



# METHODS IN CELL TRANSPLANTATION

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EDITED BY CAMILLO RICORDI, M.D.

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- SECTION A: HEMATOPOIETIC CELLS  
*Edited by Edward D. Ball*
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*Edited by John F. Hansbrough*
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*Edited by Joseph Vacanti*



Springer

New York Berlin Heidelberg London Paris  
Tokyo Hong Kong Barcelona Budapest

R.G. LANDES COMPANY  
AUSTIN

# METHODS IN CELL TRANSPLANTATION

R.G. LANDES COMPANY  
Austin, Texas, U.S.A.

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Please address all inquiries to the Publisher:  
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or  
P.O. Box 4858, Austin, TX 78765 U.S.A.  
Phone: 512/ 863 7762; FAX: 512/ 863 0081

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U.S. and Canada ISBN: 1-57059-189-X  
International ISBN: 3-540-60151-1

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## Library of Congress Cataloging-in-Publication Data

Methods in cell transplantation / edited by Camillo Ricordi.  
p. cm.

Includes bibliographical references and index.

ISBN 1-57059-189-X (alk. paper)

1. Cell transplantation. I. Ricordi, Camillo.

QP89.M46 1995  
617.9'5 -- dc20

95-15153  
CIP

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# FOREWORD

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Some books should never be published because they are not true, and others less harmful may be merely a loss of time for the editor, the contributors, and the readers. For a scientific book to be successful (and above all this means useful) it must meet the standards of relevance, need, proper timing, and currency. Furthermore, if its preparation is not a learning experience for the contributors, it will not be a valuable tool for the readers. *Methods in Cell Transplantation* fulfills these requirements.

The relatively new field of cell transplantation is evolving with incredible rapidity. Three decades ago, organ transplantation was a discipline confined to a small club of visionary surgeons within which it was easy to communicate scientific progress and technical advances. Cell transplantation developed in a different way, like a comatose baby turned into a slumbering giant by parenteral feeding until suddenly it woke up, looked into a mirror, and was confronted by a colossus which it did not recognize.

When I learned that I had been designated an Honorary President of the new Cell Transplantation Society and attended the First International Congress in 1992, I thought that I was going to a small party, almost a study group. Instead, there were over 500 founding fathers from some 33 countries. Their interest in many shared aspects of cell transplantation created an instant common language for workers in seemingly unrelated fields. The goals and problems were similar, as well as many of the potential research and clinical opportunities. The technologies were appearing or changing so quickly that it was necessary to invent new ways of communication and new means for information transfer.

Aside from the arrival of this gathering tide, the timing of the First International Congress was fortuitous in an unexpected way. Research to which Camillo Ricordi, the society's first president, was a key contributor had just been completed establishing a linkage between the whole organ and cell transplantation fields, which some had previously construed to be lined up at a competitive interface. Evidence was produced only a few weeks earlier that the ubiquitous traffic on non-parenchymal cells of bone marrow origin (the passenger leukocytes of the organs) and the survival of these migrants peripherally was the long sought-after explanation of organ "acceptance", and the key to the controlled induction of tolerance. Organ transplantation had turned out to be merely cell transplantation in artful disguise.

This book, which is completely up to date, will provide an invaluable resource for those who want to learn each other's techniques and concepts, and equally for those who must begin with no base at all and want to be armed with a primer. The list of contributors is comprehensive and includes familiar names as well as authors who have been identified as natural leaders of the next cell transplant generation.

A labor of love radiates warmth back to its creator in many ways. One channel will be through the appreciation of those whose work was made easier by the organizational genius and personal scientific contributions of the editor, Camillo Ricordi.

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When I first joined the University of Pittsburgh Transplantation Institute in 1989, my objective was to unify into a single program the seemingly unrelated fields of cellular transplantation. One may question a need to unify or attempt to coordinate widely different clinical applications of cellular transplantation when the trend in subspecialties seems to be leading towards micro-subspecialty societies. My view, however, on the need for a unifying initiative flowed from my own research experience in the field. Investigators involved in what could appear to be very distantly unrelated areas of cellular transplantation (i.e., pancreatic islets, bone marrow, endothelial, hepatocytes, epidermal, myoblasts, neural, stem cells, etc.) soon discover that the problems they face within the parochial walls of their laboratories are problems shared by investigators in companion areas and that have, in fact, more in common amongst them than apparent from the subspecialty motifs of so-called unrelated research.

Separation and purification techniques for cells, culture methods, cryopreservation and banking, pre-transplant immunomodulation, micro and macro encapsulation, bioartificial organs, tolerance induction, xenotransplantation, quality control and regulatory issues, manipulation of the environment at the implant site, techniques of transplantation, and gene therapy are all, in fact, very related to each other either in the field of experimental or clinical transplantation.

It was with the mission of promoting research and collaboration in these apparently unrelated fields that the Cell Transplant Society was founded in 1991. The initiative, that I first thought would be restricted to a very select group of investigators, maybe 30 to 50 worldwide, rapidly outgrew my most optimistic prediction and the Society found itself at the First International Congress in Pittsburgh in 1992 with over 500 founding scientists from some 35 countries. This first international congress was an overwhelming success and clearly indicated that there was a real need for a forum for intradisciplinary debate. The decision was made to hold this congress once every two years, and we are soon to embark on our third edition to be held in Miami, Florida in 1996.

Soon after the creation of the Cell Transplant Society, the journal *Cell Transplantation* became the official publication of the society. This book on methods in cellular transplantation is a natural and collected extension of the mission that fueled the origin of the Cell Transplant Society and the creation of the journal *Cell Transplantation*. To provide an example on why such a general book is important, I will draw on my own particular field of interest in pancreatic islet transplantation. What initially appeared to be simply a technique to reverse diabetes by providing a transferable source of insulin-producing tissue turned out to become a very important tool in co-transplant experiments to maintain hepatocytes when implanted in sites that are not perfused by portal blood. The field of hepatocellular transplantation was suffering because hepatocytes transplanted in ectopic sites would rapidly become atrophic. We then did a series of experiments proving that co-transplantation of pancreatic islets with hepatocytes was able to provide, at the transplant site, factors that would significantly enhance the survival of the hepatocellular implants. This was shown first in rodents and the observations were then extended to human hepatocyte transplants. This



provided for me one of the first examples of this intimate relation of two apparently unrelated cellular grafts.

We also showed that co-transplantation of adrenal cells could provide local immunosuppression that might enhance survival of other grafts co-transplanted with these steroid-secreting cells. This is still a relatively new field of endeavor and it is still to be determined whether, for example, the local production of steroids at the transplant site could enhance survival of other cellular and tissue grafts in vivo. But the notion that co-culturing enhances survival by paracrine effect, or so influences the transplant site so as to affect prolonged survival of allo- and xenografts is now extended to microencapsulation and bioartificial organs. Here co-transplantation of cells within the encapsulation devices could provide a means of manipulation of the microenvironment at the transplant site to affect the toxicity of small molecules, like free radicals and cytokines that can diffuse across most of the semi-permeable membranes used in immunoisolation devices.

For us, it has been extremely encouraging to realize how relatively easy it is to expand a well-organized cellular transplantation laboratory to isolate and study different cells utilized in transplantation without expensive and labor intensive development of individual laboratories for each cell of interest.

Once a group has set up a laboratory to meet quality control standards for pancreatic islet separation and purification, it was extremely easy to perfect techniques for hepatocyte isolation and isolation of hematopoietic cells, such as bone marrow, in the same laboratory.

We then started to study the use of bone marrow cell transplant as an approach to induce tolerance to other organ tissues or cellular transplants. Here, again, an apparently unrelated field of cellular transplantation, namely one that was created as a replacement transplant to salvage lethally irradiated recipients who suffer from malignancies like leukemia became the forerunner of a procedure that holds promise to assist in, regimens for tolerance induction to allografts and maybe even xenografts.

This book will be of assistance as cross reference manual for investigators in the different areas of cellular transplantation who have the curiosity to learn how other investigators, in an apparently unrelated field, are addressing similar problems, or to just start, from scratch, a new cell transplant field in a laboratory that was previously focused on a different aspect of cellular transplantation. Our personal experience, though concentrated mainly in the field of islet separation technology and bone marrow preparation for studies of bone marrow infusion in tolerance induction, have valued greatly the contributions of colleagues working in the same laboratories in fields seemingly disparate of hepatocellular and epidermal cell transplantation, for example.

The book is divided into sections. **Section A** will address methodology related to hematopoietic cell transplants, from sources for marrow transplantation to techniques for lymphocyte depletion and stem cell purification. This section then deals with cryopreservation techniques and immunomodulation in vitro and clinically-oriented procedures, such as patient selection, pretransplant assessment and immunosuppressive conditioning regimens for bone marrow transplantation. Finally, the topics of assessment of engraftment following a bone marrow transplant, and the problems related to graft-versus-host disease is covered.

In **Section B** the topics related to the methodology in epidermal cell preparation and transplantation are covered in detail.

**Section C** will provide an overview of some recent advanced methods in selected fields of gene therapy, including hematopoietic progenitor cell

gene therapy and somatic gene therapy in brain cells, vascular cells and synovial cells.

**Section D** will cover hepatocytes and gastrointestinal cells methodologies and extends from the description of larger scale isolation and in vitro culture of primary hepatocytes to enterocyte transplantation for tissue engineering of neointestinal mucosa.

**Section E** will address the problem related to lymphocyte preparation in immunotherapy of cancer, detailing procedures from the technique for lymphocyte harvest and purification, the selection of lymphocyte subsets and culture, and the microbiological assessment of lymphocytes for immunotherapy. In addition, morphologic, phenotypic and functional assessment of lymphocytes used in immunotherapy will be addressed in detail, together with the clinical techniques of both the harvesting and infusion of cultures or activated lymphocytes, respectively. Intravenous transfusion of lymphocytes is described for protocols in the mouse and in human studies. The section concludes with a chapter on assessment of lymphocyte function after immunotherapy.

**Section F** describes methods in neural and paraneural cell transplantation, beginning with cell preparation, assessment and culture techniques from neural tissue suspensions to adrenal chromaffin cells. Further studies on labeling and identification of cells for CNS transplantation are described, together with the technique for behavioral assessment of cell graft function. Transplantation techniques are described in small animals and in subhuman primates. Clinical approaches to neural tissue transplants in humans rounds out this section.

**Section G** is dedicated to techniques in pancreatic islet cell transplantation. The section begins with the description of quality control issues in the islet isolation laboratory. Pancreas procurement techniques, as well as islet isolation, purification and quantification are described in detail for both adult tissue and fetal tissue. The section then details culture techniques, cryopreservation and immunomodulation procedures to decrease immunogenicity of the islet preparation. A description of the transplantation techniques in the islet field is followed by a chapter on growth factors and islet neogenesis. The section concludes with a methods section on microencapsulation and hollow fibers production and macroencapsulation technology.

**Section H** includes techniques for bone, cartilage and muscle cell preparation and transplantation. An additional chapter describes procedures related to human vertebral bodies as a source of bone marrow cells and the molecular methods for assessment of microchimerism following transplantation of bone marrow derived cells. These final two chapters may better fit in Section A, but are probably justified at the end of this book, since they will undoubtedly become an independent section as an explosion of new information appears in the near future due to the increasing interest of this approach for tolerance induction. The third chapter of this section is also of general interest since the technique of immunomagnetic bead cell separation has different applications to cell transplantation. The section then continues with a chapter on tissue engineering of cartilage and bone, and concludes with methods for human myoblast culture and transplantation.

Overall, this is a very global and multidisciplinary cross reference text, that will be periodically updated and extended to newly emerging areas and techniques, to enlighten and help train interested investigators in these rapidly growing fields. All the contributors have done excellent work, especially the section editors, who had to diligently and patiently wait for the final printing of this very time consuming initiative.

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# ACKNOWLEDGMENT

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This book is the natural extension of the mission that fueled the origins of the Cell Transplant Society and the creation of the journal *Cell Transplantation*. Each chapter of the text represents a state-of-the-art report on a specific technology, that will be an updated, useful reference for scientists involved in these particular areas of cell transplant research. They are organized, as well, to provide a unique and quick reference source for researchers in other areas of cell transplantation to become acquainted with the technologic advances that may aid them in their own work. Laboratories that currently engage in different aspects of cell transplant research will find this compendium of technologic advances to be translated easily and smoothly to applications in their own specialized field of cellular transplantation research. The editors will have succeeded in their goal of providing material that is helpful to all workers in the field if this happens.

I would like to thank the authors who have thoughtfully committed their energies and current insights to ensure that this book is a timely addition to the libraries of those interested in cell transplantation. I site especially the work of the section editors, Edward D. Ball, John F. Hansbrough, Joseph C. Glorioso, Jörg Gerlach, Theresa L. Whiteside, William J. Freed, Garth L. Warnock and Joseph Vacanti for their invaluable assistance. Without the effort of these distinguished colleagues, this initiative would never have been completed.

My hope is that the progress towards new avenues of interactive communication between scientists will soon take us to the next level of contemporary communication via electronic files, perhaps through Internet, so that the second edition of this book will become rapidly available at the network level to all workers in this field. This will allow us to instantly update chapters and introduce new technologies and findings with virtually no delay in the delivery of the information to the reader.

My final word of thanks goes to those young investigators who will find in this book new avenues in cell transplantation research that will stimulate them to contribute to the future electronic additions of this text. Should that be the case, we can look back with a sense of sympathy for this editor who hopes that these "prehistoric first steps" set the stage for a new level of scientific communication between colleagues who participate in the challenges, frustrations and opportunities of cell transplantation research.

—Camillo Ricordi, M.D.

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