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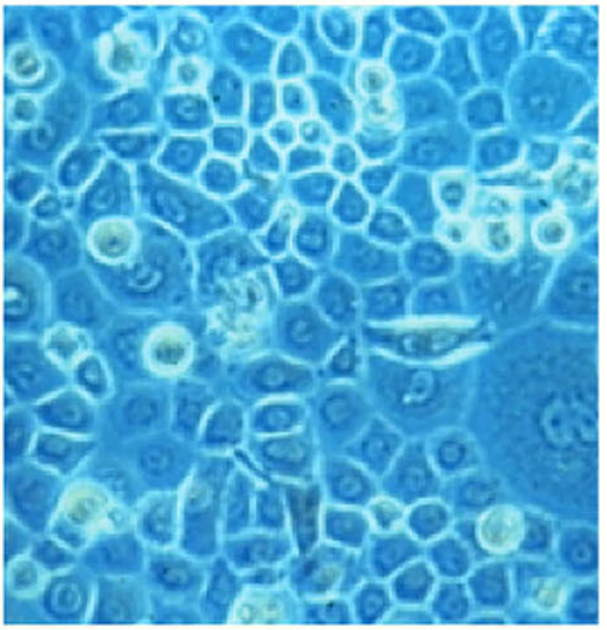

TISSUE ENGINEERING

SENIOR EDITOR
CLEMENS VAN BLITTERSWIJK

AP

Tissue Engineering

A volume in the

ACADEMIC PRESS SERIES IN BIOMEDICAL ENGINEERING

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Tissue Engineering

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Contents

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List of contributors

Anthony, Edwin, Queen Mary's University of London, UK

van Apeldoorn, Aart, University of Twente, Enschede, The Netherlands

Atala, Anthony, Wake Forest University of Medicine, Winston-Salem, USA

Azevedo, Helena, University of Minho, Guimarães, Portugal

Baaijens, Frank, Eindhoven University of Technology, Eindhoven, The Netherlands

Badylak, Stephen, McGowan Institute for Regenerative Medicine, Pittsburgh, USA

Barrère, Florence, University of Twente, Enschede, The Netherlands

van den Beucken, Jeroen, Department of Periodontology & Biomaterials, Radboud University Nijmegen Medical Center, The Netherlands

van Bezooijen, Rutger, Leiden University Medical Centre, Leiden, The Netherlands

Bianco, Paolo, Universita' la Sapienza, Roma, Italy

van Blitterswijk, Clemens Antoni, University of Twente, Enschede, The Netherlands

de Boer, Jan, University of Twente, Enschede, The Netherlands

Both, Sanne, University of Twente, Enschede, The Netherlands

Brittberg, Mats, University of Göteborg, Göteborg, Sweden

Cancedda, Ranieri, Universita' di Genova & Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy

Claase, Menno, University of Twente, Enschede, The Netherlands

D'Amore, Patricia, Schepens Eye Research Institute, Boston, USA

Dalton, Paul, University of Southampton, UK

DeRuiter, Marco, Leiden University Medical Centre, Leiden, The Netherlands

Deschamps, Audrey, University of Twente, Enschede, The Netherlands

Dhert, Wouter, Department of Orthopaedics, G05.228, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

van Dijkhuizen-Radersma, Riemke, Progentix BV, Bilthoven, The Netherlands

Ducheyne, Paul, Center for Bioactive Materials and Tissue Engineering, University of Pennsylvania, Philadelphia, USA

Feijen, Jan, University of Twente, Enschede, The Netherlands

Frey, Peter, Centre Hospitalier Universitaire Vaudois, Switzerland

van Gaalen, Steven, Department of Orthopaedics, G05.228, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

el Ghalbzouri, Abdoelwaheb, Leiden University Medical Centre, Leiden, The Netherlands.

Gibbs, Susan, VU Medical Centre, Amsterdam, The Netherlands

Gilbert,Thomas, University of Pittsburgh, Pittsburgh, USA

Gomes, Manuela, University of Minho, Guimarães, Portugal

Grijpma, Dirk, University of Twente, Enschede, The Netherlands

de Groot, Klaas, University of Twente, Enschede, The Netherlands

Habibovic, Pamela, University of Twente, Enschede, The Netherlands

Harvey, Alan, The University of Western Australia, Australia

Hierck, Beereend, Leiden University Medical Centre, Leiden, the Netherlands

Hirschi, Karen, Baylor College of Medicine, Boston, USA

Hodges, Steve, Wake Forest University Health Sciences, Winston-Salem, USA

Hubbell, Jeffrey Alan, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Hutmacher, Dietmar Werner, National University of Singapore, Singapore

Jansen, John, Department of Periodontology & Biomaterials, Radboud University Nijmegen Medical Center, The Netherlands

Janssen, Frank, University of Twente, Enschede, The Netherlands

Jukes, Jojanneke, University of Twente, Enschede, The Netherlands

Karperien, Marcel, University of Twente, Enschede, The Netherlands

Kruyt, Moyo, Department of Orthopaedics, G05.228, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

Kuleshova, Lilia, National University of Singapore, Singapore

Levenberg, Shulamit, Israel Institute of Technology, Haifa, Israel

Lewis, Jennifer, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Lindahl, Anders, Sahlgrenska University Hospital, Göteborg, Sweden

Malafaya, Patrícia, University of Minho, Guimarães, Portugal

Malda, Jos, Queensland University of Technology, Australia

Mano, João, University of Minho, Guimarães, Portugal

Martin, Ivan, University Hospital Basel, Switzerland

Meijer, Gert, Department of Oral Maxillofacial Surgery, University Medical Center Utrecht, The Netherlands

Mikos, Antonios, Department of Bioengineering, Rice University, Houston (TX) U.S.A.

Mistry, Amit, Department of Bioengineering, Rice University, Houston (TX) U.S.A.

Moroni, Lorenzo, University of Twente, Enschede, The Netherlands

Myers, Simon, Institute of Cell and Molecular Science, Bart's and University of London, London, UK

Myers-Irvin, J., University of Pittsburgh, Pittsburgh, USA

Navsaria, Harshad, Institute of Cell and Molecular Science, Bart's and University of London, UK

Ni, Ming, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, USA

Oliveira, Joaquim, University of Minho, Guimarães, Portugal

Olivo, Christina, Department of Immunology, University Medical Center Utrecht, The Netherlands

Oomens, Cees, Eindhoven University of Technology, Eindhoven, The Netherlands

Oudega, Martin, Johns Hopkins University School of Medicine, USA

Pennesi, Giuseppina, Universita' di Genova, Italy

Plant, Giles, The University of Western Australia, Australia

Poelmann, Rob, Leiden University Medical Centre, Leiden, The Netherlands

Post, Jeanine, University of Twente, Enschede, The Netherlands

Price, Richard, Institute of Cell and Molecular Science, Bart's and University of London, UK

Radisic, Milica, University of Toronto, Toronto, Ontario, Canada

Ratcliffe, Anthony, Synthasome Inc, San Diego, CA, USA

Reis, Rui, University of Minho, Guimarães, Portugal

Robey, Pamela Gehron, National Institutes of Health, Bethesda, USA

Roelen, Bernard Antonius Johannes, Utrecht University, Utrecht, the Netherlands

Rouwkema, Jeroen, University of Twente, Bilthoven, The Netherlands

Siddappa, Ramakrishnaiah, University of Twente, Enschede, The Netherlands

Silva, Gabriela, University of Minho, Guimarães, Portugal

Silva, Simone, University of Minho, Guimarães, Portugal

Sohier, Jérôme, University of Nantes, Nantes, France

Sousa, Rui, University of Minho, Guimarães, Portugal

Svalander, Peter, Swedish Biotechnology AB, Göteborg, Sweden

Tallheden, Tommi, Göteborg University, Göteborg, Sweden

Timmins, Nicholas, University Hospital Basel, Switzerland

Vunjak-Novakovic, Gordana, Columbia University, New York, NY, USA

Welin, Stellan, Professor Biotechnology Culture Society, Department of Health and Society, Linköping University, Sweden

Wendt, David, University Hospital Basel, Switzerland

Williams, David, University of Liverpool, Liverpool, UK

Woodfield, Tim, University of Twente, Enschede

Yaszemski, Michael, Department of Orthopedic Surgery, Mayo Clinic College of Medicine, Rochester (MN), USA.

Zhang, Zheng, University of Twente, Enschede, The Netherlands

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Foreword

Tissue engineering is an extremely important area. It generally involves the use of materials and cells with the goal of trying to understand tissue function and some day enabling virtually any tissue or organ on the body to be made *de novo*. To achieve this very important long-range objective requires research in many areas. This book, edited by Professor van Blitterswijk, addresses many of these important topics, and the chapters provide a foundation for the understanding and development of the cell-based systems needed for tissue engineering. One of these areas involves the cells themselves; in this regard, important chapters on stem cell biology and cell sources, and various aspects of cell culture including harvesting, selection, expansion, and differentiation, are provided. Other important themes involving cells, such as cell nutrition and cryobiology, are also examined, as is cellular signaling.

Some of the chapters deal with embryology. Much can be learned from this field as it provides the basic guide as to how tissues can form. The book also discusses morphogenesis and how tissues are generated in the embryo and tissue homeostasis. This is very important for providing vital information as to how tissues can be created. Such chapters may also offer clues for the reader as to how tissue engineering may some day be more successfully accomplished.

There are a variety of very important materials issues in tissue engineering, which are discussed in the book. Cells adhere to the extracellular matrix material in the body; this matrix has an enormous affect on how the cells behave. However, to try to recreate extracellular matrixes, is a difficult task and therefore various materials have been explored to provide substrates for cell growth *in vivo*. The physical and chemical properties of these materials are examined, and important materials that might be used in tissue engineering are discussed. These include natural polymers, degradable polymers, and bioceramics. The book also examines the important issue of tissue compatibility and biomaterials compatibility which are critical if these tissues are to be safe and integrate with the body. In addition, scaffold design and fabrication are discussed so that the reader may have a better understanding of how to develop and manufacture these systems. Ways of using controlled release from materials is examined; controlled release of different factors (e.g. growth factors to promote vascularization) can provide an important means of controlling and improving tissue function.

Once the scaffold system and the cell system are developed, they have to be put together. This is generally done through a bioreactor where the cells and materials are combined with the right type of media and flow conditions inside the reactor. By combining all of these entities, a new tissue may be created. This important issue is also discussed.

The book then turns to important areas of tissue engineering with respect to case studies on individual tissues and organs. There are chapters that specifically discuss skin, cartilage, bone, the nervous system, and various organ systems. Finally, important ethical issues in tissue engineering are discussed.

Overall, this book provides a very useful guide for those who wish to understand important issues such as cell biology, materials science, and bioreactor design with respect to tissue engineering, as well as providing specific examples of how tissue engineering is accomplished.

B. Langer

EEN PSALM VOOR VEEL LATER
Systeem, neem aan dat mettertijd
ver achter deze eeuwigheid
geen ster, geen stof, geen licht meer schijnt
en, als Gij wilt, ook Gij verdwijnt,

ook dan nog vraag ik, nu en hier
doodgedrukt op dit papier:
Verschaft ons eeuwig nietmeerzijnd
U dan nog zulk plezier?

Zo niet, laat ons dan wederkeren
als uiterst anderen, want dan
zal ik wellicht nog eens proberen
of alles anders kan

A PSALM FOR MUCH LATER
System, say that as time will flee
far past this eternity
no star, no dust, no light lives on
and, if You wish, You too are gone,

I still can ask right here and now
imprinted on this sheet and dried
so eternally: Why, how,
no, if it still pleases Thee we died?

If not, let us return some day
very different from before
and Who knows, I'll try once more
some other way

Leo Vroman, scientist, poet and artist. Homo Universalis still exists

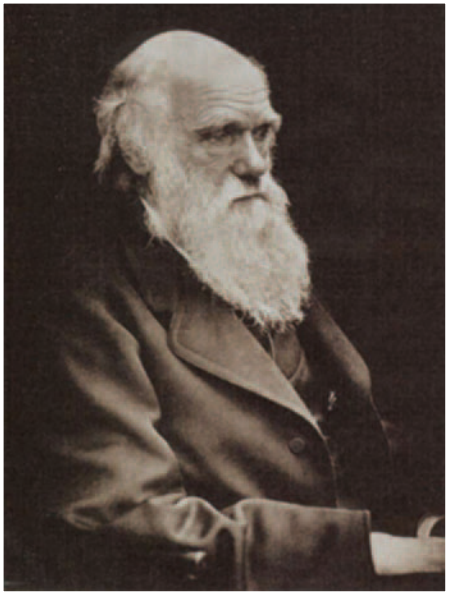
Tissue engineering – an introduction

Clemens Antoni van Blitterswijk, Lorenzo Moroni, Jeroen Rouwkema, Ramakrishnaiah Siddappa and Jérôme Sohier

LEEUWENHOEK.
London published Feb 06 1813 by C.Jones.

"Several times I wrote about bodies consisting of globules, but we must not imagine perfectly round globules, but a number of bladders of animals perfectly round, filled with water, and those bladders lying one next to the other on the earth. . . . and if a large number of these bladders were thrown in an empty barrel or packed tight therein, the round bladders would not maintain their shape but the said bladders would adapt themselves."

Anthoni Leeuwenhoeck, December 20, 1675. Letter addressed to the secretary of the British Royal Society (Leeuwenhoek, 1939)

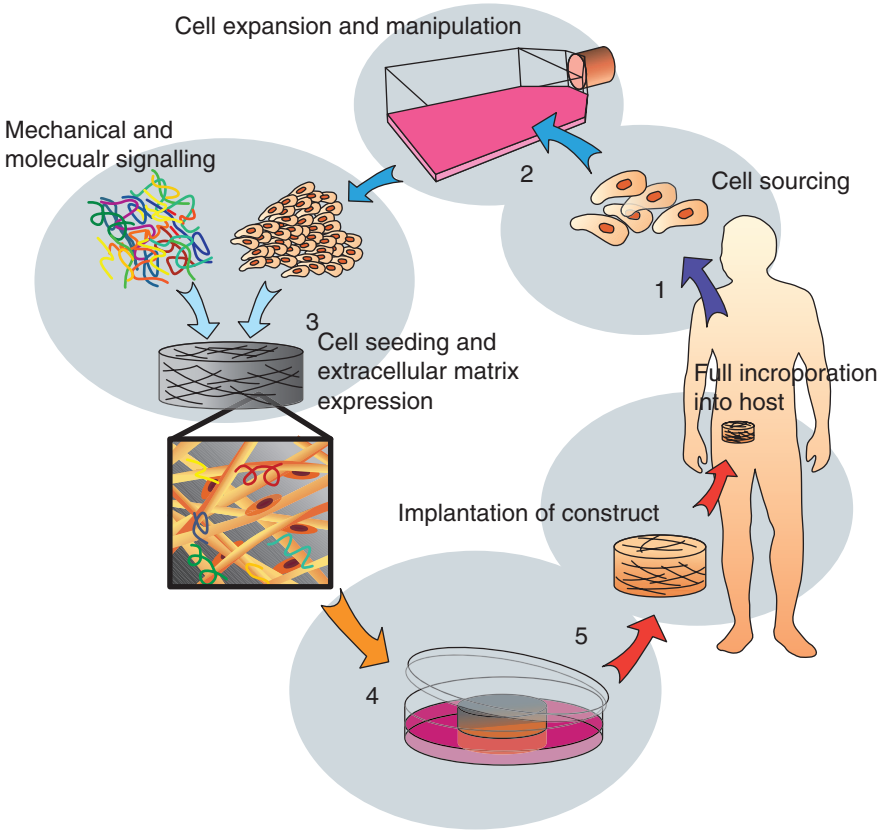


"Your aphorism that 'any remedy will cure any malady' contains, I do believe, profound truth—whether applicable or not to the wondrous Water Cure I am not very sure—The Water-Cure, however, keeps in high favour, & I go regularly on with douching &c &c:"

Charles Darwin, September 4. Letter to W.D. Fox. (Fox, 1850) *No.*

The field of life sciences moves forward at a rapid pace and many of us do not fully realize that this acceleration is a relatively recent phenomenon in the history of mankind. These days even the lesser-educated in our society are aware of the concept 'cell' and the possible benefits that applying isolated, expanded or even manipulated cells may have in healthcare. Nevertheless, the cell as a building block of organisms was unknown until scientists like van Leeuwenhoek in 1675 were able to see the first 'globules' through surprisingly powerful, though primitive, microscopes. Obviously, as they were entering a completely unknown world, where no one had gone before, many of the early assumptions these scientists made were bound to be proven erroneous today. In contrast, one can sometimes be truly impressed by the incredible abilities of these early scientists to grasp the essentials behind entirely new concepts.

It is almost impossible to imagine how van Leeuwenhoek (as quoted above) was able to translate the completely unknown microscopical dimensions of a previously imagined entity like the cell into the very correct example of bladders filled with water. Picture these bags packed tight together on the floor, or filling an empty vessel and the contemporary biologist sees the architecture of the epidermis or a gland. Even the adaptability and variability of the cell shape he already correctly foresaw. We should realize that



The central tissue engineering paradigm.

these thoughts were all triggered by data obtained through a new technology. Technology continuously shapes our society but sometimes also creates overly optimistic expectations, even among the smartest minds ever. If Darwin in his letter to Fox states that "*any remedy will cure any malady*" (Fox, 1850) this is a statement that could have been done by any ordinary person. The connotation of a 'water cure' gives the statement a comic appearance. But it also shows that even the best among scientists have a hard time to distinguish between real and fake cures. Almost 150 years later we are continuously confronted with similar expectations which are too optimistic where the influence of technology on healthcare is concerned. By the way, it is only fair to Darwin to explain that, further in the same letter, he strongly attacks alternative medicine in the following manner "*You speak about Homœopathy; which is a subject which makes me more wrath, even than does Clairvoyance: clairvoyance so transcends belief, that one's ordinary faculties are put out of question, but in Homœopathy common sense & common observation come into play, and both these must go to the Dogs, if the infinetesimal doses have any effect whatever*". If even the critical Darwin can be triggered into unrealistic expectations, then it is no wonder that healthcare hypes are a more than common phenomenon.

In 1997, media all over the world were aroused by a BBC documentary, *Tomorrow's World*, showing what is now known as the Vacanti mouse (Cao *et al.*, 1997). The term 'Tissue engineering' was no longer seen as an expression familiar only to a limited number of scientists working in the field – it had become well-known to millions of individuals worldwide. Although the Vacanti experiment (see Box 1) is truly exemplary for the discipline of tissue engineering, it is fair to say that the media upheaval was not so much caused by the actual experiment but even more by the spectacular sight of a nude mouse that had apparently grown a human ear on its back. For many, the *Island of Dr Moreau** (Wells, 1896) had become reality and media hype was born. This was not the first media hype on tissue or organ repair and most certainly will not be the last. It would not be difficult to dedicate an entire chapter in this textbook to the promising aspects of our discipline that made it to the media and had a major public impact. It is intriguing to observe that, in contrast, it would be difficult to fill an entire chapter with actual clinical successes. At first sight one would tend to say that the field has over promised and has a history of not delivering on those promises. This statement would be too simple. The eagerness of the media to report on the advances in the field of tissue engineering is not so much caused by publicity eager scientists; the actual cause is the enormous demand in our society for technologies that are able to repair, or even better regenerate, damaged or worn out tissues and/or organs.

On an annual basis many millions of patients undergo surgery for tissue reconstruction. A fair proportion are treated satisfactorily, another portion less effectively, and millions still await treatments that would help them at least in an acceptable way. A now almost classical analysis on the need and commercial opportunity was published in *Science* in 1993 by Langer and Vacanti, the abstract of which is given below.

*The *Island of Dr. Moreau* was written by HG. Wells in 1896 and is one of the early examples of a science fiction novel. On his island, Dr. Moreau applies vivisection techniques on animals to create hybrid organisms of animal origin with man-like properties. Interestingly, Wells already realizes that new technologies require new legislation and all of Moreau's monsters have to obey to the following law:

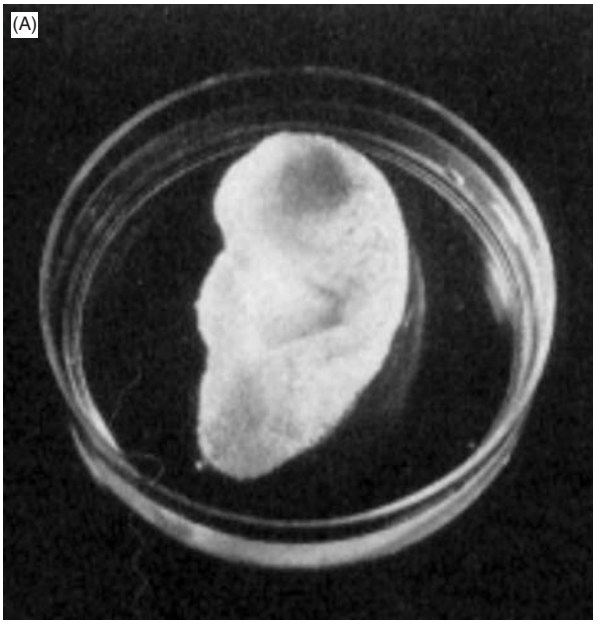
- *Not to go on All-Fours; that is the Law. Are we not men?*
- *Not to suck up Drink; that is the Law. Are we not men?*
- *Not to eat Fish or Flesh; that is the Law. Are we not men?*
- *Not to claw the Bark of Trees; that is the Law. Are we not men?*
- *Not to chase other Men; that is the Law. Are we not men?*

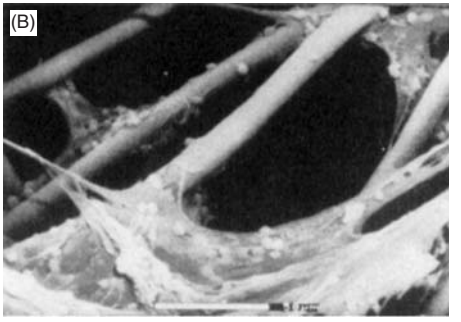
Box 1 The Mouse and the ear: tissue-engineered cartilage in the shape of a human ear

Although there have been several studies since the 1980s where the concepts of tissue engineering were first applied, a formal formulation of the discipline is traced back to the paper of J. Vacanti and R. Langer in *Science* (1993). Since then, the number of studies in tissue engineering has grown rapidly. A landmark study published in 1997 in *Plastic and Reconstructive Surgery* by Y. Cao *et al.* (1997) attracted the interest of a large audience; thanks also to a BBC service on the subject. In this paper it is shown how to successfully regenerate the cartilagineous part of a 3-year-old child's ear. The work is useful also from an educational point of view, as most of the 'ingredients' to perform a classic tissue engineering experiment are present.

To highlight the multidisciplinarity of tissue engineering the study can be divided into three parts:

- **Material Science**: first, a plaster mold of the ear of a 3-year-old child was cast from an alginate impression of the ear. Then, a 100 μm thick non-woven mesh of poly(glycolic acid) (PGA) was





Ear anatomically shaped scaffold. (a) scaffold structure before seeding; (b) SEM micrograph showing cells and extracellular matrix formed in the scaffold; (c) scaffolds implanted subcutaneously on the back of an immunodeficient mouse. Reproduced with permission from Cao, Y., Vacanti, J.P., Paige, K.T., et al., *(1997). Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear.* Plast Reconstr Surg, 100: *297–302.*

immersed in a 1% weight/volume solution of poly(lactic acid) (PLA) in methylene chloride for 2 seconds and subsequently shaped into the plaster mold.
- **Biology**: calf chondrocytes (cartilage cells) were harvested from the articular surfaces of a calf, isolated by collagenase digestion, filtered, washed in cell medium, labeled with BrdU to control their viability, and seeded onto the polymeric scaffolds (1.5×10^8 cells in total). The constructs were cultured *in vitro* for 1 week in an incubator under physiological conditions ($T = 37°C$; $p_{co_2} = 5\%$) and then implanted into a subcutaneous pocket on the back of athymic mice. Three groups of scaffolds were considered: (I) scaffolds seeded with cells; (II) scaffolds reinforced with an external stent and seeded with cells; (III) externally stented scaffolds with no cells.
- **Biochemistry**: after 12 weeks, the constructs were explanted, sectioned, and histologically stained with specific markers for typical cartilage extra cellular matrix components (hematoxylin and eosin for general tissue formation; alcian blue for glycosaminoglycan deposition; Masson's trichrome for collagen formation). Immunohistochemistry was also performed to confirm that present collagen was specific for cartilage (type II).

The results showed extensive cartilage formation in the scaffolds that were seeded with cells, while no cartilage was present in the unseeded scaffolds. Furthermore, scaffolds reinforced with an external stent for the first 4 weeks of implantation maintained the anatomical shape of the ear. In contrast, the other scaffolds lost partially their integrity and appeared of reduced size and distorted shape. From these findings the scientists concluded that cartilage formation is not mature enough in the first 4 weeks to counteract the contraction forces in the healing process.

This experiment was surely a success for those years and definitely contributed to boost the interest in the field. If we consider the state of the art nowadays, however, a number of drawbacks still characterize this study, some of which are mentioned here:

- Skin coverage is missing and is a critical element of any ear reconstruction.
- Bovine immature (young) chondrocytes were used, while clinical application thereof is of course highly unlikely and these cells are now known to be partially unrepresentative for the use of human cartilage cell sources. Such human sources tend to loose differentiation capacity quite fast and are furthermore frequently characterized by necrosis in the center of scaffolds with a clinically relevant size.
- An athymic or immunodeficient mouse model is used here. Obviously, this is nothing more than a useful screening model and large animal models are required to test clinical relevance in the presence of a functional immune system.
- Scaffolds need to provide an adequate mechanical stability to the construct at the time of implantation;
- Implications on the growth rate of the artificial ear compared to the growth rate of a 3-year-old child should be addressed before the final implantation in the patient.

The loss or failure of an organ or tissue is one of the most frequent, devastating, and costly problems in human health care. A new field, tissue engineering, applies the principles of biology and engineering to the development of functional substitutes for damaged tissue. This article discusses the foundations and challenges of this interdisciplinary field and its attempts to provide solutions to tissue creation and repair.

Langer and Vacanti (1993)

If the need is indeed so high, and the field has so extensively grown over the last decade, then why do we still lack frequent clinical successes? There are two

major reasons for this phenomenon. First, bringing a relatively simple medical device from initial idea to a widespread clinical reality frequently takes a minimum of 10 years. As the underlying technology for developing tissue engineering products is less mature, and possibly more complex, it is to be expected that clinical progress in this field will be measured in decades rather than years. Second, tissue engineering is truly a multidisciplinary field where acquired knowledge from individual classical disciplines (e.g. quantum physics, polymer chemistry, molecular biology, anatomy) no longer suffices to make substantial leaps. Individuals active in this field will have to acquire multidisciplinary skills and be willing to look over the borders of their home discipline. The relatively young age of the field does not make it easy to acquire those skills as dedicated textbooks are still scarce and frequently do not address the appropriate audience by either offering a collection of research papers or by only dealing with a selected part of the entire discipline. Without the widespread availability of such textbooks it is to be feared that the rapidly increasing number of graduate courses on tissue engineering may not be as effective as required, which may hamper the development of the field of tissue engineering into a mature scientific discipline.

With this in mind, in 2004 it was decided that it was time to bring together a representative group of internationally active scientists who would be willing to submit chapters for this book, which would address both the multidisciplinary nature of tissue engineering and the underlying base disciplines. During the process, we dedicated ourselves to offering chapters that are not so much complete overviews of the individual sub-disciplines, but rather, per chapter, would offer a small general introduction followed by more in-depth text on issues particularly relevant to tissue engineering. For instance, instead of having a general chapter on cell biology (which could automatically become superficial and partially redundant), we chose to present individual aspects of cell biology in numerous chapters, like those on Stem Cells, Morphogenesis and Cellular Signaling. Furthermore, in a chapter dealing with, for example, Cellular Signaling, we did

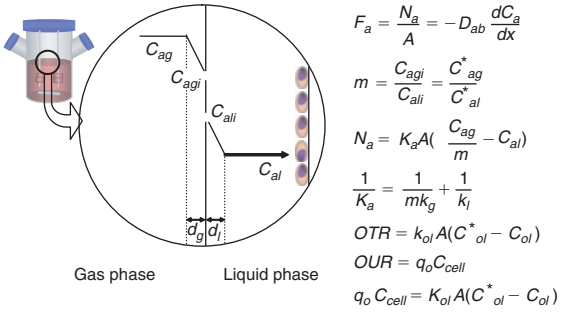


Figure I.1 A glimpse at mass transport theory.

not aim for a complete overview of all intracellular pathways. Instead, we concentrated on only a few aspects which are particularly relevant to the field of tissue engineering, and which would simultaneously provide both a general understanding for the working mechanism of most pathways, as well as offer sufficient depth for the few pathways which are discussed in some detail. Ideally, such descriptions would provide fundamental knowledge that can be used for the appropriate understanding of other chapters; like describing BMP signaling for later use in the chapter on Tissue Engineering of Bone.

This still leaves the challenge that the book will be used by students with different backgrounds and in different stages of their training. To accommodate this, we have selected a format where use is made of separate text boxes. Typically, these will address 'classical experiments', 'state of the art experiments' or other dedicated topics which a student or teacher may select at will to provide deeper insight. Sometimes, chapters will offer information that is, for instance, clearly essential for a group of biomedical engineers but might not be fully necessary for the average medical student. A clear example of that is the paragraph on mass transport in the Cell Nutrition chapter (Figure I.1). Understanding all these formulae clearly contributes to understanding the working mechanism of a bioreactor and may even be self evident for a process engineer; whereas such information may be superfluous for a medical student or molecular biologist venturing into the field of tissue engineering. For optimal use of the book,

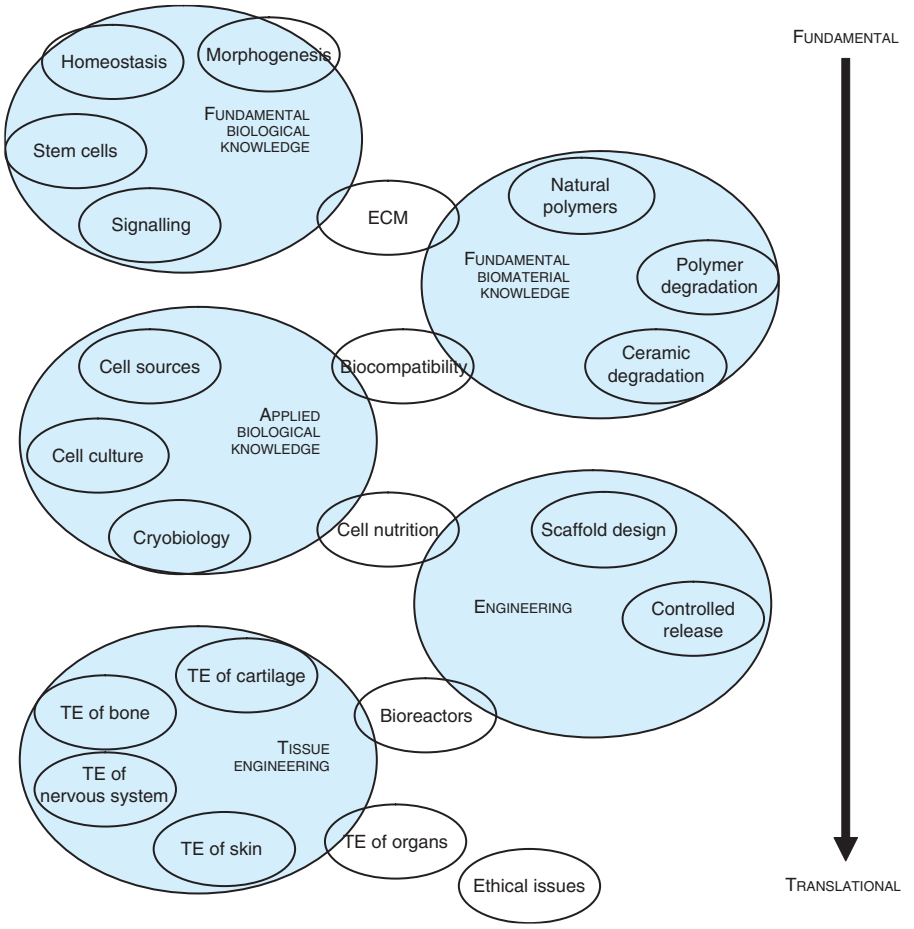


Figure I.2 Diagram sketching the layout of the book (groups biology, technology and evaluation form basic to clinical practice.

both teacher and student will have to make the appropriate selection of what is offered to them. In all instances we have tried to make the text accessible as educational material as opposed to standard scientific publications.

At this stage we were faced with the issue of which subjects to select for publication in this particular book. In essence, in a very basic approach, tissue engineering can be divided into both biological and engineering parts. In principle, most chapters can be placed into one of these categories. However, we decided to group the chapters in such a way that these related topics are presented while being alternated by clusters from the other disciplines. This avoided one part of the book being completely biological while another part focused only on engineering aspects. In general, the structure of the book moves from fundamental to translational or even clinical (Figure I.2). In this way, the reader will first be confronted with the fundamentals of the cell type that makes tissue engineering truly a part of regenerative medicine: the stem cell. **Stem Cells** (Chapter 1) gives insight

into the different aspects of this cell and will show that the stem cell is not a single cell type but, in reality, encompasses different categories of cells ranging from the multipotent embryonic stem cell to the apparently less potent adult stem cell. With this knowledge, the reader will subsequently be guided to **Morphogenesis** (Chapter 2) where the formation of tissues in the embryo is discussed. Although most tissue engineers do not venture into the realms of developmental biology and research with a strong focus on developmental biology is all too scarce in tissue engineering, we feel this is an omission and hope that this chapter will urge young scientists to enter into such an essential area. Obviously most tissue engineering constructs will not be implanted into embryos but into human beings after birth. As tissue formation is essentially different in a post-natal tissue when compared to that in the embryo the authors of **Tissue Homeostasis** (Chapter 3) provide insight into how tissues are maintained after birth. Tissues in both the embryo and in an individual after birth usually, although not always, contain multiple cell types. Understanding how these cells interact is recognized as pivotal for the success of tissue engineering. **Cellular Signaling** (Chapter 4) provides such insights. Furthermore, in spite of all media attention that befalls stem cells, in reality cells represent only a small part of the dry weight of living tissue. All, or at least most, cells interact with an extracellular matrix, which, in contrast to the errant opinion of some engineers and even biologists, presents much more than mechanical support and adds substantially to the biological interactions in our body. As tissue engineering typically combines scaffolds with biologically active components as cells or growth factors we felt that a chapter on the biological equivalent: **Extracellular Matrix** (Chapter 5) could not be missed.

At this point in the book a shift is made to the more fundamental engineering aspects. Since not all scaffolds and matrices are completely synthetic, a chapter on **Natural polymers** (Chapter 6) seemed in place as well. Most tissue engineers, actively involved in the design of both synthetic and natural scaffolds or matrices, prefer to have these degrade after implantation. This is with good cause as the prolonged presence of foreign material in the body may induce a variety of unwanted effects such as implant-associated infection or mutagenesis. Two groups of authors discuss the aspects of implant degradation and this is done in two related chapters: **Degradable polymers** (Chapter 7) and **Degradation of Bioceramics** (Chapter 8). These chapters provide deeper insight into the interactions between materials and a living system; particularly in relation to their function and the release of degradation products. These properties are some of the crucial aspects of influence for the biological performance of a material that interacts with a living system and are discussed with other relevant aspects in the chapter on **Biocompatibility** (Chapter 9), a true interface between biology and engineering.

After considering the above, more fundamental, aspects, the text now gradually moves to knowledge that bears direct practical relevance for the actual process of tissue engineering. In addition, chapters start to increasingly show the multidisciplinary nature of the field. As already explained in the chapter on Stem Cells (Chapter 1), there are different cells that can be used for generating tissues. The chapter on **Cell Sources** (Chapter 10) elaborates further on this subject and also touches on the issue of using autologous versus donor tissue. Having selected a cell source, the researcher now has to obtain these cells in appropriate numbers and sufficient purity and if necessary trigger the cells into the right differentiation, **Cell Culture: Harvest, Selection, Expansion and Differentiation** (Chapter 11) deals with this subject. In order to achieve these goals one will always have to bring sufficient nutrients to the cells. **Cell Nutrition** (Chapter 12) focuses on the various aspects that are pivotal for the understanding of this subject. Theoretically, after having obtained sufficient cells of the right type and state of differentiation, one now has a choice: immediately continue the process towards implantation or first postpone. Choosing to postpone may have several reasons: it would allow a surgeon to set a different date of surgery or, in view

of logistics, might provide an 'off the shelf' product. The most frequently used method for facilitating the postponing of implantation is freezing, as discussed in **Cryobiology** (Chapter 13).

Although some applications would involve direct injection of cells into the tissues that one intends to regenerate, most researchers active in the field of tissue engineering, and working with cultured cells, strive for implanting combinations of cells and scaffolds. These so-called hybrid constructs will usually have three dimensions and need to give access to both cells and nutrients or have to allow an out flux of active ingredients and waste products. The chapter **Scaffold Design and Fabrication** (Chapter 14) presents the reader with the different ways to manufacture scaffolds and explains the criteria these scaffolds have to fulfil. The design of scaffolds may also involve incorporating biologically active ingredients that can then be made available to either the cells in the construct or be released to initiate effects in the tissues of the organism into which the device was implanted. The various aspects of such release systems are presented in the chapter on **Controlled release strategies** (Chapter 15). Ideally the researcher working on a hybrid construct and following the chapters, as they have been presented so far, now faces one of the main obstacles of tissue engineering: how to initiate cell growth and extracellular matrix formation in three-dimensional constructs of clinically relevant dimensions (usually centimeters versus millimeters in many *in vitro* and animal studies). These challenges, fundamentals and some solutions are discussed in **Bioreactors for Tissue Engineering** (Chapter 16).

Although not complete or covering all of the subjects, for which one could argue would be essential, the editors feel that these first 16 chapters (as well as this chapter), provide a solid basis for students who wish to acquire an understanding for tissue engineering. Much of the international research effort based on tissue engineering is along the lines presented in these chapters. But it is also fair to say that some of the major challenges are still to be found into making tissue engineering a widespread clinical reality. These clinical aspects are treated in the remaining six chapters.

In making a choice on which clinical applications to discuss, the editors faced a difficult task. Researchers worldwide are currently focusing their attention on the repair or regeneration of many tissues and organs. Almost certainly most, if not all, of them deserve to be presented in this book, but this would lead to an unacceptably thick volume or a lack of sufficient depth per chapter. In selecting the subjects we felt that those tissues that historically moved first into the tissue engineering arena, and have actually found relatively widespread application, certainly deserve to be dealt with here. We feel, therefore, that most would agree that **Tissue Engineering of Skin** (Chapter 17) and the **Tissue Engineering of Cartilage** (Chapter 18) chapters warrant addition to this book on the basis of such historical observations. Another way of selecting which chapters to include involves the shear size of the tissue research field. After all, some of the students using this volume will actually find employment in tissue engineering and chances are that most will end up in the biggest segments. Over the years, it has become clear that both musculoskeletal- and cardiovascular diseases attract many tissue engineers who work towards overcoming the negative affects of these diseases in our society. Characteristically, they are always well-represented at tissue engineering conferences and this explains our choice for **Tissue Engineering of Bone** (Chapter 19). Relevance could be another selection criterion. Neural diseases would be an example of this approach. Not only is this a relevant research area, but the prospect that one day tissue engineering might contribute to relieving the problems of those suffering spinal cord lesions is truly inspiring – hence a chapter on **Tissue Engineering of the Nervous System** (Chapter 20 and Figure I.3). Any scientist, of some reputation, working in the field of tissue engineering will be frequently confronted with a question like: *When will you grow a complete heart or liver?* Many of us may feel that these are not the biggest priority; after all it would be much better to regenerate the damaged part of an organ rather than substituting it fully. Nevertheless, substituting organs is a challenge, if only since it forces us to combine

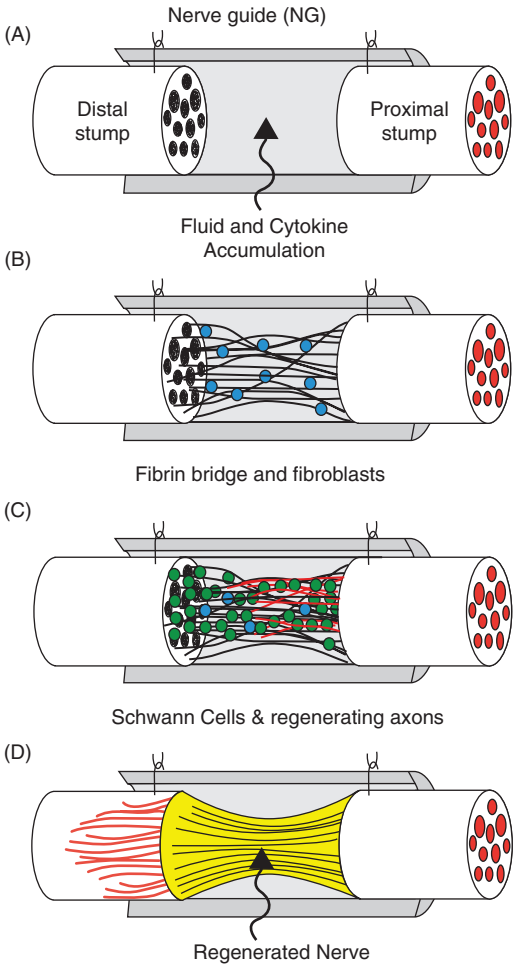


Figure I.3 Guided nerve regeneration.

multiple tissues into one system. In a sense one might say that tissue engineering can be compared to say, a house where the elements provided (i.e. windows, doors, etc.) need to fit together to create a home. This convinced us to contribute a chapter on **Tissue Engineering of Organ Systems** (Chapter 21).

There are some areas that may not directly involve actually generating a tissue or organ substitute, but are still pivotal to the overall development and acceptance of the field. Therefore, the editors decided to add one more chapter. Ultimately, tissue engineering constructs have to be placed into a human recipient. This puts major responsibility on the researchers and clinicians that are actively involved in this process. Naturally, a minimum requirement would be that the construct is safe and may not harm the patient; preferably it would be more effective than existing treatments. Apart from this, an important issue for any new technology, which is frequently underestimated by scientists, concerns the ethical aspects of such technologies. It requires little imagination to see that tissue engineering would certainly rank among those disciplines most prone to ethical scrutiny. Not only should we be sincerely interested in the ethical aspects of our field but we should thoroughly realize that public acceptance, and the ethical debate related to that, will be an important determinant for the widespread application of the technology we develop. In view of this, **Ethical Issues on Tissue Engineering** (Chapter 22) has been included. A careful reader may have noticed that this chapter does not start by giving a definition on tissue engineering, but a schematic overview. This was a deliberate choice. Very often students are confronted with definitions which they are expected to accept as the ultimate truth. In reality, any definition has both supporters and opponents, and this is certainly true in the field of tissue engineering. Therefore, instead of expecting everybody to agree to the same set of definitions, we have endeavored to encourage the reader to use the definitions in which he or she believes.

Finally, there are two further chapters which have not made it into this book, but which are equally as interesting and important as those found here. Both of these chapters can be found on our website by going to: http://books.elsevier.com/companions/9780123708694. Attention is given to a chapter called **Physico-chemical Properties of Synthetic Scaffolds and Matrices** in parallel to the biological extracellular matrix. This chapter provides a general overview of the types of synthetic matrices and scaffolds in tissue engineering and their properties. A further chapter, **Cardiovascular Tissue Engineering**, deals with musculoskeletal- and cardiovascular diseases and the negative effects of these diseases in our society.

It is the hope of the editors that the chapters that have been included both in this book, and online, will provide a solid basis in tissue engineering. Apart from explaining what the purpose of this textbook is, and how we tried to achieve this goal, this introduction chapter might further be useful in that it should exceed the purpose of the other individual chapters. While each of the remaining chapters focuses on a particular element of tissue engineering, they are not specifically dedicated to giving an overall view of the field. More particularly, all those areas where developments take place are both exciting and crucial for the further development of our discipline.

In treating some of these aspects it may become clear where the field is moving towards. Not only will it reveal that indeed in the years to come tissue engineering may likely contribute to the well-being of millions, which gives justification to the field being there at all, but it will also provide something else: *Tissue Engineering is fun!* This is a subject frequently forgotten but not justifiably so. It is relevant that researchers not only find justification in their efforts leading to the benefits of society but have fun on the way as well. After all, a subject like tissue engineering is quite adventurous and usually involves combining knowledge of different disciplines into one research project. The complexity of such combinations makes the field intellectually challenging and the possibilities are such that there is ample space for individual efforts still to be recognized. The fact that a successful experiment may bring the technology closer to clinical reality subsequently provides further rewards. That this opinion is also shared by those not professionally active in our field was illustrated by a *Time* Magazine article, around the turn of the century, ranking the 'Tissue Engineer' among the hottest future professions of the twenty-first century (Time Magazine online, http://www.time.com/time/magazine/article/0,9171,997028,00.html).

Let us look back at some of the early statements at the beginning of this chapter. Many people active in the field feel we are progressing too slowly and wonder if we have not been over-promising. We should realize that comments on the slow progress of the field can only concern the apparent lack of clinical success.

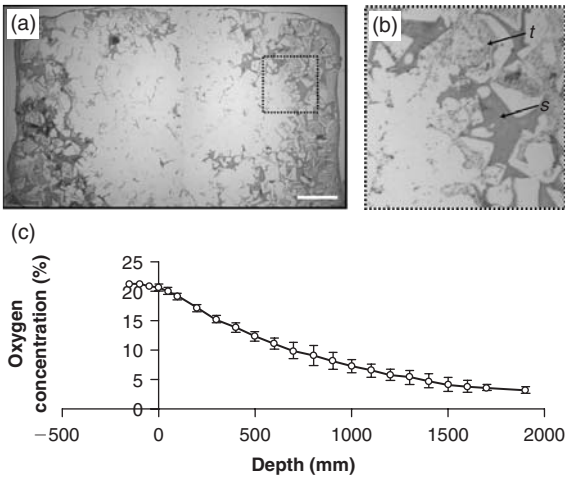


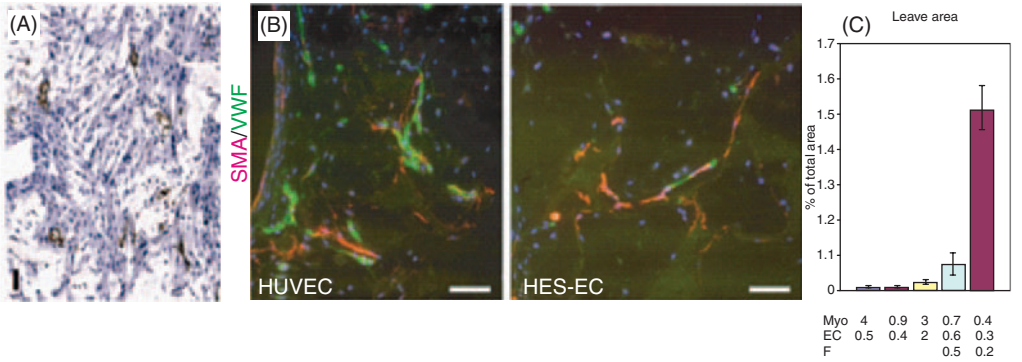
Figure I.4 Insufficient nutrition transport is one of the main problems that Tissue Engineers have to face (scale bar = 1 mm).

Science is progressing and at a rapid rate. In this perspective, it might be useful to look again at the Vacanti mouse (Cao *et al.*, 1997, Box 1). At the time this was a state of the art experiment. A cast of a human ear formed the fundament for a porous nonwoven poly(glycolic acid) (PGA)/poly(lactic acid) (PLA) scaffold which was subsequently filled with cultured cartilage cells. Then the hybrid construct was placed under the skin of an immuno-deficient mouse. Irrespective of the outcome of the specific Vacanti experiment, we now know that such an approach would, in general, present major problems. One reason being that implants of a clinically relevant size in general do not allow optimal nutrient transport to the cells in the center of such scaffolds, as illustrated in Figure I.4. After implantation this lack of nutrients is directly related to poor and late ingrowth of blood vessels into the scaffold. At the time, this was a fundamental obstacle and even today it still presents a significant challenge. However, we are progressing. To illustrate this we should introduce the Levenberg mouse (Levenberg *et al.*, 2005) (Box 2) – less well-known than that of Vacanti, the Levenberg mouse is perhaps even more interesting. The concept of this study was that a carefully generated hybrid construct of which the pores

Box 2 Cells nutrition: engineering vessels

One of the major limitations of tissue engineering is the inability to provide a sufficient blood supply in the initial phase after implantation. As long as a proper vascularization has not been established, the implant has to rely on diffusion for the supply of nutrients and the removal of waste. This can lead to nutrient limitations, which can result in improper integration or even death of the implant. Since tissue engineering has primarily been performed on implants of small dimensions for research purposes, the impact of this problem has so far been limited. However, in order to grow tissues on a larger, clinically relevant, scale, this problem has to be dealt with.

Several strategies to enhance early vascularization, for instance the targeted delivery of angiogenic growth factors, have been studied. However, these strategies still rely on the ingrowth of host endothelial cells and therefore vascularization will still take a considerable time. This paper describes a novel approach to enhance vascularization after implantation. Endothelial cells (HUVEC or endothelial cells from embryonic stem cells) were added to engineered muscle tissue, which lead to the formation of a vascular network inside the muscle tissue *in vitro*. The prevascular network was stabilized by adding smooth muscle precursor cells (mouse embryonic fibroblasts, MEF).

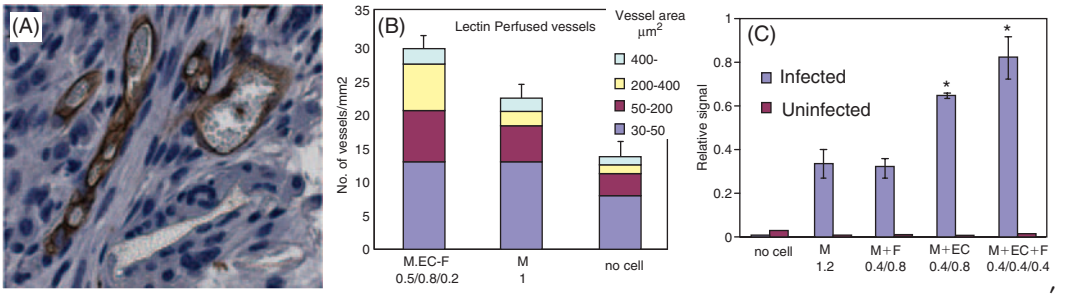


In vitro results after 10 days. A, Endothelial cells (brown) organize into vascular structures containing lumen; B, MEF differentiate into smooth muscle cells (red) and colocalize with endothelial cells (green); C, effect of different seeding ratios on the formation of vascular structures. Myo = myoblasts, EC = UVEC and F = MEF. Number of cells in millions seeded per scaffold. Note the positive effect of the addition of MEF. C, Reproduced with permission from Levenberg, S., Rouwkema, J., et al. *(2005). Engineering vascularized skeletal muscle tissue.* Nat Biotechnol, 23: *879–884.*

After implantation, the muscle tissue integrated properly with the surrounding tissue, indicating that the added vascular structure did not have a negative effect on the differentiation of the muscle tissue. Moreover, the prevascular network fused with the blood vessels of the host and became functional vessels transporting blood. This leads to enhanced vascularization and better survival of the implant.

This study shows that *in vitro* prevascularization can be a successful strategy to improve vascularization after implantation. Which means that this technique could be a way to solve the problem of nutrient limitations in large tissue constructs after implantation. It is likely that this strategy does not only

work for muscle tissue engineering, but could be used as a more general technique to successfully engineer diverse tissues with clinically relevant sizes.



In vivo results after 2 weeks. A, Human vascular structures (brown, human-specific CD31 staining) have connected to the vasculature of the host and carry blood; B, quantification of the amount of perfused vessels after 2 weeks of implantation. Note that prevascularized samples (M + EC + F) contain more perfused vessel; C, survival of cells in the implant determined with a luciferase assay. Note that prevascularized samples (M + EC and M + EC + F) display a better survival (confirmed with TUNEL assay). B and C, Reproduced with permission from Levenberg, S., Rouwkema, J., et al. *(2005). Engineering vascularized skeletal muscle tissue.* Nat Biotechnol, 23: *879–884.*

were filled with cultured tissue still faces a major risk after implantation. Where, during culture nutrients can be forced through the scaffold by processes such as agitation or perfusion, this usually is no longer the case after implantation. In most cases the scaffold will be dependent on nutrients coming from blood vessels. In an average natural tissue, cells will typically find such a source of nutrients (a capillary) within roughly 200 microns. As an implant of clinically relevant size may measure centimeters, it becomes clear that most cells in such a scaffold may quickly starve to death by lack of vascular supply. After all, it may take a week before blood vessels have sufficiently penetrated a hybrid construct after implantation. A week is really a long time, as surgeons who have to operate on patients whose bowel was constricted for only a day can tell us, as much of the tissue may have become necrotic by then. Levenberg had an appealing idea: rather than waiting for spontaneous vascular ingrowth after implantation, she would provide a cultured vascular network prior to implantation. If Vacanti, with his mouse, provided the walls, taps and sanitary equipment in a house, Levenberg would now provide the plumbing to make it all work *and* connect it to the water supply and sewer system. The Levenberg mouse is discussed later in more detail, but for now we will describe the experimental design briefly.

The goal was to grow a muscle substitute, supplemented with a vascular network. The cells of a muscle cell line were grown in co-culture with endothelial cells (the cells which line our blood vessels) from the human umbilical cord. As it is known that in natural capillaries endothelial cells are lined by supporting cells, in part of the experiment murine smooth muscle precursor cells were added to perform this function. The cells were grown in a non-woven PLA/PGA scaffold and after culture placed in immunodeficient mice. The authors investigated the functionality of the graft in several ways and the overall outcome was truly interesting. Not only had the endothelial cells formed structures that resembled a capillary network, but even better, mouse red blood cells were now found, within the construct, in

capillaries made from the human cells. The graft had connected to the host vascular network and the network was functional, as proven by superior cell survival for those hybrid constructs that contained the human endothelial network. In an editorial this was described as a landmark paper (Jain *et al.*, 2005).

Critics may still state that this is an interesting concept that nevertheless bears little clinical relevance as nobody (in the foreseeable future) is likely to implant a mouse cell line into a human being. Furthermore, only for very few individuals, would clinicians have access to, preferably autologous, umbilical cord endothelial cells. Frankly, even the most optimistic researcher would find these arguments compelling. Therefore, quite a few parameters will have to be changed and optimized for this technology to become a clinical reality. This all fits in with the earlier remark that it easily takes a decade to get from early concept to clinical reality. The next example will show that it may take even longer than a single decade before a concept is translated into a clinical and commercial success.

In the 1960s and 1970s, Urist pioneered in the field of bone morphogenetic proteins. His classical paper of 1965 (Urist, 1965) is described in the Chapter 5 (Box 1). Urist had discovered that the demineralized fraction of bone contains a protein fraction that is able to induce bone formation in a non-species specific way, even in non-bony sites. He first hypothesized that the bone formation was induced by a non-collagenous protein acting as bone morphogen. Obviously, this was a finding of great importance and researchers worldwide started to further investigate the phenomenon of bone induction. It was not until 15 years later that the bone morphogenetic proteins (BMPs) were isolated and their existence definitely proven by Sampath and Reddi (1981, 1983). Nevertheless, their role in osteoinduction mechanisms has been debated – and is still today – although it has been clearly demonstrated over the last 25 years that BMPs alone could induce bone formation in non-bony sites of many different mammals. The lack of consensus around the BMPs action further delayed the development of BMPs as a potential tool for bone regeneration and it was not until the beginning of the 1990s that industries and researchers really saw the interest of the protein and went on the way to develop and commercialize it for clinical use in fracture, non-union and spinal fusion treatment. During the 10 years necessary for the protein to be approved by the regulatory organisms, it became the most extensively studied orthobiologic product in history. Researchers would focus on how to translate their findings into clinical practice by optimizing, standardizing and industrializing the processes. In parallel, quality and regulatory protocols had to be developed as well. Finally, in 2001, BMP-7 was approved for spinal fusions and in 2002, BMP-2 followed. The protein is mixed with collagen sponges that allow its delivery to the site of surgery, from which it will induce bone formation and fusion of two vertebras. The treatment of tibial fractures by BMP-2 was further approved in 2004. Since then, the number of patients successfully treated with BMPs has not ceased to increase, making it one of the most successful commercial and healthcare successes in the field of orthopedics and in the field of tissue engineering. Overall, it took 30 years from the concept of BMPs expressed by Urist to the successful commercialization of a beneficial product. Interestingly, the availability and subsequent sales of the product now reveals that the market size for bone replacement might well be larger than ever expected, stimulating researchers in both industry and academia to try to do a better and, hopefully, faster job, than those who went before them.

The example above clearly illustrates the difficulty that tissue engineers have to face. As much fundamental knowledge is still lacking, they have to conjointly investigate and unravel the basic mechanisms taking place during tissue homeostasis and regeneration, and at the same time develop the tools to exploit and enhance these natural mechanisms. This dichotomy becomes evident when one considers the range of matrices used for tissue engineering applications.

One of the first tissue engineering paradigms was the use of supportive matrices that provide associated cells with a substrate appropriate for attachment, multiplication, and differentiation towards the desired tissue. Further than the necessary degradability

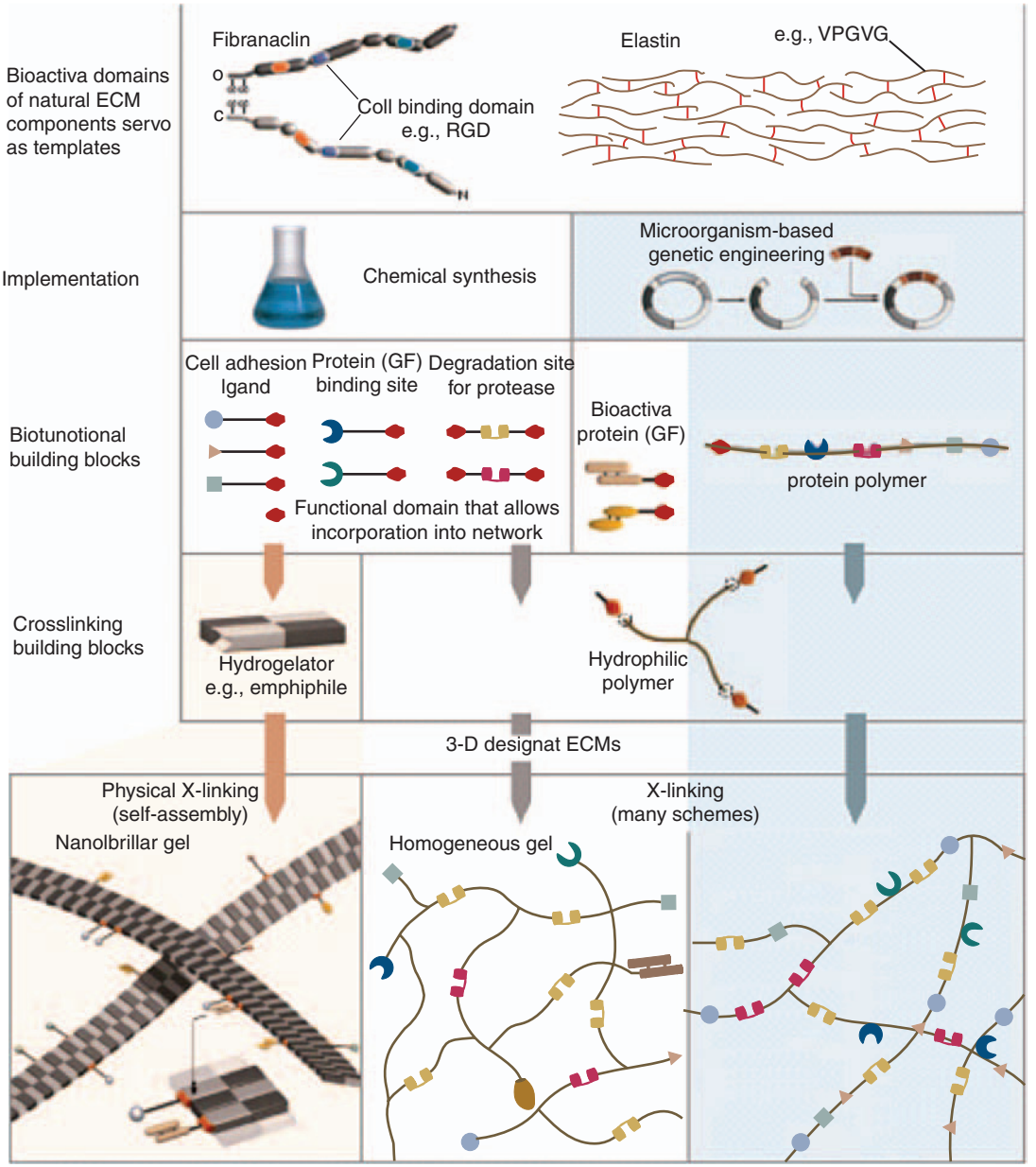
of such a substrate and eventual remodeling, the exact requirements of the matrix were not known with regard to cell interaction and response. Therefore, as a first step, many different matrices were evaluated to determine the most prone to replace the cell environment, from natural or synthetic origin. Collagen sponges made from purified bovine collagen fibers and poly(α-hydroxy) acids (such as poly(lactic-co-glycolic) acids, PLGA) scaffolds appeared suitable at first. The former is still widely in use and provides the cells with a nature-derived matrix familiar to them. However, the non-optimal control and reproducibility of the properties of this material combined with the potential transmission of diseases raises concerns for clinical applications. Polymeric scaffolds such as PLGA on the contrary can be produced in a variety of reproducible shapes and properties such as degradation and mechanical properties while being free of any pathogenic substances. However, the scaffolds produced with such polymers are foreign to the cells, thus creating important drawbacks. The lack of interaction with the polymer results in uncontrolled response of the cells, among which insufficient cell attachment (which cause tremendous difficulties during the seeding of the cells), or undesired changes of cell morphology. These non-physiological responses are the result of the material non-compliance with the necessary cell requirements. But what are these requirements and what do the cells find in their natural surroundings that they do not in a polymer scaffold? This knowledge is still not completely available and it is the task of tissue engineers to obtain and use it. Following this track, other materials have been introduced that try to more closely reproduce some key aspects of the extracellular matrix (ECM) (Box 3).

Box 3 Mimicking the extracellular matrix

One of the latest and most important realizations in the design of supportive structures for tissue engineering is the importance of mimicking the natural cell environment. Native tissue exist within a three-dimensional (3-D) viscoelastic milieu, the extracellular matrix (ECM), with which cells interact constantly and which guides their development or homeostasis. Accordingly, there is an increasing agreement that 3-D matrices such as hydrogels provide valuable systems that are closer to physiologic situations than most conventional biomaterials.

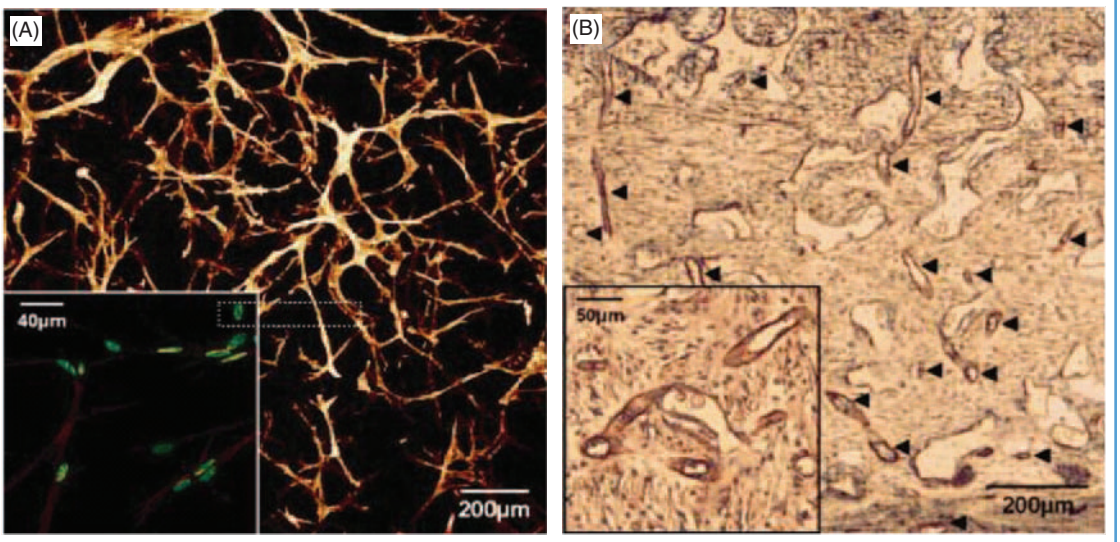
In addition to the imitation by hydrogels of the biochemical composition, structure or mechanical properties of the ECM, the creation of synthetic matrices that can as well mimic the natural interactions occurring between cells and ECM provides the tools to better understand, control, and guide the tissue regeneration. With this in mind, a novel line of research has been conducted by Hubbell and collaborators ((1995) and Lutolf and Hubbell (2005) over the past two decades, focusing on the incorporation within hydrogels of key functions of the ECM). This was attained by different strategies, based on the functionalization and modular design of synthetic hydrogels with bioactive domains of natural ECM components recognized as interacting with the cells. For instance, the biological recognition between the cells and their 3-D milieu can be achieved by incorporating the RGD tripeptide of fibronectin in the hydrogels design during synthesis. The local remodeling by the cell of their immediate surrounding, or in other words the cell-controlled degradation of the hydrogels and the cell migration, is achieved by the inclusion within the hydrogel of sites that are sensitive for proteases usually excreted at the cell surface during tissue repair (such as hyaluronidase, plasmin and matrix metalloproteinases-MMP). Finally, the presentation of growth factors to the cells is elegantly obtained by covalently attaching to the matrix material recombinant growth factors containing a protease cleavage site. Doing so, the cells induce a controlled and localized release by proteases active at their surface.

A good example of this approach can be found in the publication of Lutolf *et al.* (2003) in which proteolytically-sensitive networks bearing adhesion peptides and entrapping or bounding growth factors are presented. Hydrogels containing cells were prepared using vynil sulfone-functionalized



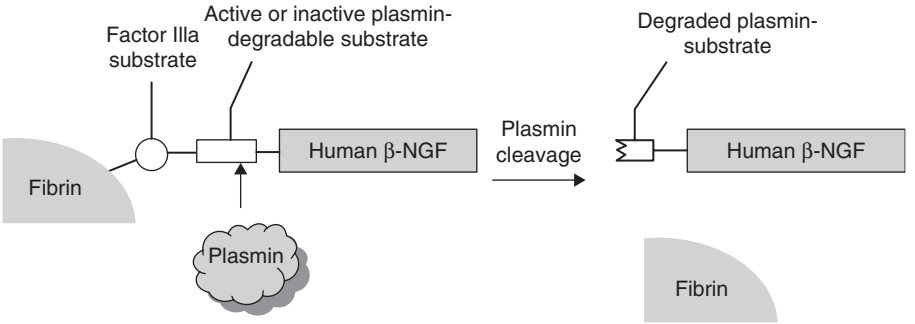
Design strategies of hydrogels mimicking ECM key functions. Bioactive domains of proteins are identified and synthesized by chemical strategies or by recombinant technologies. They include cell-adhesive ligands, growth factor binding sites or modified growth factors with cleavage sites and domains for protease degradation. Hydrogels can then be obtained by physical cross-linking of the selected components, either by physical or chemical mechanisms. Reproduced with permission from Lutolf, M.P. and Hubbell, J.A. (2005). Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol, 23: *47–55*.

poly(ethylene glycol) macromers that were conjuguated by a Michael-type addition to cysteine-containing peptides at physiological temperature and pH. The peptides selected contained a cell adhesion motif based on the RGD peptide and a MMP sensitive sequence. The network supported adhesiveness of the cells (fibroblast) which spread and migrated within the gel. The migration was controlled and regulated by the MMP-sensitive sites repartition and by the level of secretion of MMP by the cells, which is increased in necrotic or healing tissues. The migration and cellular multiplication allowed the formation of complex morphologies *in vitro* and *in vivo*, in which the material was remodeled without loosing its integrity. *In vivo*, the incorporation of vascular endothelial growth factor (VEGF) allowed the complete remodeling of the material by infiltration of connective tissue and extensive new vascularization.



The cell-controlled degradation and migration of the hydrogels allowed the formation of complex network morphologies while retaining the material integrity and mechanical properties. (A), The inset shows the cell nuclei (green) and actin cytoskeleton (red). When implanted subcutaneously in the rat, a complete remodeling of the hydrogel into native tissue was observed; (B), with the formation of new blood vessels (arrowheads) and connective tissue infiltration. Reproduced with permission from Lutolf, M.P., Raeber, G.P., Zisch, A.H., et al. *(2003). Cell-responsive synthetic hydrogels.* Adv Mater, 15: *888–892.*

The ability of such functionalized hydrogels to provide cell with localized growth factors release by protease was further demonstrated in another publication from Sakiyama-Elbert *et al* (2001). There, a recombinant β-nerve growth factor (β-NGF) variant that expresses a domain substrate for factor XIIIa, which cross-links the growth factor into fibrin has been produced. A protease cleavage site was included between the growth factor and the fibrin-coupling site to enable a localized release by cell surface proteases. The use of this functionalized gel induced an increase of dorsal root ganglia nerve ingrowth up to 100% compared to non-functionalized gels or gels containing unbound growth factor, demonstrating the advantage of an on-demand delivery of growth factor to cells.



β-NGF variant incorporated to fibrin hydrogels and allowing a cell-controlled release of the growth factor under protease action. Reproduced with permission from Sakiyama-Elbert, S.E., Panitch, A. and Hubbell, J.A. (2001). Development of growth factor fusion proteins for cell-triggered drug delivery. FASEB J, 15: *1300–1302.*

The development of synthetic matrices that mimic the natural functions and structure of the ECM, although not completely and perfectly, gives the tools to tissue engineers to selectively and modularly refined their materials in view of providing an environment that dictates and guides cells to tissue regeneration.

The first improvement is to provide the cells with a three-dimensional watery environment (hydrogels) instead of a flat surface on which to attach (at the cell scale, a polymeric scaffold is a flat surface). Furthermore, these hydrogels can be functionalized with moieties that interact in a defined way with cells. For instance, the attachment and spreading of the cells within such matrices can be achieved by the incorporation of specific peptide sequences known to play a role in cell adhesion (as the RGD sequence for instance). The ECM being constantly remodeled by the cell *in vivo*, the hydrogels can also include protease sensitive sites that will allow the cell-controlled degradation of the matrix during cell migration. Finally, as ECM is a natural reservoir for signaling molecules and growth factors (present in minute quantities but of high potency) the hydrogels should provide the signals controlling cell fate.

Although these key elements might not be sufficient to reach the final goal of fully mimicking the ECM, they do represent a significant first step that should allow researchers to further identify crucial needs of the cell.

Aside from the importance of understanding and creating the most optimal environment for the cells, another open question of possibly higher importance concerns the cells themselves: what are the most suitable cells to use for any given tissue? Pivotal knowledge regarding this fundamental question is also lacking. One could logically think that the most suitable cell source for cartilage or bone, for instance, is cartilage and bone, respectively. This is logical indeed and was the first approach to be followed. However, many hurdles render this strategy not as straightforward as expected. The first difficulty was seen in the number of cells necessary to provide a basis for tissue growth. The extraction of cells from native tissue is generally done in humans by biopsy and usually results in an insufficient cell number (Figure I.5). The cells so collected therefore have to be multiplied. This step is generally done by culturing them on polystyrene culture flasks that allow cell attachment. Although almost any cell types can be expanded, provided the right and specific conditions are supplied, their multiplication immutably induces the loss of their particular phenotype; in other words, they

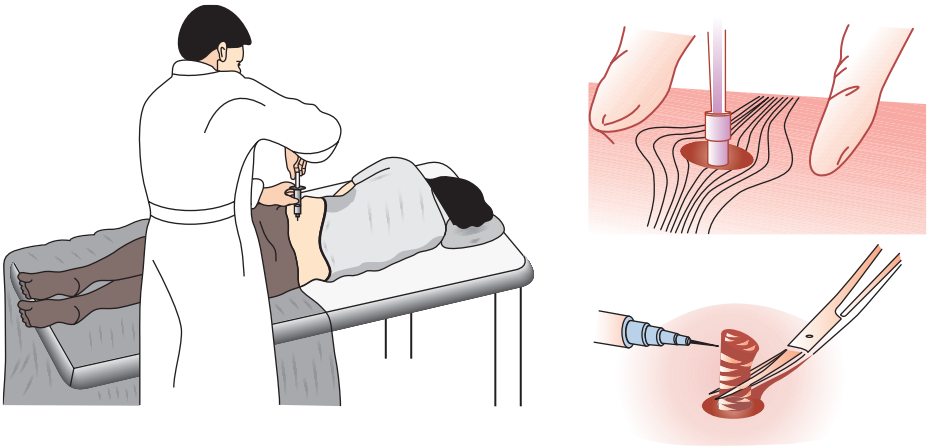


Figure I.5 Although human biopsies are the main cell source, they do not provide a sufficient number of cells.

dedifferentiate. For instance, a cartilage cell of round morphology will turn to a fibroblastic-like cell (of stretched and elongated shape) after some divisions (von der Mark *et al.*, 1977). As a result, once a sufficient number of cells have been reached, they have to be re-differentiated to the desired phenotype, which is not easily attainable.

Another approach, which has gained a constant increase in interest, consists of using undifferentiated cells as starting material. After all, a complete organism consisting of billions of highly specialized cells originates only from a single undifferentiated one. Even in adults, there are pools of undifferentiated cells which allow self-renewal of the organism over a life-span; in other words, cells that form the stem of all the others. The first of these stem cells to be identified after the Second World War were the hematopoietic cells that allow the renewal of blood (Till and McCulloch, 1961). Since then, other stem cells have been discovered in the bone marrow, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, heart, epidermis, mucosa of the digestive system, cornea, liver, and pancreas. These cells, once isolated, can be differentiated in highly specialized tissues (multipotency) if provided the right conditions, which are not entirely established. This is one of the issues that tissue engineering has to face in order to collect the fruits promised by the use of stem cells.

Even though the apparent slow progress of the field can be understood, it should not hide the fact that successful applications already exist. With current techniques, which may be qualified as crude by some, the effectiveness of tissue engineering for some applications can already be demonstrated clinically, as was done recently by Atala *et al.* (2006) in a milestone article (see Box 4). This research group focused on the application of tissue engineering to urological diseases and, more specifically, bladder-related diseases. In this particular study, young patients suffering from an invalidating congenital malformation that induces high pressure in the bladder and, as a result poor and low compliancy, were treated with a tissue engineering approach. Native bladder cells were isolated and expanded *in vitro* prior to seeding on a composite scaffold of collagen and polyglycolic acid. The scaffold was designed to replace or augment the bladder size to improve its compliancy and patient continence. After a short period of *in vitro* culture – to allow cell attachment to the scaffold – the engineered constructs were implanted for a period up to 61 months. The main outcome of this study was the definitive improvement of the bladder compliance and capacity while restoring physiological

function. In addition, no side effects caused by the tissue engineered construct were found.

Although this study treats a particular organ that can already be considered as simpler than, for instance, bone or skin (which are still by far surpassed in complexity by a kidney or our brain), it still shows the potency of the tissue engineering strategy. It should serve to convince those who do not see tissue engineering evolving fast enough that, although its progress is slower than all of us would wish, it has already reached the stage of treating numerous patients and adding valuable quality of life.

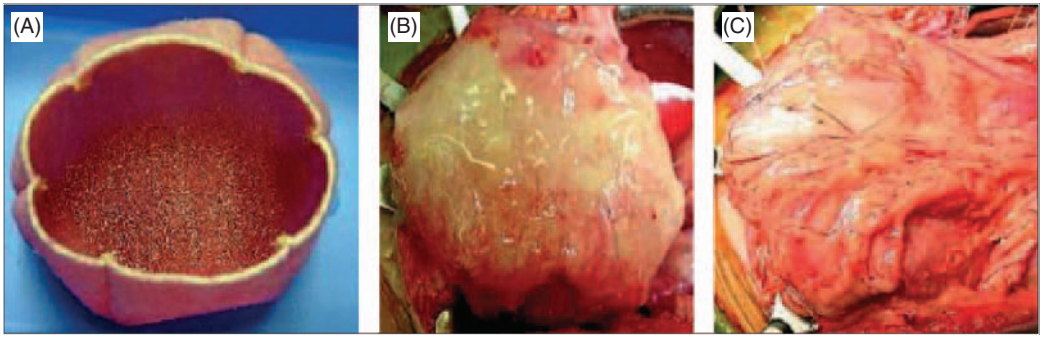
Box 4 Tissue Engineered Bladder: A seminal clinical study

Generating an organ is the holy grail of tissue engineering. In contrast to the classical grail ours will actually be found. As a matter of fact, there will be many grails and a small but interesting one has been described by Atala *et al.* (2006) which has made a seminal attempt in applying the basic research in bladder tissue engineering into clinical application.

Traditionally patients with end-stage bladder disease are treated with cystoplasty using gastrointestinal segments. However, such segments results in many complications such as metabolic disturbances, urolithiasis, increased mucous production, and malignant disease.

The authors successfully used an alternative approach using autologous engineered bladder tissues for reconstruction. Their earlier animal model experiments, using autologous cells in combination with biodegradable matrix from normal and diseased bladders, demonstrated similar functional properties encouraging the authors to engineer human bladder tissues by seeding autologous cells on different matrices in patients with end-stage bladder diseases requiring cystoplasty. The authors, based on their initial preclinical studies, decided to use collagen matrix derived from decellularized bladder submucosa. Their additional animal experiments with a collagen and polyglycolic acid (PLG) with omental coverage improved tissue vascularization and performed better in long-term.

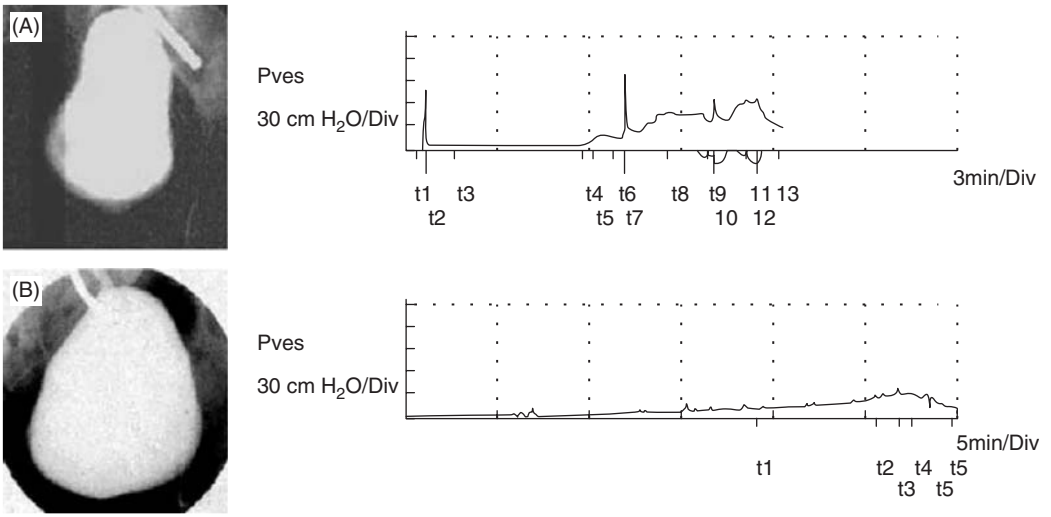
The study included seven patients with patients being implanted with collagen scaffold without omental wrap and four patients with collagen-PLG with omental wrap. A bladder biopsy sample (1–2 cm^2) was obtained through a small suprapubic incision. The initial size of the bladder mould ranged



Construction of engineered bladder *Scaffold seeded with cells (A) and engineered bladder anastamozed to native bladder with running 4–0 polyglycolic sutures (B). Implant covered with fibrin glue and omentum (C). Reproduced with permission from Atala, S., Bauer, S.B.,* et al. *(2006). Tissue-engineered autologous blassers for patients needed cystoplasty.* The Lancet, 367: *1241–1246.*

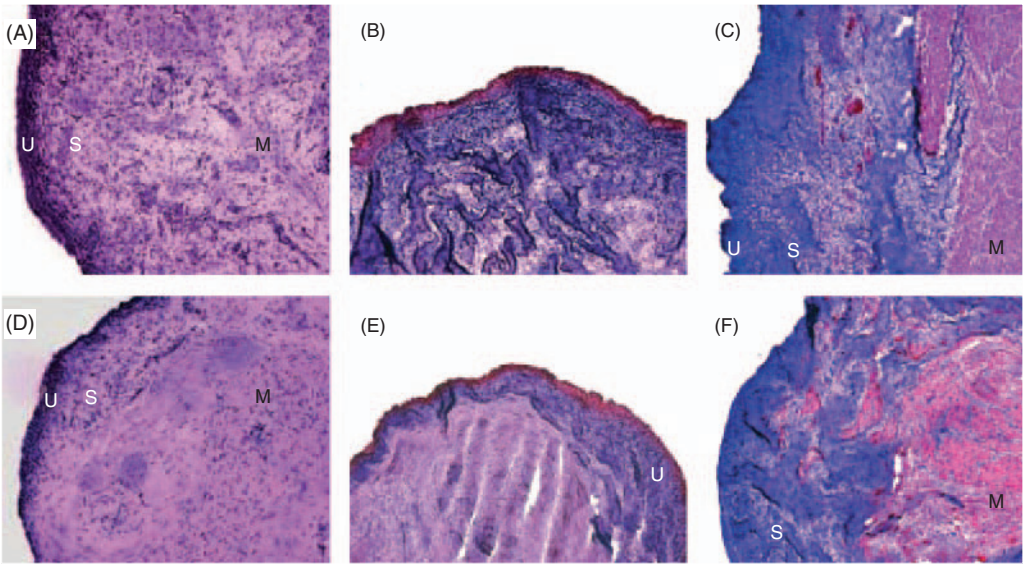
from 70–150 cm^2 with a thickness of around 2 mm.The exterior surface of the scaffold was seeded with smooth muscle cells at a concentration of $50 \times 10^6/cm^3$. After 48 hours, urothelial cells were seeded by coating the inside of the scaffold at a concentration of $50 \times 10^6/cm^3$ and maintained at 37°C until implantation. The patients were followed up to five years postoperatively.

All of the patients urodynamic studies demonstrated that the mean leak point pressure decreased postoperatively by 13% and 29% in collagen and collagen-PLG scaffold with omental wrap, respectively. Further, the bladder capacity was found to have decreased by 30% in collagen scaffold. However, the collagen-PLG scaffold with omental wrap showed 1.58-fold increase in the bladder capacity. The postoperative compliance found to be increased by 15% and 67% in collagen and collagen-PLG with omental wrap respectively. The irregular bladder pressure found preoperatively was substantially improved postoperatively.



Preoperative (A) and 10-month postoperative (B) cystograms and urodynamic findings in patient with a collagen-PGA scaffold engineered bladder *Note irregular bladder on cystogram, abnormal bladder pressures on urodynamic study preoperatively, and improved findings postoperatively. Reproduced with permission from Atala, S., Bauer, S.B.,* et al. *(2006). Tissue-engineered autologous blassers for patients needed cystoplasty.* The Lancet, 367: *1241–1246.*

Further, all of the patients had normal serum sodium, potassium, chloride, phosphorus the and arterial blood gases postoperatively with a normal mucus production. Morphological analysis of the implanted engineered bladders demonstrated a tri-layered structure consisting of a urothelial cell-lined luman surrounded by submucosa and muscle indicating the implanted bladder was anostmosed to host tissue. This study not only demonstrates that reconstructed engineered bladders showed improved functional parameters that were durable over a period of years but also stands as milestone towards successful tissue engineering in other disciplines.



Morphological analysis of implanted engineered bladders. (A, B, C): Cystoscopic biopsies of implanted engineered bladders 31 months after augmentation shows extent of regeneration. Engineered bladder tissue showed tri-layered structure, consisting of lumen lined with urothelial cells (U) surrounded by submucosa (S) and muscle (M). Haemotoxylin and eosin. A, immunocytochemical analysis with anti-pancytokeratin AE1/AE3 antibodies; B, and anti-α smooth muscle actin antibodies; C showed presence phenotypically normal urothelium and smooth muscle; (D, E, F): native bladder tissue. Magnification 100×. Reproduced with permission from Atala, S., Bauer, S.B., et al. *(2006). Tissue-engineered autologous blassers for patients needed cystoplasty.* The Lancet, 367: *1241–1246.*

These final considerations will undeniably persuade the reader of the long way that Tissue engineers have already progressed and of the long way that still remains. A journey where unexpected hurdle will certainly appear, always making room for renewed excitement and fun. Indeed, Tissue engineers will have to become as multiple as the tissues they wish to regenerate and as pluripotent as the cells they use. We hope this textbook will contribute to this and to a faster progress of the field towards a clinical reality by enlightening young researchers to the state of the art of tissue engineering and to the challenges still lying ahead.

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Chapter 1
Stem cells

Jojanneke Jukes, Sanne Both, Janine Post, Clemens van Blitterswijk, Marcel Karperien and Jan de Boer

Chapter contents

Chapter objectives:

- To recognize the defining properties of stem cells
- To identify the major differences between embryonic and adult stem cells
- To understand that the mechanisms that regulate self-renewal are complex
- To understand how stem cells can differentiate into a more specialized cell
- To learn how researchers can isolate and characterize embryonic stem cells
- To know where adult stem cells can be found in the body
- To understand the challenges for tissue engineers when using stem cells

"The essence of knowledge is, having it, to apply it; not having it, to confess your ignorance"

Confucius

"Nobody said it was easy"

From the song The Scientist by Coldplay, from the album A Rush of Blood to the Head

1.1 What defines a stem cell?

Stem cells can be defined by two properties: the ability to make identical copies of themselves (self-renewal) and the ability to form other cell types of the body (differentiation) (Figure 1.1). These properties are also referred to as 'stemness'. Stem cells may potentially provide an unlimited supply of cells that can form any of the hundreds of specialized cells in the body. It is because of these properties that stem cells are an interesting cell source for tissue engineers.

Stem cells can be divided into two main groups: embryonic and adult or somatic stem cells. Embryonic stem cells are responsible for embryonic and fetal development and growth. In the human body, adult stem cells are responsible for growth, tissue maintenance and regeneration and repair of diseased or damaged tissue.

1.1.1 Stem cell self-renewal

During a stem cell division, one or both daughter cells maintain the stem cell phenotype. This process is called self-renewal. Stem cells can divide symmetrically or asymmetrically. It is the balance between symmetrical and asymmetrical divisions that determines the appropriate numbers of stem cells and differentiated daughters.

During a symmetric cell division, both daughter cells acquire the same fate; either undifferentiated (new stem cells) or differentiated.

During an asymmetric cell division, one daughter cell becomes a new stem cell; the other differentiates into a more specialized cell type (see Figures 1.1 and 1.2). Asymmetric cell divisions are controlled by intrinsic and extrinsic mechanisms. Intrinsic mechanisms rely on the asymmetric partitioning of cell components, such as cell polarity factors or cell fate determinants. In the extrinsic mechanism, the two daughter cells are positioned asymmetrically in their environment and receive different external signals (Morrison and Kimble, 2006).

The past 25 years of research have given some insight into the mechanism by which a cell maintains its undifferentiated fate. Since self-renewal involves both proliferation and the maintenance of an undifferentiated phenotype, multiple pathways are involved. Stem cells from different tissues or at different stages of developmental potential (pluripotent or multipotent) use different mechanisms to regulate self-renewal. The pathways regulating self-renewal are depending on the context. Factors that might stimulate differentiation of one cell type, might be involved in the maintenance of self-renewal of another stem cell. Some mechanisms and interactions are still unknown, some are debatable, and others are well described. Self-renewal of embryonic and adult stem cells is described in section 1.2.3 and 1.3.3.

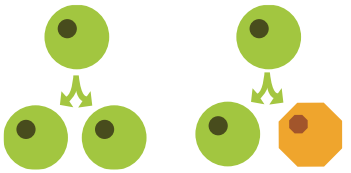

Figure 1.1 Stem cell characteristics. Upon cell division, a stem cell (green circle) can produce a new stem cell (self-renewal), and a differentiated daughter cell (orange hexagon). On the left, a symmetrical is shown and on the right an asymmetrical cell division.