stem cells). Because of their potential to make new cells, stem cells have for some years been seen as a very promising source of new tissue. Initial experiments were generally designed on the assumption that a useful stem cell therapy would work by the stem cells themselves making new tissue to replace that lost to injury. In recent years, though, the field has been made more complicated by the realization that some stem cell types, in particular mesenchymal stem cells, exert significant beneficial effects by secreting factors that modulate inflammation and healing by the host tissue, and that much new growth is from the cells that were already there rather than being from the stem cells. The dual mode of stem cell action, which places different emphasis on each mode according to the precise situation, makes analysis of stem cell-mediated repair complicated. On the positive side, the realization that secreted factors may be of significant, or even primary, importance in the mechanism of this repair is exciting from a clinical point of view because it may be possible to apply the factors directly without any need for patients to undergo potentially dangerous stem cell transplantation. This is not yet possible, though, and at the moment attempts at this type of therapy requires the use of stem cells themselves.

Development of effective stem cell therapies can be divided into two sub-problems; that of finding the most suitable type or source of stem cells to use, and that of applying them to the injured body in the most effective way possible. For convenience, section 2 of this book has been divided along precisely these lines, chapters 8-11 covering sources of stem cells and chapters 12-16 covering their application.

Arguably the most convenient source of stem cells for therapy is the patient himself. Bone marrow is a rich source of stem cells. Many attempts to use them have involved mechanical recovery of marrow followed by purification of stem cells, perhaps with additional steps of proliferation and reprogramming, followed by injection into systemic blood or directly into a site of damage. In chapter 8, Christian Drapeau and colleagues discuss an alternative approach that involves much less invasive manipulation Their strategy is to use the fact that endogenous bone marrow stem cells can be mobilized by cytokines, and they describe experiments that involve injecting pure cytokines into animals that have suffered cardiac infarction, in an effort to encourage mobilization of the animals' own stem cells to effect a repair. The authors also describe preliminary studies of this approach in humans, to treat stroke and kidney failure.

A novel source of pluripotent stem cells, capable of making any body cell, is the testis. Spermatogonial cells are the stem cells that naturally maintain production of sperm. Within the testis, they are constrained by their environment to have only the simple choice between self-renewal and spermatogenesis. Taken outside that environment, though, the cells can differentiate into a large range of cell types, making them effectively pluripotent. Liz Simon and colleagues analyze the abilities of these stem cells in chapter 9, and evaluate their potential for therapeutic use compared to the potential of other stem cell types. While spermatogonial cells can be obtained only from men, pregnant women can be a source of fetal membrane-derived mesenchymal stem cells. Shin Ishikane and colleagues describe the isolation and properties of these cells in chapter 10, and summarize their ability to modulate immune activity and to become a useful tool in regenerative medicine. The theme of mesenchymal stem cells is explored further by Arshak Alexanian in chapter 11, with a special emphasis on their ability to improve central nervous system repair.

Wherever and however they are obtained, stem cells have to be applied in a way that optimizes their ability to effect repair. Five chapters in this book focus on the application of stem cells to different clinical problems in the circulatory and the musculoskeletal systems. José Lamas and colleagues (chapter 12) address the potential of mesenchymal stem cells to treat osteoarthritis, a common and debilitating disease of joints, and outline current knowledge and future prospects for this important field. Namath Hussain and colleagues address another common and important chronic problem in chapter 13; that of debilitating lower back pain caused by damage to intervertebral discs. They explain the basic pathology of the disease and then review the results from the (very small) studies that have so far been conducted into the efficacy of stem cell treatment for disc damage in humans. Marianna Karagianni and colleagues consider a different problem in orthopaedics; the healing of defective bone. In chapter 14, they also examine the regulatory frameworks that govern the use of 'advanced therapy medicinal products' and consider how these frameworks shape research and development. Regeneration of bone is also addressed by Dilawhare Khan, Arnaldo Santos and their colleagues (chapters 15 and 16) . Khan *et al.* use an unusual source of stem cells - teeth - and having demonstrated that these show promise, they make the suggestion that milk tooth stem cells could perhaps be banked for a patient's later use. Santos *et al.* provide a wide-ranging review of different approaches to the regeneration of bone including, but not restricted to, the use of stem cells. Their chapter could have appeared in almost any section of this book: it was included as a final chapter of the stem cells section to highlight the need to compare stem cell approaches with the best of other techniques, because there is arguably a tendency, at the moment, to place undue emphasis on some ways of effecting repair perhaps to the detriment of developing others that may even show more promise in existing clinical trials.

One theme that emerges from a large number of these chapters is that studies of the effects of stem cell treatment in humans are few, and tend to be small. Perhaps because of their small size and consequent low statistical power, these studies frequently produce contradictory results. This is not helped by the lack of standardization in how experiments are performed and assessed, which makes meta-analyses problematic. Overall, it is clear that, in most areas of application, it is still far too early to decide whether stem cell treatments really are a means of effective cure and repair, or whether other approaches, such as those described in section 3 below, will actually prove more useful.

4. Construction of scaffolds

Where there are large-scale defects in tissues, caused either by injury or by congenital abnormality, simple stem cell treatments - however well they can be made to work - are unlikely to be able to make a proper repair. In terms of directly producing new tissues, stem cells are expected to work by recapitulating the processes of natural development or tissue maintenance. Embryonic development tends to take place at small scale and tissues then grow; when an embryo first makes a trachea, for example, it is less than a millimetre long, not the many centimetres it is in adult life. Also, many embryonic events depend on signals from other embryonic tissues that move or disappear by birth. There are therefore good reasons that a stem cell, even in a state that corresponds perfectly to the cells that would make a tissue in an embryo, would not be able to make it in an adult. Similarly, the stem cells concerned with tissue maintenance are regulated by the environment of the tissue in which they are situated and there is no reason to assume that they can rebuilt tissues across a large gap or scar, in which this environment is missing. In the case of genetic abnormality, the cells may be incapable of making the body part normally anyway. For all of these reasons, there is a strong argument that large scale regeneration requires the construction of scaffolds to bridge gaps and to control cell behaviour.

Bone tissue, which in its mature form is mostly inorganic matrix, lends itself to a scaffold-based approach. In chapter 17, Magdalena Cieślik and colleagues compare the ability of different matrices, based on the natural structure of bone with additional components such as bioglasses, to promote effective bone repair. On a related topic, Peter Emans and colleagues propose, in chapter 18, the use of scaffolds designed with the normal developmental process of endochondral ossification in mind. The chapter includes a critical review of clinical trials (which, as with much regenerative medicine, are less effective than original experiments gave hope to believe).

Tissue engineering of soft tissues involves different considerations, such tissues generally being much more flexible and much more cellular in terms of the ratio of cells to surrounding matrix. Chao Feng and Yue-min Xu illustrate this in chapter 19, where they explore techniques for reconstructing the lower urinary tract. They compare different scaffold materials, such as fleeces, sponges and advanced materials that include signalling molecules, and consider techniques for populating them with cells before their use. The chapter includes a review of clinical data on reconstruction of human bladder and urethra, with an encouraging rate of success.

In the last chapter in this section, chapter 20, Abir El-Sadik connects the rapidly developing field of tissue engineering with another 'hot topic': nanotechnology. Nanotechnology is young and still raises significant safety concerns so there is little clinical data, but the chapter explains the ways in which, in principle, nano-materials can modulate the function of stem cells and other tissue cells. It also illustrates, using nerve regeneration as an example, how scaffolds that incorporate nano-materials can promote useful neuronal growth and decrease glial scarring in experimental systems.

5. Assessing tissue regeneration

Having ideas about how to improve tissue regeneration is all very well, but it is essential both to the process of research that evaluates these ideas, and also to the proper care of a patient undergoing regeneration, that progress can be monitored, evaluated and perhaps even predicted. The two chapters in the last section of this book address this specific issue.

Magnetic resonance imaging is an excellent non-invasive technique that can provide high-resolution images of any part of the body. In chapter 21, Miyata illustrates how the ability of advanced MRI systems to provide quantitative information, particularly on the state of water and whether it is a free liquid or mainly bound to glycosaminoglycans, can be used to monitor the state of cartilage. This offers both researchers and clinicians an opportunity to monitor the progress of regenerative treatments over a long time-course, optimizing care and leading the way to patient-specific treatment regimes. It is possible that this approach will be extended in future to monitoring events in soft-tissue repair.

In the final chapter, Vermolen and van Rijn take an approach very different to most other authors in this book: they describe mathematical models of the processes involved in the 'healing' (by scar formation) of accidental wounds, particularly burns. The interest in the biophysics of scar formation is not simply academic and 'basic science' because, as the authors point out, creating tools that can *predict* the natural outcome of a patient's specific wound could be very useful to the design of a patient-specific programme of treatment designed to resolve that wound with as little aesthetic impact as possible. In particular, it can help physicians apply the right boost to regeneration (by whatever method) for each part of the wound, navigating successfully between the Scylla of overgrowth and the Charybdis of under-regeneration and consequent scarring, neither of which would be aesthetically satisfactory.

6. Using the chapters of this book

The whole philosophy of this multi-author book, and of its publisher, has placed unusual demands on both the authors and the editor. Unlike a conventional volume, which can only be bought or borrowed in its entirety, this book can be viewed two ways. The first is the traditional one - purchase of a single bound volume containing everything in order. The second is downloading of individual chapters from the Internet, using the Open Access model. There is much to be said for this, not least by clinicians and researchers who do not work in rich institutions that are blessed with a large library.

The knowledge that the authors are writing both for a traditional, whole-book-owning readership and for readers who may view just one chapter has, however, presented the Editor with an unusual problem: that of judging how much introductory material is necessary for a chapter to be readable in its own right, and how much can be left to the authors of other chapters. I hope that a sensible compromise has been struck, but am aware that there has had to be some repetition of some introductory material from chapter to chapter. In a book that could only be obtained as a whole, this repetition would have been removed. The editor and authors trust that readers will understand why some has had to remain, given the necessity for each chapter to stand alone.

The chapters in this book were written only a short time before publication and represent a very up-to-date overview of the field. Regenerative medicine is moving so quickly, however, that the authors expect some details of their material - especially reviews of human trials - to become out of date in only a few years. Depressing as a work's rapid obsolescence might seem to be for an author, really we rejoice in the fact, for it is the mark of a vibrant and fast-moving field that promises to have a significant impact on twenty-first century medicine. It is our hope that this book may inspire some of the new work that will one day make it obsolete.

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