REGENERATIVE PHARMACOLOGY

Edited by

GEORGE J. CHRIST ▪ KARL-ERIK ANDERSSON

CAMBRIDGE

REGENERATIVE PHARMACOLOGY

Regenerative medicine is broadly defined as the repair or replacement of damaged cells, tissues, and organs. It is a multidisciplinary effort in which technologies derive from the fields of cell, developmental, and molecular biology; chemical and material sciences (i.e., nanotechnology); engineering; surgery; transplantation; immunology; molecular genetics; physiology; and pharmacology. As regenerative medicine technologies continue to evolve and expand across the boundaries of numerous scientific disciplines, they remain at the forefront of the translational research frontier with the potential to radically alter the treatment of a wide variety of disease and dysfunction. The goal of this book is to draw attention to the critical role that the pharmacological sciences will undeniably play in the advancement of these treatments. This book is invaluable for advanced students, postdoctoral Fellows, researchers new to the field of regenerative medicine and its companion field, tissue engineering, as well as experienced investigators looking for new research avenues. This is the first state-of-the-art book in this rapidly evolving field of research.

GEORGE J. CHRIST is Professor of Regenerative Medicine and Translational Science and head of the Program in Cell, Tissue and Organ Physiology at the Wake Forest Institute for Regenerative Medicine. He is an affiliate faculty in the Molecular Medicine and Molecular Genetics Programs, as well as the Virginia Tech–Wake Forest University School for Biomedical Engineering and Sciences. He also holds appointments in the Departments of Urology and Physiology and Pharmacology and the Sticht Center for Aging. He is the former director and founder of the Institute for Smooth Muscle Biology at the Albert Einstein College of Medicine. Dr. Christ is an internationally recognized expert in muscle physiology. He is the past chairman of the Division of Systems and Integrative Pharmacology of the American Society of Pharmacology and Experimental Therapeutics (ASPET) and past president of the North Carolina Tissue Engineering and Regenerative Medicine Society. He currently serves on the Executive Committee of the Division for Integrative Systems, Translational and Clinical Pharmacology of ASPET. He is on the editorial board of five journals and has authored more than 200 scientific publications. Dr. Christ has served on both national and international committees related to his expertise and has also served on NIH study sections in the NIDDK, NICHD, NCRR, and NHLBI. He has chaired working groups for both the NIH and the World Health Organization. Dr. Christ is a co-inventor on more than 24 patents (national and international), which are either issued or pending, related to gene therapy for the treatment of human smooth muscle disorders and tissue engineering technologies. He is a co-founder and directing member of Ion Channel Innovations, LLC, a development-stage biotechnology company pioneering the use of gene therapy for the treatment of human smooth muscle disorders. In addition, he is a co-founder and board member of Creative Bioreactor Design, Inc., another early-stage biotechnology company in the expanding field of regenerative medicine and tissue engineering.

KARL-ERIK ANDERSSON, MD, PhD, is Professor of Regenerative Medicine and Urology at Wake Forest Institute for Regenerative Medicine. He also holds appointments in the Departments of Physiology and Pharmacology and Molecular Medicine at Wake Forest University School of Medicine. He has Swedish Specialist Degrees in internal medicine and clinical pharmacology and a PhD in pharmacology from the University of Lund, Sweden. From 1978 to 2006, he was Professor and Chairman of the Department of Experimental and Clinical Pharmacology, University of Lund, and from 1993 to 2000, he was Vice Dean of the Medical Faculty at the University of Lund. Dr. Andersson has received several awards, including a Lifetime Achievement Award from the Society for Urodynamics and Female Urology. Dr. Andersson is a member of many international societies, including the American Urological Association, the European Association of Urology, and the International Society for Sexual Medicine. He has served on both national and international committees related to his expertise in basic and clinical physiology and pharmacology and has also served on NIH study sections in the NIDDK. He has chaired working groups for the International Consultation of Urogenital Diseases, supported by the World Health Organization. He also serves on the editorial boards of several journals, including the *Journal of Urology* (section editor), *Neurourology and Urodynamics* (associate editor), *and European Urology*, and is editor-in-chief of the *Urotoday International Journal*. Dr. Andersson has authored more than 800 articles in peer-reviewed international journals. His current research interests include clinical and basic physiology and pharmacology of the urogenital tract and regenerative medicine.

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GEORGE J. CHRIST

Wake Forest Institute for Regenerative Medicine

KARL-ERIK ANDERSSON

Wake Forest Institute for Regenerative Medicine

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This book is dedicated to our parents, families, mentors, students, and colleagues.

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Contributors

Karl-Erik Andersson, MD, PhD
Professor
Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC

Anthony Atala, MD
Chair, Department of Urology
Director, Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC

Atta Behfar, MD, PhD
Assistant Professor
Marriott Heart Disease Research Program
Division of Cardiovascular Diseases
Mayo Clinic
Rochester, MN

Timothy A. Bertram, DVM, PhD
President, Research & Development
Chief Science Officer
Tengion, Inc.
Winston-Salem, NC

Stefanie Biechler, PhD
Biomedical Engineering Program
University of South Carolina School of Medicine
Columbia, SC

Peter R. Brink, PhD
Professor and Chair, Department of Physiology and Biophysics
Institute for Molecular Cardiology
Stony Brook University
Stony Brook, NY

David Burmeister, PhD
Postdoctoral Fellow
Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC

Martin K. Childers, DO, PhD
Professor
Department of Rehabilitation Medicine
Institute for Stem Cell and Regenerative Medicine
University of Washington
Seattle, WA

George J. Christ, PhD
Professor
Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC

Ira S. Cohen, MD, PhD
Professor, Department of Physiology and Biophysics
Director, Institute for Molecular Cardiology
Stony Brook University
Stony Brook, NY

Stephen A. Fann, MD
Associate Professor
Department of Surgery
Medical University of South Carolina
Charleston, SC

Harold I. Friedman, MD
Professor and Chief
Department of Surgery, Division of Plastic Surgery
University of South Carolina School of Medicine
Columbia, SC

Mark E. Furth, PhD
Chief Technology Officer
Comprehensive Cancer Center
Wake Forest School of Medicine
Winston-Salem, NC

Peter A. Galie
Graduate Research Assistant
Department of Biomedical Engineering
University of Michigan
Ann Arbor, MI

Gautam S. Ghatnekar, DVM, PhD
CEO and President
FirstString Research Inc.
Charleston, SC

Richard L. Goodwin, PhD
Associate Professor
Department of Cell Biology and Anatomy
University of South Carolina School of Medicine
Columbia, SC

Robert G. Gourdie, PhD, FAHA
Professor and Center Director
Virginia Tech Carilion Research Institute and
Virginia Tech–Wake Forest University School of Biomedical Engineering and Sciences
Roanoke, VA

Roche de Guzman, PhD
Postdoctoral Fellow
Virginia Tech–Wake Forest University School of Biomedical Engineering and Sciences
Virginia Polytechnic Institute and State University
Blacksburg, VA

Benjamin S. Harrison, PhD
Associate Professor
Wake Forest Institute for Regenerative Medicine
Wake Forest University Health Sciences
Winston-Salem, NC

Johnny Huard, PhD
Stem Cell Research Center
Department of Orthopedic Surgery
Department of Bioengineering
McGowan Institute of Regenerative Medicine
University of Pittsburgh
Pittsburgh, PA

Emily Ongstad, MS
Graduate Student
Clemson University–Medical University of South Carolina
Bioengineering Program
Virginia Tech Carilion Research Institute
Charleston, SC

Jay D. Potts, PhD
Associate Professor
Department of Cell Biology and Anatomy
University of South Carolina School of Medicine
Columbia, SC

Lola M. Reid, PhD
Professor
Department of Cell and Molecular Physiology
and Program in Molecular Biology and Biotechnology
University of North Carolina at Chapel Hill
Chapel Hill, NC

Justin M. Saul, PhD
Associate Professor
Department of Chemical and Paper Engineering
School of Applied Engineering and Science
Miami University
Oxford, OH

G. Sitta Sittampalam, PhD
National Center for Advancing Translational Sciences
National Institutes of Health
Therapeutics for Rare and Neglected Diseases
Rockville, MD

Bert Spilker, PhD, MD
Pharmaceutical Consultant
Bethesda, MD

Jan P. Stegemann, PhD
Associate Professor
Department of Biomedical Engineering
University of Michigan
Ann Arbor, MI

Andre Terzic, MD, PhD
Professor
Marriott Heart Disease Research Program
Division of Cardiovascular Diseases
Mayo Clinic
Rochester, MN

Panagiotis A. Tsonis, PhD
Professor, Department of Biology
Director, Center for Tissue Regeneration and Engineering at Dayton
University of Dayton
Dayton, OH

Virginijus Valiunas, PhD
Research Associate Professor
Department of Physiology and Biophysics
Institute for Molecular Cardiology
Stony Brook University
Stony Brook, NY

Mark Van Dyke, PhD
Associate Professor
Virginia Tech–Wake Forest University School of Biomedical Engineering and Sciences
Virginia Polytechnic Institute and State University
Blacksburg, VA

J. B. Vella, MD, PhD
Stem Cell Research Center
Department of Orthopedic Surgery
Department of Bioengineering
University of Pittsburgh
Pittsburgh, PA

M. Natalia Vergara, PhD
Postdoctoral Fellow
Wilmer Eye Institute
Department of Ophthalmology
Johns Hopkins University, School of Medicine
Baltimore, MD

Belinda J. Wagner, PhD
President
Biographic Design Consulting
Winston-Salem, NC

J. Koudy Williams, DVM
Professor
Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC

James Yoo, MD, PhD
Professor and Chief Scientific Officer
Wake Forest Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC

Michael J. Yost, PhD
Associate Professor
Department of Surgery
Medical University of South Carolina
Charleston, SC

Foreword

Regenerative pharmacology is poised to revolutionize human treatment options in medicine and define a new medical frontier. Prepared minds have recognized the convergence of discoveries in pharmacology, molecular biology, and genetics with those of nanotechnology, advanced analytical techniques, and biomaterials resulting in the ability to initiate differentiation and regeneration of cells, tissues, and organs.

Dating back thousands of years, ancient civilizations documented how they imagined being able to regenerate limbs lost in battle or trauma. For centuries, the regenerative characteristics of salamanders, chicks, and other animals were known but it was only within the past four decades that scientists began to mobilize the integrative thinkers, resources, and enabling technologies to identify and address the reality of cellular differentiation. Understanding of hematopoietic stem cell differentiation led to the first life-saving regenerative intervention for bone-marrow transplantation in the mid 1970s and, over the next 15 years, scientists refined genetic engineering to succeed at more complicated hematopoietic cell interventions resulting in FDA-approved recombinant therapies to enhance regeneration of red blood cells and granulocytes. Yet, to take regenerative therapies to the next level, where pluripotent cells could be differentiated, de-differentiated, and reprogrammed, it meant that the nature of the regenerative biomedical research community itself needed to be remodeled.

Centers of Excellence in stem-cell and regenerative research were established and now serve as welcoming institutions where creative "new alloy" scientists, who possess a wide range of interdisciplinary expertise and skills in enabling technologies, can work toward a similar goal. These multidisciplinary scientists are funded to focus on teamwork and characterizing regenerative interventions that unite specific biology, physics, genetics, chemistry, and enabling technologies in a way that was only imagined in the past. Following his discoveries of alpha and beta adrenergic receptors in 1948, and therapeutic use of beta-blockers for the treatment of blood pressure and heart disease, Dr. Raymond P. Ahlquist remarked "... at this time

being a pharmacologist is akin to being a physiologist with a screwdriver." Today, a regenerative pharmacologist must surely be equipped with a hardware store of tools.

The impending impact of regenerative therapeutic intervention cannot be overstated in considering improvements to quality of life and reductions in healthcare costs. In the near term, the pharmaceutical industry will seek the talent and technology to develop research and interventions requiring partnerships with the NIH and with the FDA for approvals. The negative long-term physical, emotional and financial impact of birth deformities, traumatic injury, and dismemberment will be mitigated with future regenerative therapies and definitive treatments for life-long illnesses like diabetes and cardiovascular disease will be part of our history. With the complexity of the human organism itself, interdisciplinary teams of biomedical scientists are now identifying and replicating the sequence and symphony of essential factors that initiate, modulate, differentiate, de-differentiate, and remodel cells and tissues for organ regeneration. Today, scientists are pharmacologically able to guide pluripotent cells to differentiate along predictable paths of development, producing various heart cells and valves, cardiac tissues, urinary bladders, and other tissues with histologically appropriate layers, differentiation, innervations, and functionally appropriate contractions.

Dr. George J. Christ and Dr. Karl-Erik Andersson are congratulated for an outstanding book, *Regenerative Pharmacology*, which should be required reading for all biomedical scientists, medical students, integrative pharmacologists/physiologists, and indeed contemporary healthcare practitioners, regardless of specialty. *Regenerative Pharmacology* is a premier foundational treatise that introduces the topic and complexities of regenerative medicine and specifically describes new major developments in regenerative therapies. The book captures the evolution of many proposed regenerative interventions and, in an unassuming manner, the authors communicate in conversational style, to deliver details of their work in extensively referenced chapters.

Regenerative Pharmacology is a milestone publication and a definitive reference work for truly state-of-the-art discussions on stem and progenitor cells, bioreactor technology, and wound healing. This reference provides for in-depth understandings of regeneration of cardiac, kidney, bladder, and muscle cells and tissues, as well as micro/nano technology for delivery of therapeutic agents, active factors embedded in biomaterials, enabling technologies, implanted materials, and tissue-engineered constructs.

Congratulations to the editors for compiling this work. Congratulations to the editors and chapter authors for sharing their world-level expertise and for the manner in which the fundamentals of their work are introduced in understandable terms and then built upon to state-of-the-art discussions and future directions. The authors are among the top experts in this new frontier of biomedical research and truly represent

the "new alloy" scientists and pioneers who will shape our lives with their regenerative research and therapies of the future.

Dennis C. Marshall, RN, MS, PhD
Immediate Past Chairperson, Executive Member,
Division for Integrative System, Translational and
Clinical Pharmacology, American Society
for Pharmacology and Experimental Therapeutics and
Subcommittee for Clinical and Translational Research,
Federation of American Societies for
Experimental Biology and
Executive Director,
Medical Affairs Ferring Pharmaceuticals Inc.

Preface

The concept for this book, although based on years of prior research and learning, was definitively established several years ago when we coined the phrase "regenerative pharmacology," and moreover, wrote our first article introducing the topic and the potential implications for pharmacologists (Andersson & Christ, Mol. Int., 2007). Since that time, the field has truly exploded, although the underlying purpose for this first edited volume on the subject remains the same: namely, to get pharmacologists more involved in this field of research by exposing them to the tools, opportunities, challenges, and expertise that will be required to ensure awareness and galvanize involvement. In addition, we hope that the excellent material provided by the diversity of experts in this volume will spark new multidisciplinary conversations among all of the stakeholders. In our opinion, the field of regenerative medicine and its companion field, tissue engineering, would benefit significantly from the more rigorous application of pharmacological sciences. Specifically, despite enormous progress and promise, regenerative medicine and tissue engineering would still profit from a greater focus on the evaluation of functional outcomes and endpoints. In particular, a more extensive characterization of basic pharmacodynamics (excitation-contraction coupling mechanisms, rigorous analysis of concentration-response curve (CRC) data using standard pharmacological analyses/methods, estimation of receptor affinity, receptor subtypes, intrinsic activity, efficacy, potency, etc.) is required. In addition, we posit that greater emphasis on the pharmacology and physiology of various regenerative medicine and tissue engineering approaches is critical to increase understanding of tissue/organ regeneration and repair processes, as well as to enhance the rate of technology development and eventual clinical translation. In this volume we have brought together diverse fields of research, ranging from materials chemistry and functionalized biomaterials to stem cells, high-throughput drug screening and bioreactors for in vitro tissue engineering, as well as in vivo studies of wound healing and tissue and organ regeneration and repair. Again, we hope that the outcome will be recognition by all parties of the importance of the cross-fertilization of ideas and

tighter integration of the pharmacological sciences into the regenerative medicine and tissue engineering translational research enterprise. In fact, the image on the cover of this book, a 3D torus, is a simile for the ultimate complexity (and beauty) of tissue and organ regeneration and repair, as well as their eventual manipulation by pharmacology. That is, once we understand the properties of the knot, we can use pharmacology to drive regenerative medicine and tissue engineering technologies toward the creation of very precisely regulated tissue and organ structures with the requisite functional characteristics. We envision this book as the first volume of a series that will grow in parallel with this exciting field of research, and moreover, describe the journey at various points along the path. We look forward to the enormous possibilities for improved human health that can result from further development of regenerative pharmacology, and remind the reader that this is only the beginning of a long voyage.

George J. Christ, PhD
Karl-Erik Andersson, MD, PhD
Winston-Salem, NC, USA

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So many people have provided the inspiration and guidance required to complete this edited volume, which reflects many years of thought and preparation. We appreciate the understanding and encouragement of all our friends and family over the years. Above all, we would especially like to thank our most immediate families: Gina, Brandon, Jamie, Bryan, and Jake (George Christ); and Dagmar, Kristian, Mikael, and Karl (Karl-Erik Andersson), who paid the greatest price, but were always supportive and saw the greater good in this effort, while sharing love and laughs and many important moments throughout the years that led to the creation of this book. In addition, we would like to thank the folks at Cambridge University Press, especially Amanda O'Connor. Peggy Rote and her team at Aptara, Inc., also did an amazing job with the production of the book. Finally, we are grateful to Donna Tucker who helped organize and coordinate the final phase of copyediting and production among all of the authors and editors.

Section I

Basic Principles of Regenerative Pharmacology

1

Introduction to Regenerative Pharmacology: A Short Primer on the Role of Pharmacological Sciences in Regenerative Medicine

GEORGE J. CHRIST AND KARL-ERIK ANDERSSON

Regenerative medicine technologies continue to evolve and expand across the boundaries of numerous scientific disciplines, remaining at the forefront of the translational research frontier with the potential to radically alter the treatment of disease and dysfunction from a variety of causes. For the purposes of this book, regenerative medicine is broadly defined as the repair or replacement of damaged cells, tissues, and organs. This interdisciplinary effort includes, but is not necessarily limited to, the fields of cell, developmental, and molecular biology; chemical and material sciences (e.g., nanotechnology); engineering; surgery; transplantation; immunology; molecular genetics; physiology; and pharmacology. The goal of this book is to draw attention to the critical role that the pharmacological sciences will undeniably play in this process. In this regard, in 2007 [1], we defined "regenerative pharmacology" as "the application of the pharmacological sciences to accelerate, optimize and characterize (either in vitro or in vivo), the development, maturation and function of bioengineered and regenerating tissues" and posited that it would be of widespread utility to the sustained growth, expansion, and translation of regenerative medicine technologies. Since that publication, there has been a robust expansion of pharmacological approaches and applications to regenerative medicine. Many aspects of that growth are captured in the chapters included in this volume.

When viewed from a broader context, the timing of the regenerative pharmacology effort is auspicious and could leverage ongoing national efforts. One example is the creation of the Armed Forces Institute of Regenerative Medicine (AFIRM; http://www.afirm.mil). The AFIRM consists of two civilian research consortia working with the U.S. Army Institute of Surgical Research (USAISR) in Fort Sam Houston, Texas. Each consortium is a multi-institutional network, together comprising more than 30 academic and 15 for-profit members. Moreover, a national strategy for regenerative medicine has been outlined by the recently established Alliance for Regenerative Medicine (http://www.alliancerm.org/), a Washington, DC–based nonprofit organization. The mission of this organization is to educate key policy makers about

the potential of regenerative medicine and furthermore to advocate for public policies that will create the favorable environments for funding, regulatory approval, and reimbursement strategies, among others, that will be required to move the field forward. In addition, the National Institutes of Health (NIH) has recently published a fact sheet on the past, present, and future of regenerative medicine research and clinical translation (http://report.nih.gov/NIHfactsheets/Pdfs/RegenerativeMedicine(NIBIB).pdf). More recently, the Regenerative Medicine Promotion Act of 2011 (HR 1862) was introduced in the House of Representatives in May. Finally, the NIH recently established a Center for Regenerative Medicine: crm.nih.gov. Clearly, these are very exciting times for expanding the role of pharmacologists and the science of pharmacology into the realm of regenerative medicine and tissue engineering.

Therefore, the explicit aim of this chapter is to provide a conceptual framework from which to view the potential impact of regenerative pharmacology on the wider fields of regenerative medicine and tissue engineering. When viewed in this context, there is an important distinction between regenerative pharmacology and the more traditional applications of the pharmacological sciences to the development of small molecules (<500 Da), delivered systemically, for the palliation and symptomatic treatment of human disease (see Chapter 9 for additional details). More specifically, regenerative pharmacology seeks not only to create a new generation of therapies for improved symptomatic treatment of disease (i.e., fewer side or off-target effects caused by improved mechanisms of action [MOAs], enhanced localization, and cellular and subcellular specificity), but rather to maximally leverage existing multidisciplinary expertise for the development of transformational curative therapies through implementation of the science of pharmacology in the domains of regenerative medicine and tissue engineering. The focus on curative pharmacological therapies represents a paradigm shift for this longstanding field of medical research that has already had an enormous worldwide impact on healthcare delivery.

Importantly, organ and tissue engineering and the application of regenerative medicine technologies to patients also have a long and distinguished history. The necessity for these technologies grows logically out of the shortage of donor organs for replacement and transplantation, as well as the need for reconstructive procedures in patients experiencing tissue loss as a result of trauma, disease, or other congenital or acquired conditions [2,3]. The historical details of the field are well beyond the scope of this chapter; therefore, interested readers are referred to several other excellent expert opinions, reports, and textbooks [4–8], as well as other chapters in this volume that review some of the key developments. Without question, though, regenerative medicine represents a continuously evolving interdisciplinary biotechnology enterprise with global roots. However, as recently pointed out by Ingber and Levin [9], interdisciplinary distinctions can become quite blurred when dealing with a subject as complex as tissue and organ regeneration and engineering. Nonetheless, this field of translational research offers tremendous potential to positively impact and

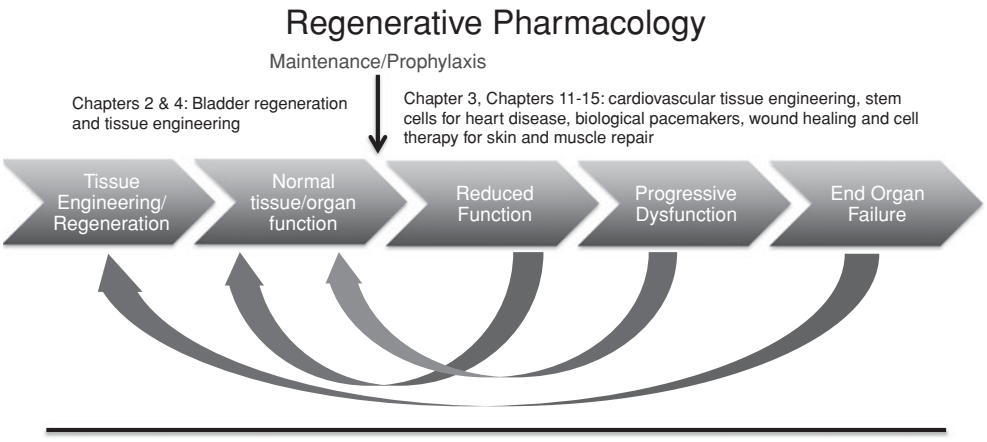

Figure 1-1. Schematic depiction of the utility of regenerative pharmacology to tissue engineering and regenerative medicine for the treatment of end-organ disease or failure. As illustrated, because of a variety of circumstances or causes, normal tissue or organ function can be compromised and transit through a series of stages starting with reduced function, eventually leading toward increasingly progressive dysfunction and finally end-organ failure. At each point along this path, demarcating the initiation and progression of tissue or organ dysfunction, regenerative strategies using or incorporating pharmacological strategies can be envisioned for restoration of function. However, at the point of end-organ failure, there is, by definition, not enough viable tissue remaining that any conventional gene- or drug-based strategy will be useful, and therefore, tissue engineering strategies would be required for whole organ replacement or alternatively, strategies for promoting endogenous organ regeneration. However, irrespective of the precise cause and degree of dysfunction, regenerative pharmacology provides an opportunity for restoration of normal organ and tissue function. Certainly, the exact strategies and technologies applied will depend on the magnitude and duration of dysfunction, as well as the organ or tissue of interest. The *arrow* denoting maintenance or prophylaxis indicates the possibility that after the process is sufficiently well understood, it might be possible to develop strategies for the maintenance of normal tissue or organ homeostasis or to slow the initiation and progression of tissue or organ dysfunction. Guidance concerning the relevance of each chapter to this overall scheme is provided. However, it is important to emphasize that the chapter denotations are the editors' (not the authors') and, moreover, are merely meant to reflect more general aspects of their relationship to the process being depicted. DDS = drug delivery system; HTS = high-throughput screening.

extend the useful lifespan of a seemingly ever-aging U.S. and world population, and the goal of this chapter (and book) is to begin to outline the numerous ways in which pharmacology can assume a primary role in this process.

The potential scope of regenerative pharmacology ranges from enhancing cellular therapy to optimizing bioengineered tissue and organ replacements to promoting endogenous tissue and organ repair. Figure 1-1 presents a conceptual framework for thinking about the application(s) of regenerative pharmacology during the initiation,

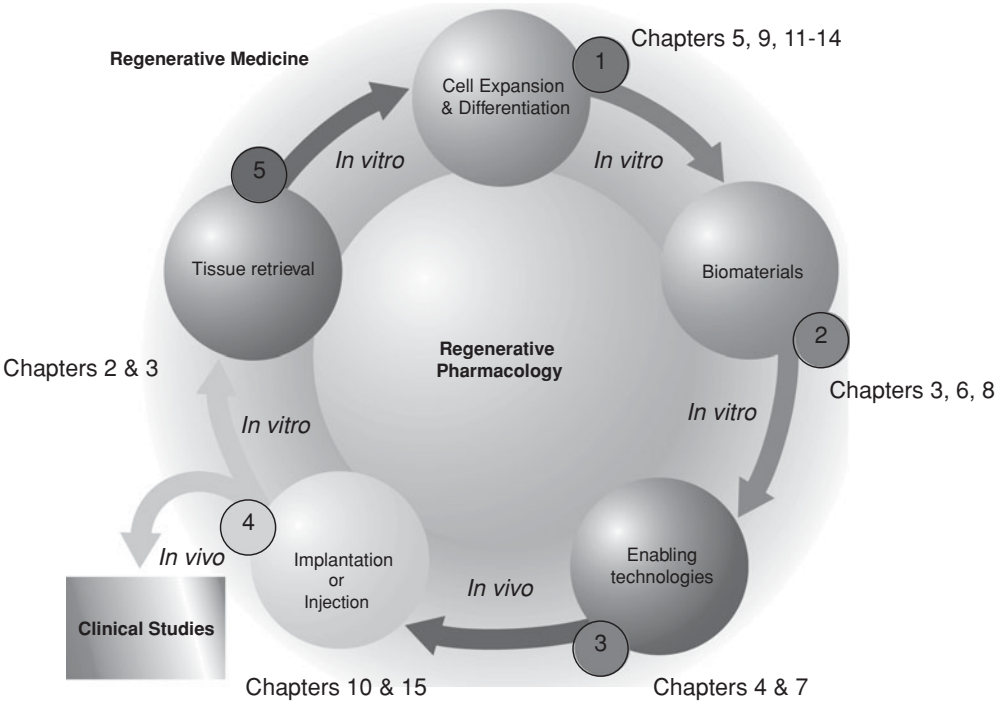

Figure 1-2. Schematic depiction of the iterative process that characterizes regenerative pharmacology. As illustrated, at all five steps along the path to clinical translation, regenerative pharmacology may be used to promote or direct the regenerative process as well as to report or dissect the impact of that process on established tissue or organ function(s). In this scenario, regenerative pharmacology is relevant to augmentation of cell expansion and differentiation (step 1) and furthermore can be combined with various nanotechnologies to create functionalized biomaterials or drug delivery systems (steps 2 and 3) as well as bioreactor technologies (step 3; note that a host of other enabling technologies, including but not limited to organ or tissue printing, vascularization, and innervation strategies might also be required) to further facilitate the tissue engineering or regenerative process before implantation (step 4) and tissue retrieval (step 5; preclinical analysis). Although one cannot rule out the possibility that at some point in the future technologies might exist to recapitulate embryonic development in adults in vivo (e.g., blastema formation, as described in Chapter 15), at the present level of technological development, this seems a reasonable research strategy for improved treatment of a variety of human diseases and dysfunctions. Regardless of the particular strategy used, regenerative pharmacology would play an important role in further augmenting or accelerating organ or tissue development at all five steps in the process. Again, an attempt has been made by the editors to position the main purpose of the various chapters in the context of the overall iterative regenerative pharmacology process. (Modified from Andersson and Christ [1].)

development, and progression of tissue or organ disease and dysfunction. Figure 1-2 provides a more comprehensive breakdown of the potential contribution of regenerative pharmacology to the each step in the iterative process that leads to advancement or creation of new regenerative medicine or tissue engineering technologies for the

treatment of organ or tissue disease and dysfunction. For the convenience of readers, the editors have noted where the individual chapters in this volume primarily impact these overarching themes. The numerous excellent contributions in this volume cover virtually the entire spectrum of regenerative pharmacology as originally described [1], with a few notable exceptions, which are described briefly in this chapter.

As illustrated, regenerative pharmacology can be used to both dissect and direct the regenerative process, and examples of this are provided in the chapters in this volume. In the former role (i.e., dissect) regenerative pharmacology is clearly more akin to "classical" pharmacology (see Chapters 2 and 3), but the latter role, that is, using pharmacological technologies to direct the development and regeneration of engineered and endogenous organs in vitro and in vivo, is clearly a more novel area of investigation, and thus, the vast majority of chapters in this volume are devoted to further exploration of this concept (see Chapters 4 to 15). Recent work from our group provides examples of how regenerative pharmacology can be used to dissect pertinent characteristics of regenerating and engineered organ and tissues in vitro and in vivo. For example, these studies have shown the utility of this approach in investigating de novo bladder regeneration, which is discussed in detail in Chapter 2. In this chapter, we briefly describe other examples of regenerative pharmacology to the in vitro investigation of bioreactor-derived tissue-engineered blood vessels (TEBVs; [10]) as well as after retrieval of implanted bioengineered vessels [11] or tissue-engineered skeletal muscle repair [TEMR] constructs [12,13]. Both TEBV and TEMR constructs were created using in vitro bioreactor technologies. TEBVs are being developed for the repair and replacement of damaged and diseased blood vessels (e.g., coronary artery bypass, peripheral artery disease, and dialysis access grafts) and were used as an interposition graft in the carotid artery of a sheep model. The TEMR constructs are being developed for the treatment of volumetric muscle loss (VML) and the associated irrecoverable functional deficits produced by these injuries. VML injuries may be caused by trauma as well as a variety of congenital and acquired conditions. To assess the utility of tissue engineering approaches to the treatment of VML injuries, we have examined the ability of implanted TEMR constructs to repair surgically created VML injuries of the latissimus dorsi (LD) muscle in a murine model (see Figs. 1-3 and 1-4 for details).

Briefly, our experience with the TEBV and TEMR constructs reveals the importance of bioreactor preconditioning in vitro to tissue formation and function in vivo and points to the current limitations of in vitro tissue engineering. More specifically, in these two instances, currently available bioreactor technology and methods produce relatively immature bioengineered tissues in vitro, with respect to both their physiological characteristics and pharmacological responsiveness [10–13]. The most salient features of these published studies with respect to their implications for regenerative pharmacology are summarized in Figures 1-3 and 1-4. A key feature of regenerative pharmacology that is emphasized in Figure 1-2 is the importance of bioengineered

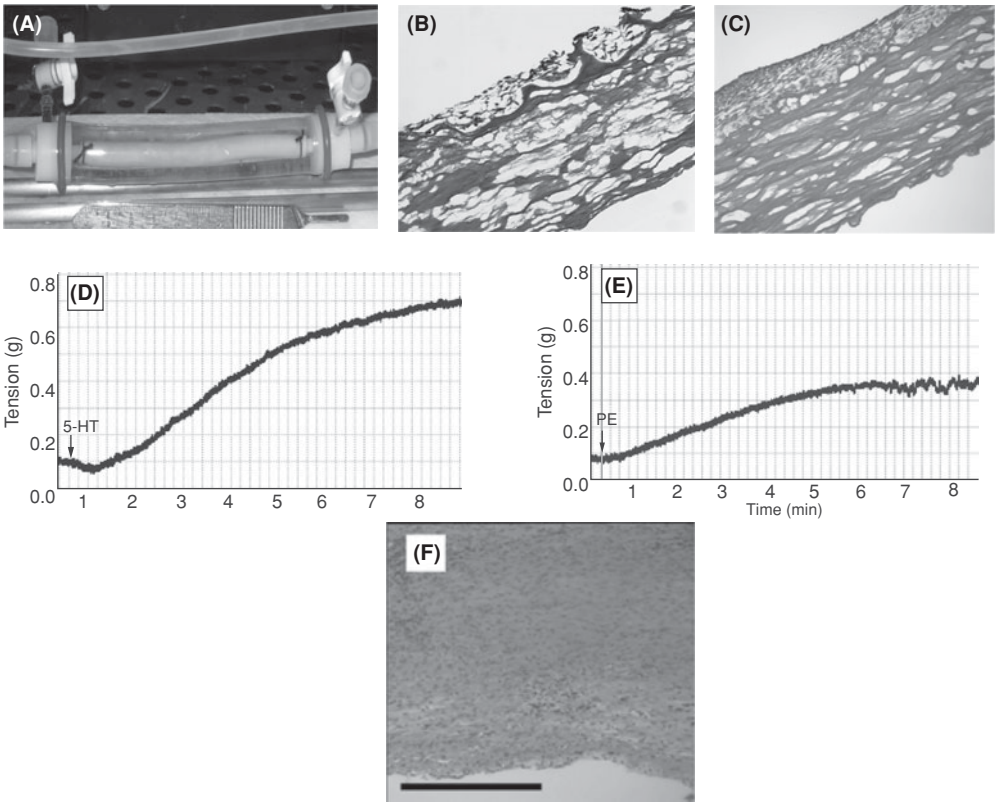

Figure 1-3. Illustration of the applicability of regenerative pharmacology to the development of tissue-engineered blood vessels (TEBVs). (**A**) Bioreactor flow system containing the scaffold seeded with endothelial cells (ECs) on the luminal side and with smooth muscle cells (SMCs) on the abluminal side. The bioreactor provides an external media bath, optical access, a bypass system, control over flow and pressure conditions, and the ability to maintain sterility. (**B**) Hematoxylin and eosin (H&E) stain of representative example of statically seeded SMCs on a decellularized construct after 48 hours and (**C**) after longer-term (3–4 weeks) bioreactor preconditioning. As shown, this period of bioreactor conditioning is sufficient to cause formation a substantive medial SMC layer. As noted by Yazdani et al. [10], Fura-2–based digital imaging microscopy experiments revealed no receptor mediated increases intracellular calcium levels. However, as indicated by the representative tracings shown in (**D**) and (**E**), retrieval of TEBV 4 months after implantation as a carotid artery interposition graft in sheep (Neff et al. [11]), revealed pharmacologically mediated contractile responses to 10 μM 5-Hydroxytryptamine (**D**) and 10 μM phenylephrine (**E**). *Arrows* indicate the application of agonists. (**F**) Representative H&E staining of a retrieved TEBV 4 months after implantation. Scale bar = 400 μM (Modified from Yazdani et al., 2009; Neff et al., 2011). (See color plate 1.)

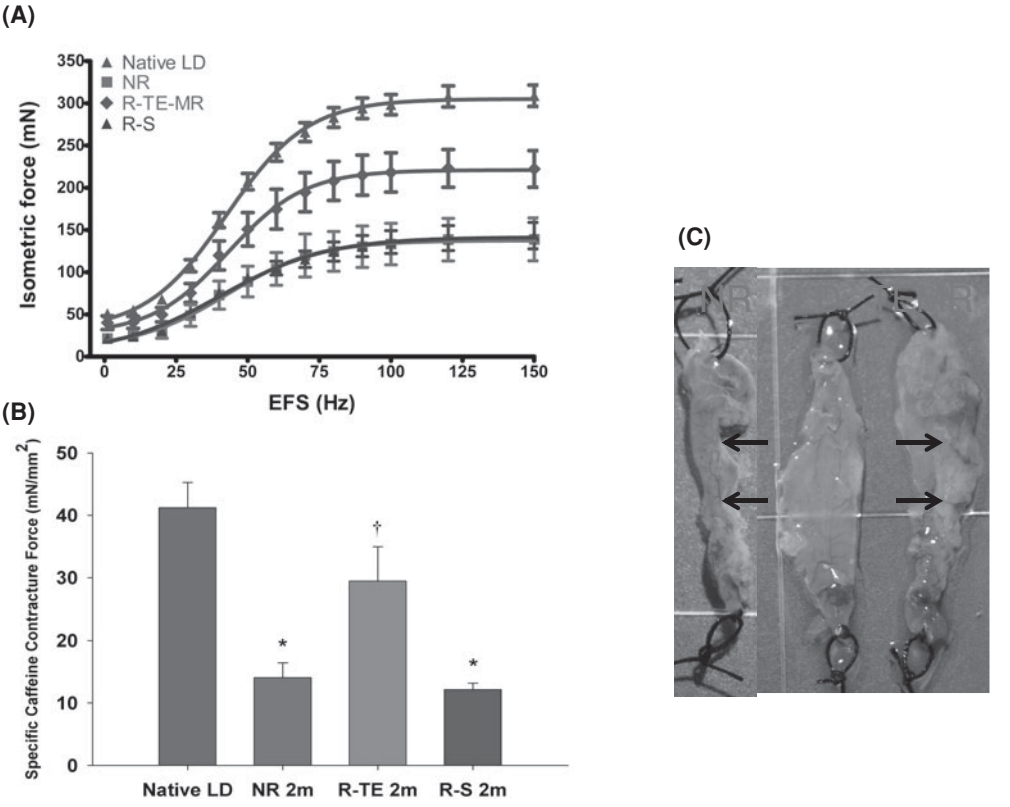

Figure 1-4. Morphologic assessment and functional recovery of retrieved tissues from the mouse volumetric muscle loss (VML) injury model. For these studies, bioengineered skeletal muscle implants were sutured into a surgically created VML injury by removal of approximately 50 percent of the murine latissimus dorsi (LD) muscle (see Machingal et al. [12] for details). (**A**) The mean values for the electrical field stimulation (EFS)–induced contractions observed on all retrieved tissues 2 months after injury or implantation. The sample sizes are native LD = 20, no repair (NR) (see C) = 5, repair with tissue-engineered muscle repair implantation (R-TE-MR) = 5, and R-S (repaired with a scaffold alone – no cells) = 5. The isometric absolute force (mN) is displayed as a function of stimulation frequency. Additionally, in (**B**) after force-frequency testing contralateral native LD muscles ($n = 6$), NR ($n = 4$), R-TE-MR ($n = 3$), or R-S ($n = 4$) at the 2-month time point were subjected to twitch contractions at 0.2 Hz in the presence of a maximally stimulating concentration of caffeine (50 mM). The *asterisk* denotes that group means are significantly different from that of control ($p < .05$). Values are means ± standard error of the mean. *Dagger* indicates that the group mean is significantly different from that of all other groups ($P < .05$). (**C**) shows representative examples of the gross morphology of retrieved LD tissues for an NR, native LD, and TEMR animal. *Arrows* indicate the original site of the surgical defect. Morphologic examination of tissue demonstrates robust tissue formation and remodeling of the TEMR construct but little or no tissue formation in the NR group. (Modified from Machingal et al. [12]). (See color plate 2.)

tissue characterization after implantation and retrieval (step 5 in the iterative process). As illustrated in Figures 1-3 and 1-4, after implantation in vivo, both TEBV and TEMR constructs produce new tissue formation and integration with host tissue, resulting in a dramatic increase in tissue physiology and pharmacological responsiveness to relevant stimuli. Nonetheless, and quite interestingly, despite quite remarkable functional recovery after implantation, both technologies reveal suboptimal physiological characteristics with respect to comparison with their native tissue counterparts. For example, the TEBVs in this study produce only approximately 20 to 30 percent of the contractile force of a native carotid artery to the same level of pharmacological stimulation. Although an improvement over prior work, which documented less than 10 percent functional recovery [14], there is clearly still room for improvement. In addition, although the TEMR-repaired LD muscles recover approximately 60 to 70 percent of native LD contractility to electrical field stimulation in the murine model, they still revealed evidence for altered excitation–contraction coupling; therefore, it appears that a component of the regenerating muscle may still be experiencing disruption in the EC coupling process, which would contribute to voltage-induced force deficits [15,16] (Fig. 1-4).

In short, with respect to both the TEBV and TEMR technologies, pharmacological studies have shed important mechanistic insight on the characteristics of the engineered and regenerating tissues (both in vitro and in vivo) that provide critical guidance for future technology developments. More data and additional pharmacological probes (with improved selectivity profiles) and bioactive agents would certainly aid in the continued development of regenerative pharmacology for vessel and muscle engineering. Of course, these represent just two examples, but they are further reinforced by the information contained in Chapters 2 and 3, which focus on the urinary bladder.

The applications of regenerative pharmacology continue with Chapter 4, which begins to examine the importance of matrix biology and mechanical forces on the differentiation of mesenchymal stem cells with specific emphasis on cardiovascular applications. Whereas Chapters 2 and 3 largely emphasize the utility of pharmacology to dissect aspects of the regeneration, this work highlights the ability of pharmacology to both dissect and direct regeneration. It is difficult to overestimate the value of this type of pharmacological data or information (to the nature of the regenerative process) and its importance to the improved understanding and clinical application of tissue engineering and regenerative medicine technologies.

Another major focus of this volume, and one to which the majority of chapters are devoted, is on the utilization of regenerative pharmacology to direct organ or tissue regeneration and engineering. Chapters 5 and 9, for example, deal with stem cells. The ability of pharmacology to modulate the behavior of stem and progenitor cells will be a key to the explicit goal of promoting the development, maturation, and function of bioengineered and regenerating organs and tissues. In this regard, stem cell source(s),

characterization, and differentiation are the subject of Chapter 5, and the development of the high-throughput screening methods for stem cell expansion and differentiation that would be required for efficient clinical translation and implementation are described in Chapter 9.

As noted in two recent articles, the field of biodegradable materials (i.e., biomaterials) represents a natural interface for pharmacology and regenerative medicine, yet there remains a paucity of successful clinical or commercial applications [17,18]. Consistent with their continuously evolving role [19], biomaterials, nanotechnologies, and their applications to the development of next generation gene and drug delivery systems (i.e., functionalized biomaterials) represent some of the most important and exciting new areas of applied pharmacology and are covered in Chapters 6 and 8. In fact, a variety of extant biomaterial-based technologies are available for tuning spatial and temporal delivery of bioactive agents. The applications of such technologies to regenerative medicine and tissue engineering are virtually endless and will undoubtedly open up new vistas of scientific enquiry.

Regenerative medicine technologies will likely need to be both organ specific as well as patient specific. For example, patients with diminished regenerative capacity may require more advanced technologies; therefore, one might suspect that more organ or tissue development will be required in vitro for successful regeneration after implantation in vivo. In this regard, significant tissue or organ maturation in vitro will require the use of bioreactors (i.e., the laboratory instruments or devices that are used to seed or precondition engineered tissues or organs by providing a biomechanical environment and milieu that mimics key aspects of the in vivo characteristics of the tissue or organ of interest) [20]. The complexity and sophistication of bioreactors may need to be enhanced to accommodate these more demanding requirements. Chapter 7 provides some examples of how this might transpire, and one can easily imagine how these devices could assume a pivotal role in regenerative medicine and the applications of regenerative pharmacology, perhaps especially with respect to the eventual transportation of the clinical product.

Another key aspect of regenerative pharmacology is the selection of animal models for studying the time course and characteristics of endogenous regeneration as well as the regenerative response that occurs after implantation of bioengineered organs and tissues. In this regard, efficient clinical translation requires the use of the most appropriate animal model for a given organ or tissue. Matching the pharmacology of the organ or tissue of interest to the corresponding human condition is therefore of paramount importance. There are many considerations and possibilities, and Chapter 10 provides an excellent summary of current knowledge and opportunities for selection of the most translational animal models.

Chapters 11 to 14 provide examples of ongoing applications of regenerative pharmacology to the treatment of a variety of diseases and disorders. More specifically, these range from the development of biologic pacemakers for cardiac disease (see

Chapter 11), stem cell therapy for cardiac repair (see Chapter 12), and pharmacology and cell therapy for wound healing and repair of damaged muscle (see Chapter 13) to regenerative pharmacotherapy for skin and heart. Not only do these chapters highlight the obstacles and promise of regenerative medicine and the impact of pharmacology from leading experts in the field, but importantly, they also identify numerous molecular targets for future consideration and development. In short, these "state-of-the-art" approaches point to the many exciting potential applications of pharmacology to regenerative medicine and tissue engineering.

Finally, Chapter 15 describes the current status of research in the amphibian kings of regeneration. Undoubtedly, there is much we can learn from the awesome regenerative capacity of the urodeles (newts and salamanders), which is characterized by complete wound healing and functional regeneration of a wide variety of tissues and organs. The molecular fingerprint and pharmacological blueprints uncovered by these investigations may one day provide important clues and novel approaches for enhanced clinical treatment of numerous age- and disease-related degenerative conditions of cells, tissues, and organs in patients.

There are clearly aspects and applications of regenerative pharmacology that are not covered in this initial volume on the subject. The most notable among these are potential applications to central nervous system (CNS) disorders. Thus, although important aspects of peripheral nerve regeneration are covered in Chapter 8, applications of regenerative pharmacology to a wide variety of CNS disorders (e.g., Parkinson's disease, Alzheimer's disease), including spinal cord injuries, is well beyond the intended scope of this book. However, the potential applications of nanotechnology, particularly to assist with transiting drugs and pharmaceuticals across the blood–brain barrier, have been well codified elsewhere [21,22]. In addition, we do not consider potential applications of regenerative pharmacology to genetic diseases, although clearly, regenerative pharmacology may provide an immediate therapeutic opportunity to slow down the decline of muscle function or loss in patients with, for example, muscular dystrophy [23]. The rationale for this particular application is that targeting key events downstream of the genetic defect can compensate, at least partially, for the pathological consequences of the disease. Finally, the use of drug-eluting stents for the treatment of vascular disease is not covered in this book, although we do recognize that there is obvious overlap between stent use and other aspects of the regenerative pharmacology applications that are covered herein. In short, these omissions are consistent with the main intent of this first volume on the subject, which is to provide readers with a reasonably comprehensive introduction and familiarity with the possibility of regenerative pharmacology but not an exhaustive recitation of all potential and current applications. We anticipate that this will be a very fast-moving field of research; therefore, application coverage can be even further expanded in subsequent updates as the field matures.

In summary, there exists an extraordinary opportunity for pharmacologists to get involved in this quickly developing research and development effort. As outlined

throughout this volume, regenerative pharmacology is a relatively recent field of endeavor, but it is one with enormous potential to move regenerative medicine and tissue engineering technologies more rapidly forward toward clinical translation. One particularly striking feature of the application of pharmacology to regenerative medicine is that it has the intrinsic potential to be curative rather than palliative, although improved treatment of symptoms would also be welcome. As noted earlier, this type of thinking represents a paradigm shift from the more traditional view of small molecule-based systemic therapeutics, which are designed to provide symptomatic relief. Regenerative pharmacology is equally applicable whether the loss of viable tissue occurs as a result of congenital anomalies, traumatic injury, inflammation, infection, or surgery or as a complication of another chronic disease. In each instance, regenerative pharmacology holds the promise of providing a curative therapeutic solution for end–organ and tissue failure, whether it is through augmentation of endogenous regeneration or enhancement of engineered replacement tissues and organs.

When considered in its entirety then, regenerative pharmacology is an enormous field of endeavor, and undoubtedly, this volume can provide only a glimpse into the huge scope and virtually endless possibilities of this burgeoning field of research. As such, we have necessarily focused on only a few examples to demonstrate the point, recognizing that, of course, there is still much below the "tip of the iceberg." This chapter, as well as prior reports [1,24], have provided some specific examples of regenerative pharmacology that begin to address the important role of the pharmacological sciences in tissue engineering and regeneration. In this book, we build on that conceptual base but expand our consideration to include a broader and more detailed discussion of the spectrum of pharmacological approaches currently being used or contemplated for regenerative medicine. In this scenario, the use of not only biomaterials but also cells as drug delivery vehicles for enhancing regenerative capacity and extending the applications of tissue engineering and regenerative medicine will also be explored.

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2

Regenerative Pharmacology of the Bladder

DAVID BURMEISTER, KARL-ERIK ANDERSSON, AND GEORGE J. CHRIST

Regenerative pharmacology can be defined as "the application of pharmacological sciences to accelerate, optimize and characterize (either in vitro or in vivo) the development, maturation and function of bioengineered and regenerating tissues" (Andersson & Christ, 2007). Generally, two approaches may be used: (a) the "active" (i.e., directing) approach, exemplified by the use of growth factors and different pharmacological agents or bioactive molecules to alter cell proliferation, differentiation, and function in a desired fashion, and (b) the "passive" (i.e., dissecting) approach, as illustrated through the use of established pharmacological methods to evaluate and compare salient characteristics of endogenously regenerated or bioengineered cells and tissues (e.g., how closely do the requisite signal transduction mechanisms of an engineered or regenerating tissue or organ compare with the native tissue or organ?). Both of these approaches are currently used in regenerative medicine, and the goal of this chapter as well as Chapter 3 is to illustrate these basic principles in detail using organ regeneration as observed in the bladder.

Why the bladder? Somewhat surprisingly perhaps, the bladder has actually been at the leading edge of clinical translation in tissue engineering and regenerative medicine. This is partly attributable to the rather extensive intrinsic regenerative capacity of this organ (Table 2-1). Regenerative pharmacology has been used as a tool to understand not only the phenomenon of endogenous bladder regeneration (with and without the use of scaffolds or cells) but also to optimize bioengineered bladder constructs for implantation (see Chapter 3 for more details). Because of the bladder's natural regenerative capacity, regenerative pharmacology not only can be used to characterize "normal" bladder regeneration (e.g., functionally, structurally, molecularly) but can also be used to identify mechanisms to improve regeneration in scenarios in which it is compromised.

In this regard, the distinction between "accelerating" or "augmenting" organ or tissue regeneration on the one hand and "characterizing" functional restoration in the regenerating organ or tissue is not trivial and indeed is at the heart of "directing"

Table 2-1. *A summary of clinical experiences with De Novo bladder regeneration*

Reference	Major clinical finding
Sisk et al. 1939	A 58-year-old man underwent extensive STC (leaving 3 × 3 cm of posterior bladder wall) and voided through his urethra 8 weeks later
Folsom et al. 1940	Eight women with interstitial cystitis underwent STC, resulting in bladder capacities up to 600 mL (one failure caused by pyelonephritis)
Richardson 1952	A 66-year-old man had removal of necrotic bladder tissue above trigone with normal cystogram and urination and a 350-mL capacity 1 year later
Bohne et al. 1957	Seven patients with carcinoma underwent STC, and bladder regeneration did occur; however, infections prevented success in some
Portilla Sanchez et al. 1958	A 65-year-old patient with bladder cancer underwent STC with a plastic mold; 3 months later, a bladder with a transitional epithelium grew larger than the mold
Baker et al. 1959	70 patients with bladder cancer underwent STC, with most resulting in sufficient bladder regeneration (∼20% incidence of asymptomatic ureteral reflux)
Liang 1962	11 patients underwent ∼75% STC without molds, suggesting a mechanical stretch stimulus for bladder regeneration
Tucci et al. 1963	A 45-year-old man underwent 80%–90% STC for bladder cancer, leaving only the ureterovesical junction and bladder neck; normal urination and 400-mL bladder capacity were observed 6 months later
Baker et al. 1965	Several patients presenting with recurring multiple transitional cell carcinomas underwent total mucosal excision; complete epithelial regeneration occurred without the incidence of cancer
McCallum 1965	A 36-year-old man had necrotic tissue (entire bladder except for part of the trigone) removed; bladder capacity increased from ∼45 mL to ∼300 mL in 6 weeks with normal bladder function

STC = subtotal cystectomy.

versus "dissecting" regenerative pharmacology, respectively. As mentioned earlier, the directing approach involves using pharmacological agents to actively modify different aspects of bladder physiology and function during regeneration and repair and could include treatments such as stem cell therapy or the delivery of growth factors as well as a host of other bioactive molecules. Although regenerative pharmacology can also be used to control, for example, stem cell growth or differentiation in vitro for eventual use in bladder repair, that topic is the subject of other chapters (see Chapters 5 and 9).

In contrast to the "directed" or "active" approach, the "dissecting" approach simply uses pharmacological methods to evaluate the characteristics of functional restoration during regeneration. As illustrated in Figure 2-1 and further discussed in Chapter 3, the regenerative process is evaluated using multidisciplinary investigations ranging from studies of bladder function in vivo (via cystometry) to cell and tissue function

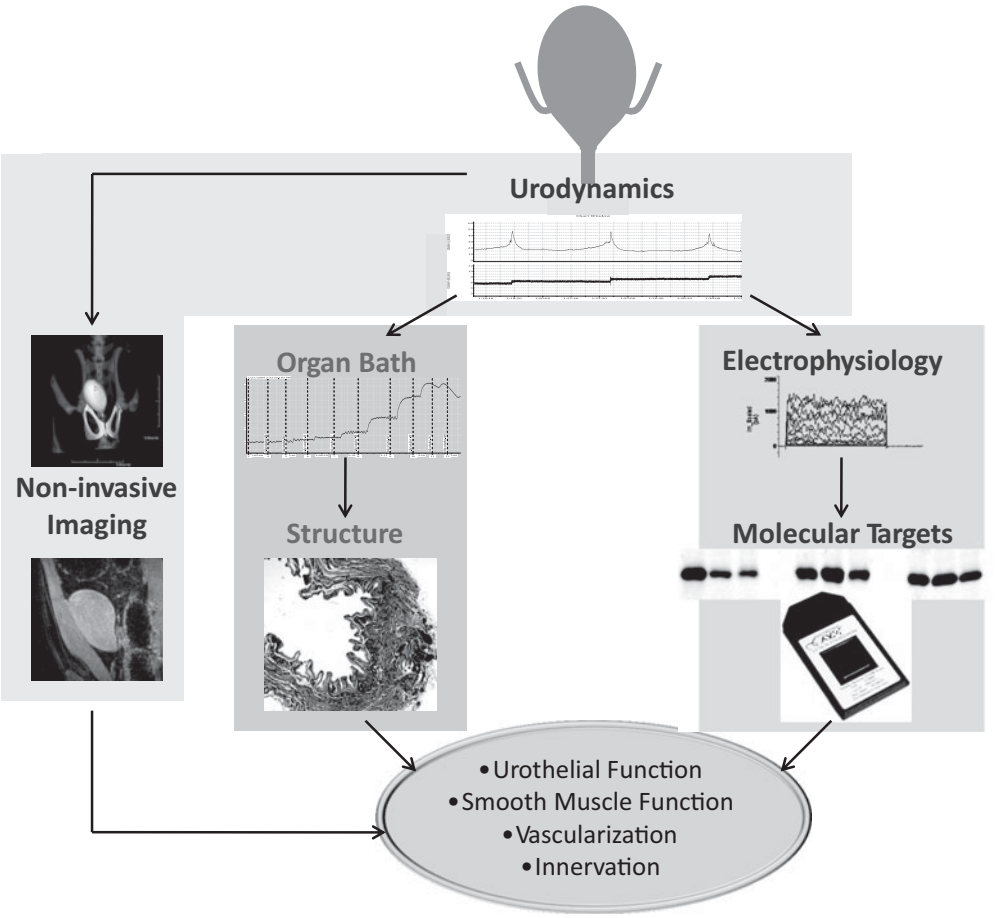

Figure 2-1. Dissecting (passive) regenerative pharmacology of the bladder. The bladder can be used as a model system that integrates multidisciplinary studies to evaluate organ regeneration (i.e., function and structure) on the whole organ (*green text*), tissue (*red text*), and cellular (*blue text*) levels. In vivo urodynamic studies can be used to examine overall bladder function. After euthanasia, bladder tissue can be cut into strips and stimulated to contract in an organ bath system (pharmacological studies), or sliced into sections for structural analysis (histological studies). Additionally, both gene and protein levels can be evaluated (molecular studies) and patch clamp methods can be used (electrophysiological studies) to study regenerated tissue. Noninvasive CT and MR imaging can be used longitudinally to examine organ morphology during regeneration, and possibly provide information on aspects of tissue phenotype gained via other methods. The information obtained from these studies can be used to design therapeutic interventions (see Fig. 2-2). (See color plate 3.)

in vitro via a variety of standard as well as state-of-the-art assays. In the remainder of this chapter, we will focus on discussion of the strategies outlined earlier with specific emphasis on animal models of intrinsic bladder regeneration. Additionally, the unique regenerative properties of the bladder will be addressed, not only in terms

of the current clinical need and available technologies but also with respect to how bladder derivatives per se can be used in regenerative pharmacology.

Regenerative Pharmacology and Bladder Disease

The aim of regenerative pharmacology is, ideally, to facilitate the restoration of normal organ function. This can be accomplished in conjunction with tissue engineering strategies to replace a nonfunctioning organ (end-stage disease). Alternatively, regenerative pharmacology can enhance endogenous regenerative capacity when sufficient viable bladder tissue remains in the face of functional impairments. From a physiological perspective, normal bladder function involves storage of urine at increasing volumes (without increasing intravesical pressure or spontaneous bladder contractions) until complete voluntary emptying is required. Diverse disease etiologies (e.g., neurogenic, congenital, trauma, infections) that compromise the low-pressure, high-volume function (decreased compliance) of the bladder lead to a number of lower urinary tract symptoms such as urgency, urgency incontinence, frequency, and nocturia. Without a doubt, there is enormous room for improved therapeutics, and regenerative pharmacology may be applicable to a number of these scenarios.

In this regard, antimuscarinic drugs (e.g., oxybutynin, solifenacin, darifenacin) are now the first-line therapy for treatment of detrusor overactivity and the overactive bladder syndrome. Lower urinary tract symptoms can also be treated with α-adrenoreceptor (AR) blockers (e.g., doxazosin) alone or in combination with antimuscarinics (Kaplan et al. 2006; Chapple et al. 2009). However, in cases of neurogenic bladder overactivity, in which one of the main aims of treatment is to prevent damage to the upper urinary tract, bladder contractility can be reduced with these treatments, necessitating the use of clean intermittent catheterization in some cases. With such diverse etiologies for bladder dysfunction and such a large demand (more than 50 million people are estimated to have some type of urgency incontinence), many different classes of drugs have been investigated (Andersson et al. 2009). These include, for example, β-3 AR agonists (e.g., mirabegron) and botulinum toxin-A, but a more detailed discussion of all current pharmacological interventions is well beyond the scope of this chapter, and moreover, these interventions are discussed in detail elsewhere (Andersson et al. 2009).

Most importantly, severe cases of bladder dysfunction are largely refractory to conventional pharmacological treatments. In this scenario, high bladder pressures may develop and lead to upper urinary tract deterioration (i.e., end-stage renal disease [ESRD]), particularly if the intravesical pressure exceeds 40 cm H_2O. Patients who display poorly compliant bladders caused by structural or neurogenic causes are at risk for ESRD and are thus candidates for surgical intervention (Reyblat et al. 2008). Currently, the gold standard treatment in these situations has been bladder augmentation. This procedure has been performed in patients with bladder diseases arising from

many different etiologies, including spinal cord injury, myelomeningocele, interstitial cystitis, idiopathic detrusor overactivity, radiation cystitis, multiple sclerosis, and schistosomiasis. Thus, the potential applications of regenerative pharmacology to the treatment of bladder dysfunction and end-organ bladder disease are truly enormous.

The purpose of bladder augmentation is to maintain low intravesical pressures while increasing bladder capacity (Gurocak et al. 2007). Attempts to increase bladder capacity can be traced back to the late 1800s and throughout the twentieth century with many different materials both natural (fascia, dura mater, intestinal segments) and synthetic (Teflon, polyvinyl) (Schwartz 1891; Kudish 1957; Bono et al. 1966; Kelami et al. 1970; Gleeson et al. 1992; Cheng et al. 1994). By the middle of the twentieth century, the use of intestinal (usually ileal) segments became commonplace, but this procedure was still associated with side effects such as urinary stones, pyelonephritis, metabolic imbalances, infections, and mucus production (Flood et al. 1995). This, along with the lack of tissue available for donor bladder transplantation, pointed to the need for regenerative medicine or tissue engineering technologies for the bladder (Aboushwareb et al. 2008). Although this approach is also covered in Chapter 3, we discuss some relevant background herein to provide important context to an improved understanding of endogenous bladder regeneration, which is the focus of this chapter.

In one of the first successful neo-organ transplants, bladders were constructed by seeding dome-shaped synthetic scaffolds (collagen or collagen–polyglycolic acid composites) with urothelial cells on the inside and smooth muscle cells on the outside that were subsequently implanted into patients with myelomeningocele (Atala et al. 2006). The regenerative pharmacology aspects of this work (which were crucial for the success of these neo-bladders) are covered in detail in Chapter 3. Although these studies indicate that an autologous, engineered tissue can be safely implanted and may have clinical utility for the treatment of neurogenic bladder, the clinical experience is still limited, and the technology is not yet ready for wide dissemination (Atala 2011). In this regard, it is clear that greater mechanistic insight of the endogenous regenerative process would be beneficial to further improve this technology for broader clinical applications. Such is the focus of this chapter.

The successful application of tissue engineering approaches to bladder augmentation in patients may not be surprising given the long known regenerative capacity of the bladder in both animal models and humans. There are numerous indications that the human bladder possesses significant regenerative capacity (e.g., after subtotal cystectomy [STC]) as outlined in Table 2-1. Sisk and Neu reported one of the first clinical experiences in 1939, describing a patient who voided through the urethra 8 weeks after STC leaving only a 3-by-3 cm patch of the posterior bladder wall (Sisk et al. 1939). Later studies described presumptive regeneration in bladder cancer patients. For example, one study reported that only 6 months after removal of the entire bladder, except for the ureterovesical junction and bladder neck, bladder capacity reached 400 mL (Tucci et al. 1963). Even though unsuccessful accounts of bladder

regeneration also exist (Bohne et al. 1957; Ross et al. 1969; Goldstein et al. 1970; Taguchi et al. 1977; Barros et al. 2006) citing complications such as infections, taken together, the extant literature clearly indicates that under certain circumstances, the human bladder can regenerate in situ. However, a more precise identification of the requirements for human bladder regeneration has not been codified, and the continued use of bladder augmentation techniques ensures that this avenue will not be pursued until further understanding of the natural process is obtained. To this end, the use of animal models allows researchers to study bladder regeneration under tightly controlled conditions. Indeed, Daniel Liang in the 1960s attempted to leverage this idea and reported similar findings of STC-induced bladder growth both in humans and rats (Liang 1962; Liang et al. 1963).

The utility of the bladder as a model organ system to study the characteristics of endogenous regenerative capacity and the role of regenerative pharmacology in characterizing this process is highlighted in Figure 2-1. As described in Chapter 1, the use of regenerative pharmacology to modulate the regenerative process (see Fig. 2-2) is iterative. Through this approach, one can identify, for example, biologically active molecules (e.g., small molecules and growth factors) that can be utilized to further optimize functional bladder regeneration.

Passive Regenerative Pharmacology of the Bladder

As noted earlier, the underlying premise for this line of research is that strategies aimed at harnessing the body's natural capacity for regeneration will undoubtedly benefit from a basic understanding of de novo bladder regeneration. Given that there is a lack of knowledge on endogenous organ regeneration per se in well-characterized and easily studied systems, it is not entirely surprising that so few strategies to enhance this process have been discovered. As a first step in this direction, we have recently published the first studies we are aware of that used a multidisciplinary approach to characterize bladder regeneration (Burmeister et al. 2010; Peyton et al., 2012). In the initial report (Burmeister et al., 2010), trigone-sparing cystectomy (STC) was performed in 12-week-old female rats. Computed tomography revealed a time-dependent increase in bladder volume at 2, 4, and 8 weeks after STC that positively correlated with restoration of bladder function. Bladders emptied completely at all time points studied, that is, we observed functional regeneration, albeit in the presence of significantly diminished contractility (see later discussion for details). Moreover, the bladder displayed urothelial, lamina propria, and detrusor muscle layers and regained normal thickness upon histologic evaluation. Immunohistochemical staining also showed expression of proliferating cell nuclear antigen and a population of CD117 (c-kit)–positive cells after STC that was not seen in control bladders.

More recently (Peyton et al., 2012), fluorescent bromodeoxyuridine (BrdU) labeling was used to quantify the spatiotemporal characteristics of the proliferative response

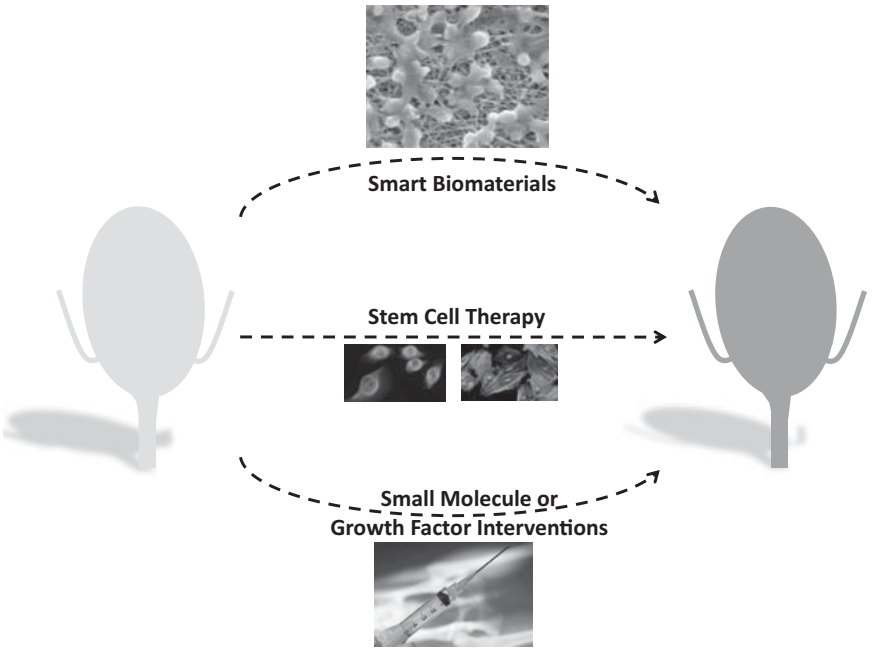

Figure 2-2. Directing (active) regenerative pharmacology of the bladder. Dissecting the process of bladder regeneration (Fig. 2-1) can identify specific processes (e.g., cell proliferation, differentiation, angiogenesis, innervation, and stem cell migration) that can be manipulated pharmacologically to direct tissue engineering and regenerative medicine strategies for the bladder. (*Top*) Tissue-engineered constructs currently used for bladder augmentation techniques can be altered to include, for example, different cell types and/or controlled oxygen delivery to enhance regeneration. (*Middle*) Supplementation of stem cells may modulate different aspects of bladder regeneration in a paracrine fashion. (*Bottom*) Pharmacological manipulation with small molecules or growth factors may be used to target specific signaling cascades involved in bladder regeneration. The ultimate goal with any of these interventions is to maximize bladder regeneration to restore normal bladder function. (See color plate 4.)

that mediates this robust functional regeneration during the first week post-STC. Less than 1 percent of cells in the bladder wall were labeled with BrdU in control bladders, but this percentage significantly increased by 5–8-fold at all time points post-STC. Specifically, the spatiotemporal characteristics of the proliferative response were defined by a significantly higher percentage of BrdU-labeled cells within the urothelium at 1 day than in the muscularis propria (MP) and lamina propria (LP). A time-dependent shift at 3 and 5 days post-STC revealed significantly fewer BrdU-labeled cells in the MP than LP or urothelium. By 7 days, the percentage of BrdU-labeled cells was similar among urothelium, LP, and MP. STC also caused an apparent increase in immunostaining for Shh, Gli-1, and BMP-4. In summary, the early stages of functional bladder regeneration are characterized by time-dependent changes in the location of the proliferating cell population in bladder wall layers, and expression of several

evolutionarily conserved developmental signaling proteins. This report extends previous observations and further establishes the rodent bladder as an excellent model for studying novel aspects of mammalian organ regeneration.

Interestingly, although we have indeed observed functional regeneration (as reflected by the fact that animals are continent, with low-pressure, high-volume reservoirs), the regeneration process still does not result in full restoration of a bladder with identical properties as native bladders. More specifically, we observed a decrease in bladder smooth muscle contractility to both muscarinic and electrical field stimulation (EFS). Cholinergic activation resulted in contractile responses that were approximately 20 percent of that observed in normal bladder tissue of age-matched control participants. Although we observed a time-dependent increase in detrusor contractility, when bladder volume was completely restored (i.e., 8 weeks after STC), maximal steady-state contractions were still only about 37 percent of normal values. This diminished contractility occurs despite the apparent recovery of bladder wall innervation as judged by the presence of contraction to EFS (and staining to protein gene product 9.5). These observations are in agreement with an earlier study by Frederiksen et al. (2004) in which whole-mount staining of acetylcholinesterase was performed to visualize the pattern of "normal" innervation after regeneration. They found that regenerating bladder tissue contained nerves on the anterior aspect of the bladder that were more slanted against the longitudinally running muscle bundles compared with control participants. This pattern of innervation was more reminiscent of the trigonal region of the bladder (i.e., the tissue that was still present after cystectomy) than in native bladder wall from control animals.

In fact, this same group had already posited that the newly forming bladder tissue after STC most closely resembles the remaining supratrigonal tissue. In 2004, Frederiksen et al. (2004) provided the first and most complete description of the pharmacology of newly forming bladder tissue (after STC) at that time. In those studies, transverse strips were excised from the bladder body 15 weeks after STC in female rats and were evaluated by investigating the possibility of regional differences in contractility. The authors used agonists and antagonists of muscarinic receptors and α_1-AR, as well as an agonist and desensitizing agent of P2X1 receptors (α,β-methylene adenosine triphosphate [ATP]). Their findings showed that contractility in response to EFS was not affected by α1-AR blockade, and strips from just above the trigone contracted similarly (in terms of percent maximal response) when muscarinic receptors were blocked. However, in more distal (i.e., equatorial) preparations, muscarinic blockade produced a greater inhibition of contractility in control bladders than that from animals that had undergone STC. The result of this investigation was consistent with prior work and supported the supposition that although the newly formed bladder smooth muscle is well innervated, the pharmacological properties are most reminiscent of the trigonal tissue from which it had developed.