Section I

Basic Principles of Regenerative Pharmacology

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

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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

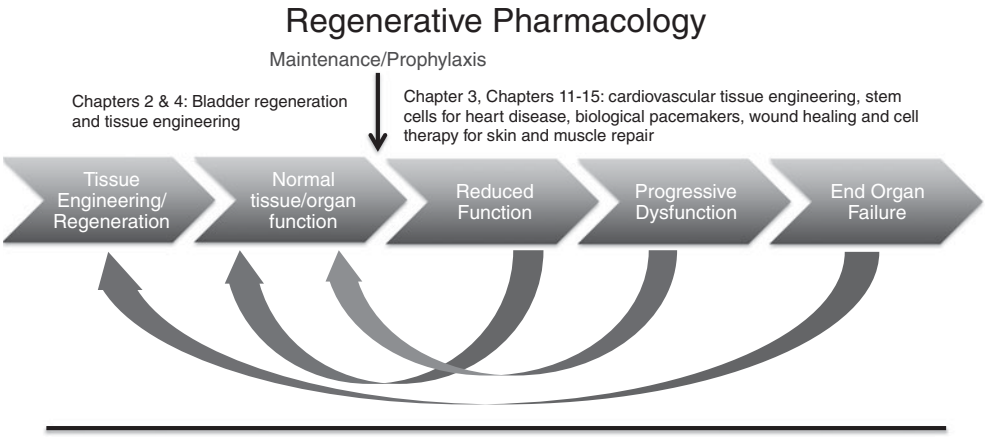

Figure 1-1. Schematic depiction of the utility of regenerative pharmacology to tissue engineering and regenerative medicine for the treatment of end-organ disease or failure. As illustrated, because of a variety of circumstances or causes, normal tissue or organ function can be compromised and transit 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,

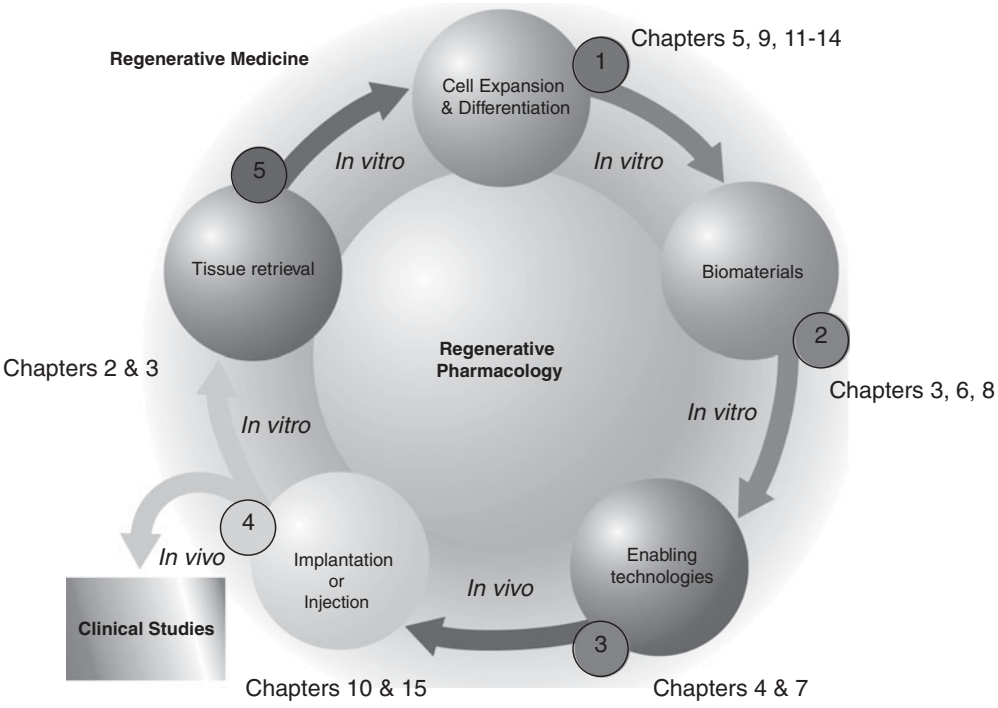

Figure 1-2. Schematic depiction of the iterative process that characterizes regenerative pharmacology. As illustrated, at all five steps along the path to clinical translation, regenerative pharmacology may be used to promote or direct the regenerative process as well as to report or dissect the impact 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

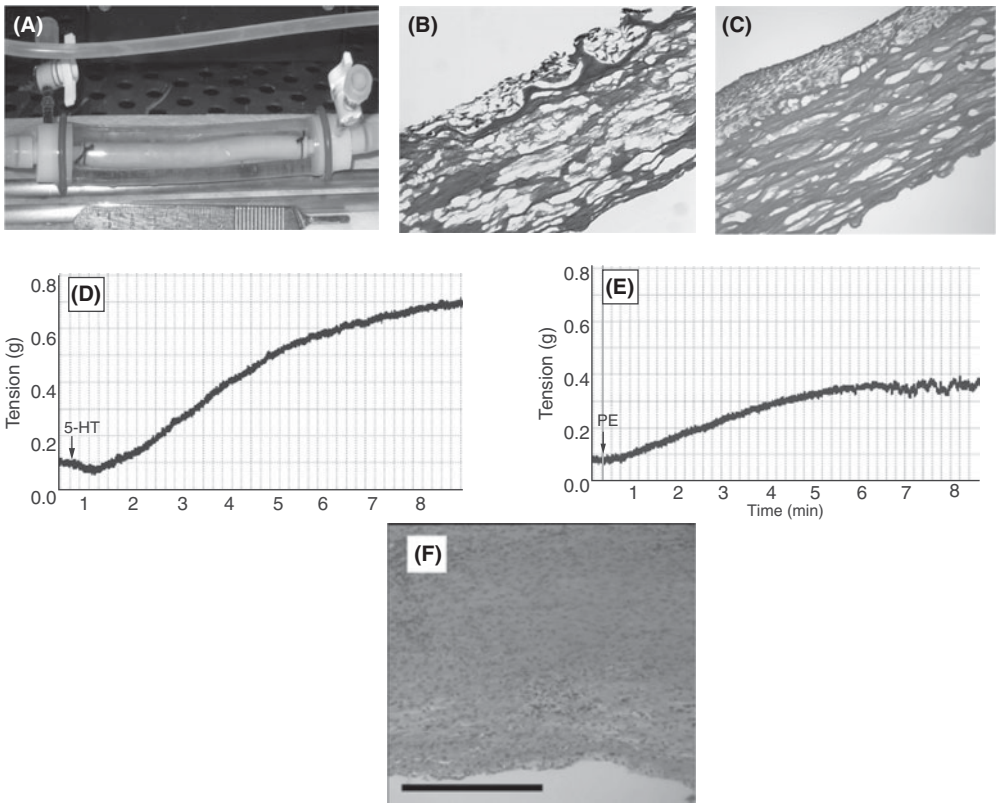

Figure 1-3. Illustration of the applicability of regenerative pharmacology to the development of tissue-engineered blood vessels (TEBVs). (**A**) Bioreactor flow system containing the scaffold seeded with endothelial cells (ECs) on the luminal side and with smooth muscle cells (SMCs) on the abluminal 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.)

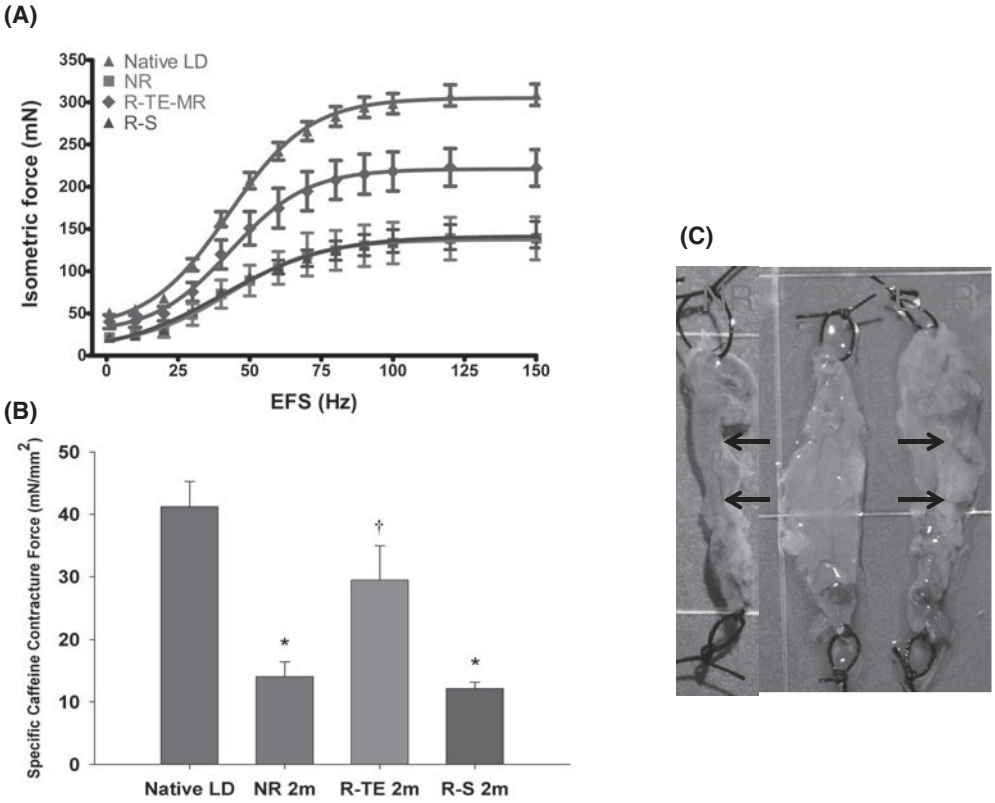

Figure 1-4. Morphologic assessment and functional recovery of retrieved tissues from the mouse volumetric muscle loss (VML) injury model. For these studies, bioengineered skeletal muscle implants were sutured into a surgically created VML injury by removal of approximately 50 percent of the murine latissimus 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),