



Introduction to Regenerative Medicine

1

Timothy Ganey and H. Thomas Temple

Regenerative medicine has been one of the frontiers for understanding human biology for centuries. Long before it fell under the category of medical research, or was assigned the regenerative moniker, or even considered medical practice, humans have sought to understand the basic context of how the body emerges with such complexity and near errorless synchrony to produce the dividends of interdependent function. When that elegant system fails and disease or degeneration breaks into perfection, however, the challenge then becomes to isolate the weakness and either replace or regenerate the affected tissue. As physicians and biologists seek to “unbrick” the wall, to isolate the piece or pieces from the whole, the evolution of knowledge has shifted the balance of understanding to seek the indivisible rather than to reintegrate the fragments as a functional system.

Medical practice also comes under the aegis of governing approvals and oversight that ensures that both safety and efficacy are attained. With both health and commercial practices collaborating and competing to accommodate patient care without compromising the economics of reimbursement, technologies have been developed that are broadly reductionist and guided by the trajectory of regulatory approval. Seeking “mechanism of action,” strategies aligned to identify niche assets of a biological process that are economically viable and scientifically accurate, companies have strived for therapeutic advantages for patient care and have evolved along with the principles of action–reaction understanding.

It has been clarified that with respect to cell-based matrices, the market and regulatory bodies have accepted that living cells can be included in allograft for use in repairing bone. With a source that is allogeneic, i.e., from a donor that

will be used for homologous use, several products have come to market that have living cells and are marketed under FDA guidelines that regulate them as Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). To meet the threshold that defines that categorization, there are several criteria that must be met [1]. Without broad discussion, four tenets have defined the cornerstone of dialogue with the FDA and the discussions for product development for commercial purposes:

- The HCT/P is minimally manipulated;
- The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer’s objective intent;
- The manufacture of the HCT/P does not involve the combination of the cells or tissues with another article, except for water, crystalloids, or a sterilizing, preserving, or storage agent, provided that the addition of water, crystalloids, or the sterilizing, preserving, or storage agent does not raise new clinical safety concerns with respect to HCT/P; and
- Either:
 - The HCT/P does not have a systemic effect and is not dependent upon the metabolic activity of living cells for its primary function; or
 - The HCT/P has a systemic effect or is dependent upon the metabolic activity of living cells for its primary function, and:
 - Is for autologous use;
 - Is for allogeneic use in a first-degree or second-degree blood relative; or
 - Is for reproductive use.

If the definition of the cells, in particular, does not remain within the margins imposed by this standard, then the proposed product is regulated as a “Biologic” and requires different stringency for FDA approval prior to marketing in the United States. For companies trying to commercialize regenerative medicaments for therapeutic use, such additional

T. Ganey (✉)
Vivex Biomedical, Inc., Miami, FL, USA

BonePharm, LLC, Tampa, FL, USA
e-mail: tim@bonepharm.com

H. T. Temple
Department of Orthopaedic Surgery, HCA Healthcare Inc.,
Miami, FL, USA

regulatory approval often creates an economic barrier that is insurmountable.

With regard to viable cell allografts, cell-based matrices, and other living tissues, the FDA to date has permitted the use of cells having metabolic function in the transplant. In the field of regenerative medicine, stem cells have been shown to be able to self-renew but also give rise to daughter cells committed to lineage-specific differentiation. To achieve this remarkable task, they undergo an intrinsically asymmetric cell division whereby they segregate cell fate determinants into only one of the two daughter cells. Stem cells have been typed with a specific “cluster of differentiation,” “cluster of designation,” or “classification determinant” that is usually abbreviated as **CD** for identification. Once identified, extensive in vitro investigations have been carried out to determine specific cell functions under precise conditions in the scientific method of changing single variables and measuring outcomes. Elaborate cellular mechanisms that orchestrate the processes required for asymmetric cell divisions are shared between stem cells and other asymmetrically dividing cells. These cells demonstrate that asymmetry/polarity is guided by varying degrees of intrinsic and extrinsic cues and intracellular machineries that divide the desired orientation into a balance of asymmetry/polarity.

Regenerative medicine has utilized cells with the CD designations from a variety of sources, including bone marrow, adipose tissue, peripheral blood, placental tissues, etc. to produce cell lines that have purposeful uses and specific cell phenotypes. When isolated and expanded cells (called A in this example) are steeped in science (called B), cells with the same phenotype present in cellular matrices (labeled as C) are then presumed supported by the same science. In this case of transitive equivalence, $A = B$, and $A = C$, so therefore $B = C$. The argument, empiric in nature, may be essentially accurate but likely understates the science and underestimates the integration of cell, cell factors, cell polarity, asymmetry, immune integration, and a myriad of yet undiscovered complications needing further explication.

A colleague offered me insight into this context a few years ago stemming from his appreciation of music. Although our discussion focused on spectrums of incident light and electromagnetic frequency on adaptation, its analogous value to music was the space between the notes. His awareness was fresh and reverent to an understanding widely ascribed to the French composer Claude Debussy, a prominent musician in the style commonly referred to as “Impressionist Music.” Neither likely intended to link a harmonic guiding a psychophysical phenomenon, remarkable in part because the perception of periodicities, namely pulse and meter, arise from stimuli that are not periodic [2]. One possible function of such a transformation is to enable synchronization between individuals through perception of a common abstract temporal structure (e.g., during music performance). Understanding

that the underlying brain processes are a fundamental aspect of human perception enables communication between neural areas such as auditory and motor cortices. Should we think that the organization and integration of tissue interfaces are any less driven by a similar message? I think that is one of the keys that accelerates the interest and defines the concept of regeneration—essentially aligning that the periodicity of the asymmetry of the tissues and cells is a more orchestrated construct than one relying on the simplicity of “**CD**” designation and tissue composition.

Curiosity drives the human mind to find out more and to look for additional factors, but each evolving inner analog offers less information that contributes to a better understanding of the whole. Using bone as an example of a tissue that for the most part retains restorative potential throughout life, it remains opportune that regenerative medicine engages the subsets of understandings that have been found in reducing its parts as we make attempts to further the regenerative techniques we have gleaned from this reduction (Fig. 1.1).

The concept is straightforward; for every point on a line, there is a space between, and within that space exists something unmeasured, something assumed to be average or represented by the adjacent known entities, but still vastly unknown. From a classic perspective of molecular metrics first demonstrated by Kees Boeke in 1957 [3], the lay public was offered that insight in the seminal work of *Powers of Ten* by Philip and Phyllis Morrison [4]. Both depictions collapse a logarithmic trek from the cosmic outer limits to the ocean of the universe within a carbon atom, with humans serving as a mere intercept along the journey, a placeholder, or milestone to a personalized awareness. Coupling the musician’s awareness of the silence between the notes that brand the music, the challenge to biologists is to understand the space between the defined but arbitrary scales of life and investigate the depths of the dark space to differentiate determinants of illness from measures of health. A better sense of that space should help facilitate understanding and translate an unknown into a meaningful therapeutic intervention.

A Holy Grail of modern stem-cell research is the recreation of a functioning organ. The vital importance of achieving this goal is all too clear. In the United States alone, nearly 9% of patients with liver failure die waiting for a new organ. An example of a much broader need is the organ transplant services, where from December 1988 through February 7, 2019, more than 758,000 transplants have successively been performed [5]. With the demand for transplantable organs far exceeding supply, the need for regeneration therapies has never been greater. This translates into a significant opportunity to repair, restore, and regenerate organs before the need for replacement imposes a life or death mandate.

Among the earliest attestation to regenerative medicine emerges from the Greek literature in the myth of Prometheus. Each day, an eagle would feast on his liver, and each night

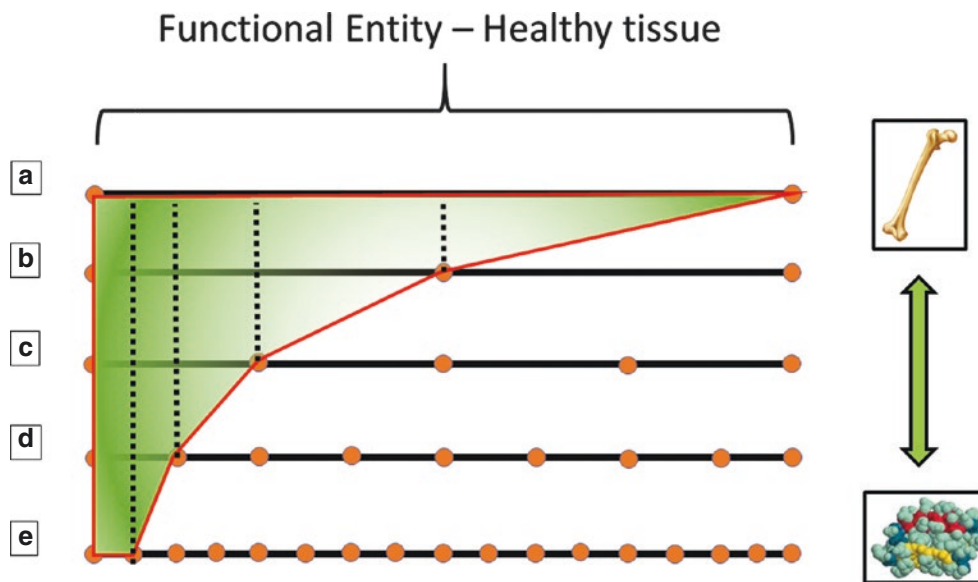


Fig. 1.1 Functional entity—healthy tissue. While it is possible to know ever more distinct areas of a system, it is more challenging to fully integrate individual aspects of their actions into a predictable scheme. The science of regenerative medicine has been paved in individual bricks that appear to offer both dimension and direction. This cartoon depicts the ever-increasing complexity that defines a sector, but at the same time independent of the connection might not fully characterize the science attending the conclusion. (a) Bone is a living tissue that provides skeletal support. “Bone” is the whole. (b) Skeletal support is dynamic and interdependent on mechanical stimulation for modeling. Interdependent and analog spaces are “bone” and “load.” (c)

Mechanical modeling of bone depends on adequate blood supply, endocrine interaction, and nutrition. “Bone” has now four derivatives: load, blood supply, endocrine, and nutrition. (d) Each Linnean reduction comes with a subset of its own reductions, and if a fraction of blood supply is further divided, the logic of asymptotic understanding is assured. In the instance of blood supply, the additions of endothelial lining, sympathetic tone, growth factor activity, endogenous regulation, and repair are just the start. (e) Furthering those strands of knowledge, say fibroblast growth factor as an example, is it possible to extrapolate FGF in vascular homeostasis as a meaningful prediction of the whole organism?

his liver would regrow in time for the eagle’s return. When hearing this tale, it is tempting to consider that the ancient Greeks had witnessed the amazing capacity of the liver to restore itself and noted the cruel and incremental penance as a substantiation of the immortality of the gods as, in fact, it was Zeus who had deemed this his punishment. This possibility fascinates those engaged in regeneration research, and for some, it is the seminal reference to a cultural understanding of regenerative powers by the Greeks [6–8]. Authors assume that the Greeks knew about the liver’s regenerative powers [9] or adopted an agnostic attitude through uncited logic in exceptional journals [10, 11]. An extensive discussion of the regenerative awareness of ancient civilizations suggests that early human anatomists trailed the myths by more than 1500 years and that the more likely scenario of culinary prowess, a belief in organ vitality, and the subsequent blurred lines of myth and time perhaps have led to more confusion than convincing evidence [12].

The literature is replete with notations of what constitutes attempts by the body to make the system whole. Since the time that it was observed and long before it was documented that limb regeneration occurs in amphibians, inquisitive individuals sought a remedy for loss and a solution to the need for restoration. There is little argument that regenerative medicine harbors the potential to restore tissues and organs

and reconstitute their function, yet the tenets of agreement rapidly diverge with broad tentacles that tack an immense number of strategies. Limitations of technology did not blur early insight but reduced many of the scientific merits to musing. In what is a limitless framework of observation, experimentation and communication, key elements can be drawn together to formulate a basic understanding of the potential for regeneration and how it can be utilized in legitimate medicine. It is also possible to append the analog between the cardinal points to better perceive, if not correct, the trajectory of pathology.

Modern therapeutic remedies are guided in the framework of regulation and under the auspices of what is safe and efficacious and what the main risks are. Perhaps a more rigorous evaluation would engage an overview of how regeneration differs from generation. Ernst Haeckel coined the phrase that each acolyte in the sciences is exposed to—*Ontogeny recapitulates Phylogeny*, which is akin to “The Biogenetic Law” that assigns a context where evolution added new stages to produce new life forms. Thus, embryonic development became a record of evolutionary history. The single cell corresponded to amoeba-like ancestors, developing eventually into a sea squirt, a fish, and so on.

By the turn of the century, discoveries were made that defied Haeckel’s so-called law. Initially cast as exceptions, the

rise of genetics and the modern synthesis has since explained the rate and direction of embryonic development. Individual genes can mutate and cause different changes to the way embryos grow, either adding or taking away new stages at any point along their path or altering the speed of development. This science of epigenetics is the foundation of regenerative medicine, and although somewhat guided through the tiers by a Lamarckian notion that evolution has direction, the challenges of regenerative integration compared with generative development are vastly different (Fig. 1.2).

In the framework intersecting science and experience as this book touts, there are foundations that assure certainty and others that remain to be conquered. It is not richly imaginative to appreciate the fact that all life on Earth shares a common

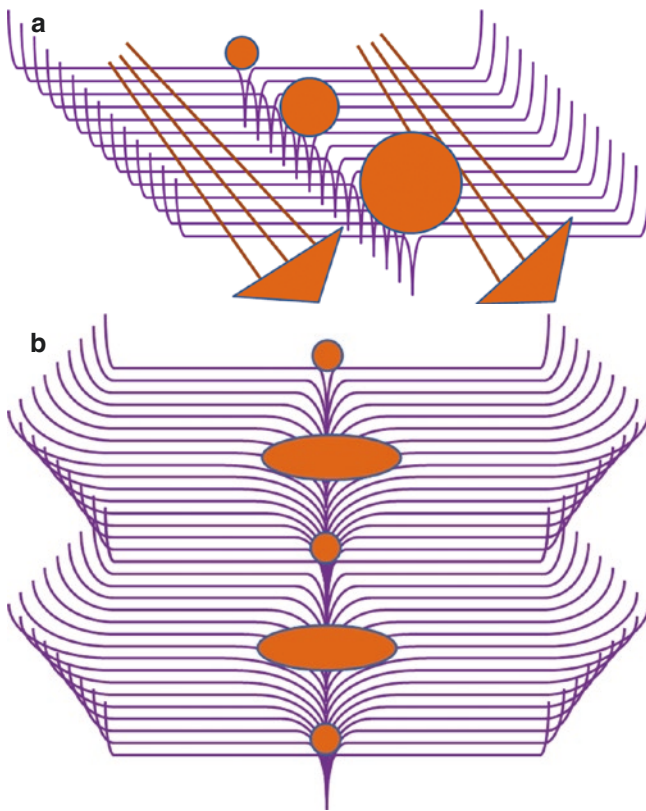


Fig. 1.2 The concept of ontogeny recapitulating phylogeny is rooted to the attribution of Ernst Haeckel, who suggested that an individual organism's biological development parallels and summarizes its species' evolutionary development. (a) Represented are the stages of development an organism proceeds through, with the orange sphere denoting the path of development. In this illustration, the course is singular and successive and directional as growth. (b) During a regenerative event, development, integration, and achieving appropriate size decorate an existing grid rather than establish a new one. In this example, the epigenetic influences of the existing scaffold, cell activity, and organism age serve as architects of the new potential, and the distortion or the variation between the generative and regenerative dimensions is illustrated as orange spheres that are at once both inconstant and responsive to the morphogenic field by which they are imposed. (a) Linear development isometric; (b) epigenetic and shaping influences

ancestor, a cell that arose from bacterial progenitors nearly 4 billion years ago. Whether it was from a freak accident, divine intervention, or the perseverance of a change that remained while other experiments failed remains to be determined. It is an interesting exercise to wonder how many attempts were made to unify the efficiency of a colony as a resonating single cell that could divide, diversify, and then reassemble the colony with singular and plural cell versatility expressing physical characteristics and traits that were diversified within the organism. Those cells emanating from a common ancestor have become a fundamental aspect of the science of biology and the core foundation of regenerative medicine. Distinguishing the cells as a core feature, it is important to determine the cues that shift the diversity and sort the reaction to stimulus and symptomatic change. Are the subtle signals standardized to the single cell, or does a synchrony dictate the cross-talk and exchange that can be part of the translation? Better tools, more extensive thought, shrinking dimensions of the space between the dots of knowledge bring us into a nexus that allows cells to be nearly infinitely sorted as a taxonomy in a style that Linnaeus would envy [13].

An argument could be made that the genesis of DNA discovery contributed to an evolving increase in gaps that are parallel in scope and number to the points learned. Erwin Schrodinger made two key points in his 1944 book *What is life?* [14]. Relevant to the topic of regeneration, he noted that life somehow resists the universal tendency to decay, a process that is otherwise known as entropy and stipulated in the second law of thermodynamics and second, that the secret to life's evasion of entropy lied in the genes. Years before Crick and Watson inferred the sequence of bases carried the genetic information, Schrodinger proposed that the lack of nonrepeating bases could act as a "code-script"—the first use of the term in the biologic literature [12, 15], which has become the basis of modern biology. The realms of code, 3 billion letters in our case, reads like a novel of enchanted, coherent stories and vast swaths of repetition that result in a 2% coding for proteins, a larger portion for regulatory functions, and the remainder still assigned to the cliché of needing a better understanding. Understanding the structure of the code has created the ultimate conundrum for regenerative therapeutics as genomes do not predict the future but recall the past. They reflect the exigencies of history and the containment of the environment.

What does regeneration look like in the context of tissue where information previously in equilibrium finds itself not only disrupted but unconstrained? Are those tissues able to recapitulate the origin, pass through, and return via a stable state that is differentiated to function, facilitated to anatomy, and fostered with sufficient receptors that will balance and check re-integration? As a starting point, and short of the replacement of entire extremities and organs, what features have guided the science, established a hierarchy of ethical

domains, and regulated the industry? In this chapter, the goal is to establish basic tenets of regenerative approaches, in particular, a potential that cells maintain for self-replacement, lineage multiplicity, and informed exchange to guide the function of complex tissues. In that regard, all tissues require a metabolic supply, and nearly all function from a vascular supply. Recognizing the regulation of the vascular system in and of itself is incomplete science, but given the appearance of angioblasts and a cardiac beat at 21 days from conception, its role must be carefully calibrated in the morphogenesis of tissues. From the simplest of consideration of pressure and shear forces in vessels, or as advocated by the mathematician and computer scientist Alan Turing, in 1952, it was the molecular diffusion of nutrients [16]. While none of these hypotheses were absolutely wrong, continuing work has demonstrated that factors released affect activation, perfusion, dimension, and flow dynamics that are paracrine, cytokine, and hormonal. It is safe to note that vasculogenesis is the formation of early vessels laid down by programming that is genetically deep and that satisfies a quorum of conditions to ensure competent and controlled inherent expression. At the periphery is another consideration that demonstrated mindfulness can change the response and that placebo invigoration is in itself a medicine. This consideration that functional metabolic responses follow that course is interesting but perhaps a bit peripheral. A concept that likely warrants a brief mention is the current understanding that the placebo response and gene variants in catechol-O-methyltransferase (*COMT*) gene may act as risk factors for psychopathology [17].

This is interesting for several reasons, two of which are noted here. For some time, it has been known that the brain and matter are inextricably linked by brain peptides, emotions, and physical expression of symptoms. The mind–body connection has been known in academia long before it has developed into a mainstream awareness. This is critical to understanding regenerative medicine as the psychophysiological manifestations might offer avenues of insight into repair, variation of response, and restrictions to healing that are inherent to various disease processes [18, 19]. In some instances, individuals with multiple personality disorders display symptoms that vary with each personality such as allergy to cats, diabetes, and so on. This suggests that what we know of matter and mind are surely integrated in a regenerative frontier. What are the primal signals for degeneration that guide the scope of recognizing a need for regeneration, and if it is simply insufficiency, why does the body not respond and compensate?

Regenerative medicine is a field that involves replacing, engineering, or regenerating human cells, tissues, or organs to establish, restore, or enhance normal function. It is an area with great promise that includes cell therapies, therapeutic tissue-engineering products, human cells, and scaffolds upon

which cells can grow [20]. Recently, there has been much interest specifically in the potential of adult stem cells to address a wide variety of conditions. A process of renewal, restoration, and growth regeneration allows genomes, cells, organisms, and ecosystems to attain resiliency to natural fluctuations or events that cause disturbance or damage. Every species is capable of regeneration, from bacteria to humans. Regeneration can either be complete, where the new tissue is the same as the lost tissue, or incomplete, where in the process of repair, the lost tissue is replaced by fibrotic tissue or scar formation.

At its most elementary level, regeneration is mediated by the molecular processes of gene regulation, adequate proliferation, and balanced structuring of tissues with an accompanying metabolic support. Regeneration in biology, however, mainly refers to the morphogenic processes that characterize the phenotypic plasticity of traits allowing multicellular organisms to repair and maintain the integrity of their physiological and morphological states. Everyone is familiar with the concept of debridement, the process of removing unhealthy tissue from the body. The affected tissue may be necrotic (dead), infected, damaged, and contaminated, or there may be a foreign body in the tissue that requires removal. In the context of regenerative medicine and tissue regeneration, how does the body recognize the boundaries of healthy tissue and preserve and annotate the morphogenetic field for integrated replacement. How does the body understand sufficiency and not overreach and replace an entire area during what might be intended to be a focal repair?

Regeneration somehow balances the extant or existing tissue, recognizes the errant or injured tissue, and in some primal manner determines what is repairable and what is expendable and then aligns a paradigm of repair mechanisms to make the tissue whole. Deposition, modeling, cues of repair, charge, density, permeability, porosity, and morphology are all factors that are considered.

The goal is one of normalizing self, recognizing limits of volume, cellularity, cell density, cell inhibition, adequate metabolic demands, and sympathetic restoration. How are the setpoints for the repair integrated and satisfied on the whole? What is the equilibrium that measures and weights the variants that differentiate absence and abundance? At what point does the relevance of metabolism merge into the controlled moderation genetic hierarchies, immune and injury response, tissue repair, and remodeling until function has been restored to a pattern that is more physiological than pathological.

It is common to think of tissue replaced in vast definable anatomies. Apart from large tissue regeneration or repair, the ongoing focal replacements should also be understood as regeneration. Humans and animals lose tissues and organs due to congenital defects, trauma, and disease at all times. The human body has a low regenerative potential as opposed

to the urodele amphibians commonly referred to as salamanders. Traditionally, transplantation of intact tissues and organs has been the treatment method to replace damaged and diseased parts of the body. Today variations of that technique expand the capabilities of traditional tissue banking and transplantation options. One asset of the human tissue allograft has been the inclusion of cells as a viable allograft. Through refinements in technology, techniques for sorting and collecting cells that afford renewed vigor to a host needing tissue have been developed. Variations in methods, cell sources, and carrier scaffold imbue not only viability and vitality but bioavailability as well. That availability has the paracrine factors that enable cell–cell communication at a cellular and subcellular level. This understanding is not a new one as paracrine and cytokine communication has been known for quite some time. Chapter 9 discusses exosomes as the depth of exosome understanding deserves a greater depth of discussion. With the clear evidence that it offers the basis of epigenesis, exosomes perform the transfer of genetic cargo from cell to cell. Stem cell populations can secrete various bioactive compounds, including exosomes, extracellular vesicles, and an entire secretome that effects change and, in some cases, restores equilibrium. Successful isolation of these complexes (which contain a variety of active signaling agents) and their subsequent administration might be an alternative strategy to stimulate the functions of host cell populations in damaged tissue sites. Properly delivered active signaling molecules could subsequently facilitate ECM deposition, the tissue remodeling process, and tissue regeneration.

Strategies for repair including resorting the tissue to its previous ability to take stress and strain along with the neural integration and regaining function are challenging but connected components of any therapeutic strategy. Many studies on the mechanisms of regeneration have led to the identification of cytokines, growth factors, and signal transducers that are produced by cell types within the organ being replaced or transported to the tissue repair site by vascular, lymph, and interstitial transport. These cytokines and growth factors are thought to cause cell expansion and proliferation, resulting in functional recovery. The details of such mechanisms, however, have not been sufficiently elucidated, and the practical applicability of regeneration based on the action of cytokines and growth factors is still unclear. Various cells and organs are involved in the regeneration process, which proceeds as a result of the coordination of many factors. Exposure to cytokines alone might be a trigger, but it would be naïve to assume that exchange is dormant and waiting for physiologic inspiration to activate.

Adult stem cells are important for the normal maintenance and repair of wounded tissues through their ability to differentiate, remodel the extracellular matrix (ECM), modulate the immune response, and secrete growth factors

and cytokines that stimulate cell migration and neovascularization [21, 22]. Mesenchymal stem cells (MSCs) originate in many tissues, but bone marrow and adipose tissue-derived stem cells (ADSCs) are the most available for harvest. Mesenchymal stem cells are known to differentiate down several cell lineage pathways to form cartilage, fat, muscle, and connective tissue, but they are also actively involved in the regulation of wound healing [23]. Mesenchymal stem cells have also been shown to regulate the immune response and inflammation in wounds through the secretion of immunomodulatory cytokines. These cells also cause enhanced proliferation, migration, and secretion of biologically active molecules by a process known as paracrine signaling [24]. Studies suggest that the paracrine activity of MSCs significantly enhances responsiveness and migration of macrophages, epithelial cells, and endothelial cells [25]. As a result, autologous and allogeneic stem cell therapies have been considered a form of treatment to stimulate healing of wounds, both chronic and acute. In consideration of what a wound is, every surgery is technically a wound requiring a healing event. It is not surprising that cellular, peripheral vascular, and other adjunctive assets have been employed in the mechanisms of aiding healing and hastening the process of regeneration. Coming back to the conjunction of the viable allografts/cellular bone matrices, the activity of the cells is one of the measurable metabolic parameters and one of the catalysts that produces byproducts of cell disintegration that sends the sum of its parts as a signal. In regenerative medicine, there is little evidence to associate autologous cell activity with grafting placement, and a similar abbreviated understanding needs further elucidation.

In addition to humoral factors, the autonomic nervous system is also involved in the regeneration process as noted in human liver repair [26]. Studies examining the direct feedback relationship between the liver and brain are transmitted via the afferent sympathetic nervous system to the ventromedial region of the hypothalamus and then to the lateral region of the hypothalamus. They then pass through the dorsal nucleus of the vagus nerve in the medulla oblongata, after which they return to the liver [27, 28]. It appears that the autonomic nervous system first activates the afferent sympathetic nerves in the damaged liver, which transduces the signal to the center of the autonomic nervous system in the brain and then to the efferent vagus nerve. This results in the activation of cell proliferation in various organs inside the abdominal cavity, such as the liver, gastrointestinal tract organs, and pancreas. Despite this known process, no study has focused on the effect of this system on liver regeneration. Therefore, the system as an effector of liver regeneration plays as much of a role as the local tissue. Given the large focus on cell therapy intended for applications in mesenchymal-derived tissue, is it likely that vascular,

endocrine, immune, or innervation also plays an important role and is no less involved in constitutive repair.

As the body and tissue bend to breathe the autonomic nervous system as an integrated response is attuned to both cellular and biochemical functions, how does knowing the margins of whole reintegrate the control, posture, and strength in accord with the demands of a challenging tissue like musculoskeletal? What are the signals that interpret flexibility and stiffness as sufficient, or what connotations of electrophysiology affect a membrane resonance that confers a loss of polarity and presses for proliferation and migration over matrix attachment, cell grounding, and matrix elaboration? How do the cells that might have been transplanted attenuate their placement, attach to the matrix, and reintegrate appropriate function and location? What guides the polarity of cells during asymmetric division and resonates a functional status that comprises rather than compromises that biologic expression? These questions challenge scientists, biologists, and tissue engineers to better appreciate the complexity and authorize process identity that nurtures the repair not as an alternative but as an expectation.

Fate of Transplanted Cells

The human body consists of billions of cells that exist together as an intricately organized and mutually supportive community. This cell community is a dynamic system that is maintained by a well-regulated balance between cell proliferation and death. Medical science surrounding regenerative

intervention speaks to stem cell delivery and identifying the cell lineages, but little discussion goes beyond the perception of viability and appropriate markers that suggest pluripotential before placement. Is it conceivable that by placing a bolus of cells into a defined tissue it will provide immediate integration and sustain the ligands and cell markers that have been validated in the process or is it more likely that the cascade of response directly results from the cytokine exchange and evolving phenotype in both the cells delivered as well as in the cells in the host tissue? It seems unlikely as well that cells remaining viable after placement would assemble as might be analogous to a 4-segment “Tetris” puzzle (Fig. 1.3).

Do cells migrate after they are placed and what evidence that has been defined in vitro can be applicable to in vivo validation? It is clear that cell migration plays a central role in a wide variety of biological phenomena such as in embryogenesis where cellular migrations are a recurring theme in important morphogenic processes ranging from gastrulation to development of the nervous system. Migration remains prominent in the adult organism as well as is seen in both normal physiology and with pathologic processes. In the inflammatory response, leukocytes immigrate into areas of insult, where they mediate phagocytic and immune functions [29]. Migration of fibroblasts and vascular endothelial cells is essential for wound healing and, to the extent that even the finest surgical interventions result in wounds, many considerations of the wound field are applicable. Finally, cell migration is crucial to technological applications such as tissue engineering, playing an essential role in colonization of biomaterials scaffolding. While under the aegis of regenera-

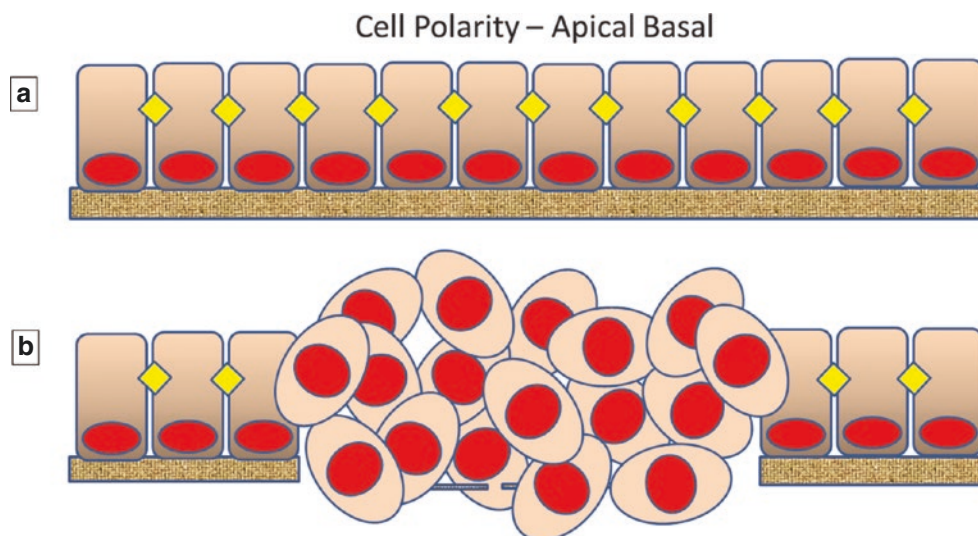


Fig. 1.3 Organizing. (a) Normal simple epithelium is comprised of a monolayer of individual cells that display distinct apical-basal polarity. Cells are tightly packed and connected to each other by the apical junctional complexes (yellow), which separate apical (light pink) and basolateral attachment to the basement membrane domain. (b) With

deposition, delivery, or wound healing, individual cells devoid of polarity must achieve a dimension defining basement membrane repair to guide their attachment and integrate as an aligned tissue within the extant tissue

tion, the restorative requirement of volume replacement in some injuries will require a scaffold that protects the space of the injury. However, when this balance is skewed by injury, repair, dysregulation of activity, or even cell accumulation, predictable outcomes are less assured. Individuals citing theoretical risks turn to the possibility of tumor development or the potential death of the entire cell community due to inflammation or uncontrolled biologic processes.

As with many other cellular processes, the molecular components involved in cell migration are being identified at a rapid rate, including the determination of how they participate in migration. The manner in which these components work together, like most other cell functions, as a dynamic integrated system to give rise to migration is only beginning to be studied. Understanding cell migration as an integrated process requires an appreciation of chemical and physical properties of multicomponent structures and assemblies, including their thermodynamic, kinetic, and mechanical characteristics, because migration is a process that is physically coordinated both spatially and temporally. Only when it is understood as an integrated system will its alteration via genetic, pharmacologic, or materials-based interventions acquire a truly rational basis where placement, scaffold, density, identity, and intention are balanced by the initial delivery—much the same as noted by the analogy of music and perception. A sense of assembly hears an orchestra where science is still recognizing the instruments.

From experiments performed more than two decades ago, a compilation of existing data emerged that maximal cell migration speed tends to correlate inversely with contractile force [30]. Further refinement of those observations contrasted contractual-migration interfaces that would imply that the optimal cell–substratum adhesive strengths yielding maximal migration speeds for fibroblasts, neutrophils, and keratocytes, respectively, would be in descending order with approximately tenfold interval decreases [29]. This dependence of cell locomotion speed on overall cell–substratum adhesive strength and the degree of spatial asymmetry suggests one means by which the various molecules regulating adhesion complexes can effectively control migration. The mechanical strength of protein–protein bonds is logarithmically related to their biochemical affinities, so alteration of the affinities of linkages within adhesion complexes by covalent modifications can “tune” overall adhesiveness as well as a spatial adhesiveness differential [31]. Regarding regenerative cells, the variations in migration, the association with ligand, and the course of spatial appropriation all appear to be time dependent as well as signal reliant.

More current discovery has identified a cell migration-dependent mechanism for releasing cellular contents, wherein a cell will leave retraction fibers behind it and vesicles at the intersections of retraction fibers. Coined “migrasomes” by the investigators, these migration nodes contain

numerous smaller vesicles, with diameters of about 50–100 nm [32]. During migrasome biogenesis, an initial phase of rapid growth and extension is followed by a relatively stable period. Most compelling to the observation is the subsequent integration and involvement following the retraction. Migrasomes are released into the medium or directly taken up by surrounding cells. The migrasome formation is an integrated conglomeration of specific integrin-coupled microvesicle exosomes that depend on integrin pairing with tetraspanin identities. The idea of trailing edge vesicle release as a primer or as a footprint for other cells to connect is not a new one [33]. It is unique that vesicle release of cellular contents renders location-specific footprinting that other cells can home to. This authenticates the possibility for spatial and biochemical information from outgoing cells, or from host cells to direct regenerative effort, or for cells delivered with extensive potency to map in time and space a scaffold that is at once both connected and polarized by attachment that spatial and biochemical information from outgoing cells can be acquired by incoming cells. Given that many important physiological functions, such as the formation of neuronal networks and innate and adaptive immune responses require localized communication between cells to achieve not only polarity but morphogenic integrity, this importance of this process becomes obvious.

Summarizing a few points in this discussion, cell polarity is a driver of activity and dissociation of charge can activate a differentiation of cell phenotype. In the context of multicellular organisms, and in particular to the essential goal of regenerative medicine, it is critical to keep in mind that cells communicate with each other utilizing chemical messengers and that the pharmacology of release is dependent on membrane polarity, which in turn affects transcription, which in turn generates exosome release that constitutes a connection if not a scaffold. For many of these messenger molecules, the membrane is an insurmountable barrier. New techniques have been developed to examine binding to surface proteins that can measure ligand binding with high temporal resolution and on a single cellular level [34]. With insights into the change of membrane, the ability to reciprocate voltage changes or imbue a capacitive coupled inference to the membranes might be possible. This insight has been similarly guiding strategies to not only define but to detect system change in many tissues by many attempts [35–37]. Possibly, it is accommodated by inherent growth factors accompanying cell and concentrated plasma products, but this connection and intention to regenerate is nourished by factors not yet completely understood. Strategies for repair are to recognize, respond, resolve differences, regenerate material, and/or supplement a scaffold that enhances generation and to provide recognition that allows integration.

Although it is likely that the assurance of composition trumps the eventual continuity of geometry, observations of

simple wound healing on skin are obvious to any who have seen restitution of tissue following a cut or scratch where pigmentation and surface erase any obvious trace of the wound. It is clear that it takes more time to guide the remodeling than it does the replacement. Biodynamics and basic physiology including the pharmacology of receptors, half-life of molecular forms, and the interaction and migrations of cells have guided the course of regeneration from the foundations of embryology. Other chapters within this book broadly discuss current regenerative therapies such as platelet-rich plasma that are not unexpectedly replete with platelet-derived growth factor (PDGF). For some time, it has been known that PDGF has a positive effect on the stimulation of mesenchymal stem cells [38]. Within a short time following that report, further studies of PDGF elucidated its receptor and biochemistry and defined the temporal context of its binding half-life [39]. Not surprisingly, the receptor half-life is less than that of the growth factor, as one might imagine the imposition of locked stimulation that was in any way errant in the magnitude of response. Using just this single growth factor as an example and considering the discrete families of growth factors and the large number of variants, the ability to responsibly expect cause and effect never strays from predictive variability. Strategies emerging from the example of PDGF have sought to block action, selectively repress signal, or in other ways to sequence activity in the time-space coordination of tissue development [40, 41]. Determining the fate or defining the relative contributions of therapy presents different challenges.

A revolutionary insight at the time was the tracking of cells using quail-chick chimeras [42]. Nicole Le Douarin, in many ways, pioneered the science defining cell fate, origin in the context of migration, and demonstrated with unusual clarity cell fate, cell migration, and anatomical emergence of distinct tissues. Her prescient observation was that the nucleolus was particularly large and conspicuous in quail mesenchymal cells. Although her work was initially given to evaluating hepatocyte cells and liver development, one characteristic she observed in the differentiation of hepatocytes was the enlargement of the nucleolus. What became intriguing to her and informative to the many who have looked at cell fate during development was that the large nucleolus was evident not only in the hepatocytes but also in the mesenchymal cells of the chimeric liver lobes that developed in culture. These observations were aided by histochemical techniques such as the Feulgen-Rossenbeck's procedure that stains DNA and a method for staining the RNA components of the nucleolus. Thus, as far as the structure of its nucleolus was concerned, the quail species appeared as an exception. This particularity made quail cells easily recognizable from chick cells at the single-cell level and at any developmental stage. Using this method of identification, determinants from early differentiation to mature tissues could be tracked—an

example of the chimeric tissue where the quail nucleoli of the neural crest region are stained and the subsequent migration led to the reconstitution of contributions of cell source to mature tissue (Fig. 1.4). The quail-chick marker system enhanced significantly the value of the avian embryo as a model for embryological research in developmental biology, combining the advantages of the availability of the embryo with observation and manipulations during the entire period of development with molecular methods [43–45].

Findings from the experiments provided some of the first evidence of pluripotency and of multiple potential contributions to various tissues including skeletal, nerve, and endocrine tissue. A demonstration of the considerable contribution of the neural crest to the vertebrate head—to the facial and visceral arch skeletal and connective structures, the skull, and the cardiovascular system were furthered beyond the likely considerations that the peripheral nerve system was sourced as well. These notions were new, and the notion of plasticity of the neural crest cells fated to build up the ganglia and nerves of the peripheral nervous system (PNS) largely depended upon environmental cues arising from the tissues in which they differentiate at the end of their migration [46]. A striking feature of the neural crest observation work was the fact that it gave rise to a large number of different cell types and that the feedback during development was regionally and spatially cued. Although the neural crest is regionalized into several distinct areas yielding different PNS structures in normal development, spatial disturbances of this preexisting order did not result in major abnormalities in PNS ontogeny, meaning that one neural crest area can be substituted for another to provide the embryo with sensory, sympathetic, parasympathetic, and enteric ganglia. In the context of regenerative medicine, coordinated repair, cell plasticity, and a sense of morphogenetic patterning seem to process an inexact but equilibrated dimension where tissue emerges not only where the anatomy would dictate but in a form that is appropriate for functional resonance with the surrounding tissue. This underscores the uncertainty of predictability in the morphogenesis of form but may not fully resonate with regenerative capabilities.

What is the remedy for random? Does order dictate and define the direction and dimension of the repairs? Accepting the context of biological “clay,” does the science of regenerative medicine have sufficient scope of understanding to predict not only the physical but the deeper and less predictable odds of the analog between the digits—what is the primal biologic utterance? Does the overarching structure impose a simplicity that is amplified in alignment rather than forcing an alignment based on an offset of constraints that are likely triggered by a symphony of cells, growth factors, cytokines, charges, cell surface kinetics, and even the polarity of the individual cells within the emerging tissues? Based on common pharmacology understanding, the location of ligands, or

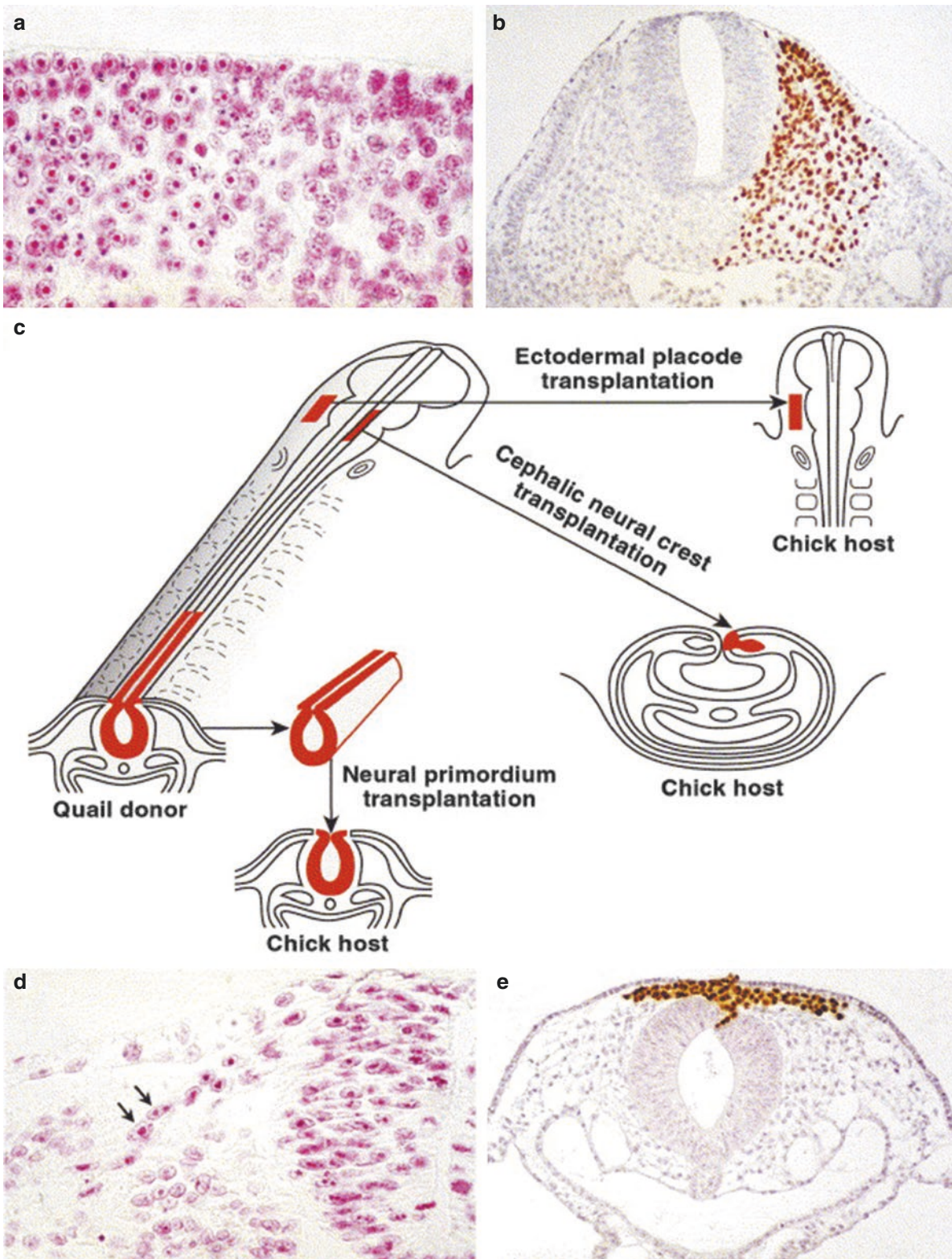


Fig. 1.4 The quail–chick marker system (a, b): two means for recognizing quail from chick cells. (a) Feulgen staining of DNA shows a large mass of heterochromatin in the center of the nucleus, which is associated with the nucleolus in quail cells (left). In chick cells, the heterochromatin is evenly distributed (right). (b) Staining of quail cells (half a somite on the right) grafted into a chick embryo with a monoclonal antibody raised against a quail nuclear antigen (produced by Carlson and Carlson, University of Michigan). (c) Different types of grafts from

chick to quail (or vice versa) embryos at the same developmental stages. The graft may involve the placodal ectoderm, the neural fold at the head level, or the neural tube including the neural folds prior to the onset of NCC emigration. (d, e) NCC migrating from a neural tube quail graft at the trunk level (d) or from a neural fold (right) graft at the cephalic level. Note that a unilateral NC fold graft expands on both sides during migration. (Le Douarin [48]. Used with Permission from Elsevier)

the ability for ligands to promote attachment are not surface agnostic and are highly ordered and directed to polarity. In terms of polarity, regenerative medicine still has to define the context of where the lead in active regeneration is precipitated and reactive, what is prescribed and responsive to inner cues, or what is defined in terms of static spaces that offer analog life as part of the procession.

Precipitation Viewed as a Nonsolute Quality

The alteration of proteins and changing the biologic charges and dynamics are some of the most important changes that can alter the course of the living organism. The interactions between linear polysaccharides and proteins can be regulated by additional modifications such as sulfation or branch formation of polysaccharide chains. Although the atomic details have been reported for certain heparin-binding proteins including antithrombin, FGF2, and annexin, 3D structural information on the recognition mode of sulfated polysaccharides remains limited. The effect that stabilizing the environment of a living cell has on the activity of the tissue in terms of differentiation and growth that results from regenerative integration has to do with what drives the fit and guides the morphology. Whether a cell forms a tumor or repairs itself appears to be generative at every step, but somehow the restitution to sameness is lost, and insufficiency becomes the faux of excess.

Mechanical vs. Medical

One of the inherent challenges of regenerative medicine is considering a closed system where all the factors are identified and defined in the hopes of achieving biologic closure. One might argue that accepting that science as defining the dots of understanding does not define the analog space between the concepts and that the mystery forces guiding living organisms to maintain cell membrane voltage, define material space, and evolve against all entropic predictions is a sufficient impetus to guide regenerative efforts even if they are yet insufficiently understood or explained.

The guiding light of medical treatment has always enlisted the applied principles of tissue engineering for years, transplanting and shifting matrices within patients to promote regenerative potential. The advent of new technology offers even greater promise and brings unbridled enthusiasm that full regenerative potential of tissue and whole organ systems can be achieved in the near future.

The implicit goals of regenerative medicine are to achieve restitution of space, mechanical solidarity, and functional continuity. Often, the biological signals do not provide sufficient stimulus to attain a full repair. The therapeutic goal is

to omit compliance features such as strain tolerance, reduced stiffness, and attenuated strength and instead promote primary tissue formation within the physical approximation of a wound or diseased tissue. Future bio-engineering strategies will combine several favorable properties of identified biologic processes in an effort to support tissue differentiation without shielding capacity for integrated modeling. Ideally, tissue compatibility that minimizes patient morbidity is optimal for healing. In scope, regenerative implantation will offer structurally enhancing solutions that are inductively optimum for predictable tissue formation. What is clear from the literature and multiple congresses that are held on an annual basis is that the prescriptions for the ultimate answer to regenerative medicine are as numerous as the opinions presenting them. Unlike the six blind men of Indostan who were unable to recognize an elephant except for the terms under which they had been exposed, the words of the Russian playwright and physician, Anton Checkhov, should be considered. Noting in the *Cherry Orchard*, “If many remedies are prescribed for an illness, you may be sure that the illness has no cure” [47]. Based on the broad use of regenerative adjuncts of cells, matrices of both autologous and allogeneic, platelet concentrates, and placental tissue products, the remedies themselves have become the numerator over a lesser number of denominating potentials. Being able to not only accept the promise but the performance of these therapeutic adjuncts is critical. Forced to seek commercial opportunities that provide meaningful clinical applications, much of the patient risk is deferred to testimonials or personal recommendations. When meaningful data have been synthetically collected rather than abstracted without regard to the number of participants, regimen of therapy, functional improvement, then regenerative medicine will be able to shed the cloak of the alternative and tailor itself to an impressive fit in various clinical applications. In the acceptable practice of physician oversight and practice with a data-driven understanding of performance, expectations should meet the intentions of regeneration as a science and tool for clinical care.

References

1. <https://www.fda.gov/downloads/biologicsbloodvaccines/guidance-complianceregulatoryinformation/guidances/cellularandgenetherapy/ucm585403.pdf>
2. Large EW, Snyder JS. Pulse and meter as neural resonance. *Ann N Y Acad Sci.* 2009;1169:46–57.
3. Boeke K. *The universe in forty jumps.* New York: John Day Company, Inc.; 1957.
4. Morrison P, Morrison P, Office of Charles and Ray Eames. *Powers of ten: about the relative size of things in the universe.* Stuttgart: Scientific American Books, Holtzbrinck; 1982.
5. Organ Procurement and Transplantation Network; Transplants by Organ Type January 1, 1988—December 31, 2018, Based on OPTN data as of February 7, 2019, Rockville.

6. Koniaris LG, McKillop IH, Schwartz SI, Zimmers TA. Liver regeneration. *J Am Coll Surg*. 2003;197:634–59. [PMID: 14522336].
7. Michalopoulos GK, DeFrances MC. Liver regeneration. *Science*. 1997;276:60–6. [PMID: 9082986].
8. Spyridonidis A, Zeiser R, Follo M, Metaxas Y, Finke J. Stem cell plasticity: the debate begins to clarify. *Stem Cell Rev*. 2005;1:37–43. [PMID: 17132873].
9. van Gene D. Prometheus, Pandora, and the myths of cloning. *Hum Life Rev*. 2006;32:15–27.
10. Schneider MD. Regenerative medicine: Prometheus unbound. *Nature*. 2004;432:451–3. [PMID: 15565135].
11. Rosenthal N. Prometheus's vulture and the stem-cell promise. *N Engl J Med*. 2003;349:267–74. [PMID: 12867611].
12. Power C, Rasko J. Whither Prometheus' liver? Greek myth and the science of regeneration. *Ann Intern Med*. 2008;149:421–6.
13. Linnaeus, Carl (1755) [1751]. *Philosophia botanica: in qua explicantur fundamenta botanica cum definitionibus partium, exemplis terminorum, observationibus rariorum, adiectis figuris aeneis*. originally published simultaneously by R. Kiesewetter (Stockholm) and Z. Chatelain (Amsterdam). Vienna: Joannis Thomae Trattner (1755) [1751].
14. Schrodinger E. *What is life?* Cambridge: Cambridge University Press; 1944.
15. Watson JD, Crick FHC. Genetical implications of the structure of deoxyribonucleic acid. *Nature*. 1953;171:964–7.
16. Fleury V. Branching morphogenesis in a reaction-diffusion model. *Phys Rev E*. 2000;61:4156–60, PMID 11088210.
17. Servaas MN, Geerligs L, Bastiaansen JA, et al. Associations between genetic risk, functional brain network organization and neuroticism. *Brain Imaging Behav*. 2016;11(6):1581–91.
18. Marmar CR, Weiss DS, Schlenger WE, Fairbank JA, Jordan BK, Kulka RA, Hough RL. Peritraumatic dissociation and post-traumatic stress in male Vietnam theater veterans. *Am J Psychiatr*. 1994;151:902–7.
19. Putnam FW. The psychophysiologic investigation of multiple personality disorder. *Psychiatr Clin N Am*. 1984;7:31–9.
20. Mason C, Dunnill P. A brief definition of regenerative medicine. *Regen Med*. 2008;3:1–5.
21. Maxson S, Lopez EA, Yoo D, Danilkovitch-Miagkova A, Leroux MA. Concise review: role of mesenchymal stem cells in wound repair. *Stem Cells Transl Med*. 2012;1:142–9.
22. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One*. 2008;3:e1886.
23. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, Park JS. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci*. 2007;48:15–24.
24. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood*. 2005;105:1815–22.
25. Hocking AM, Gibran NS. Mesenchymal stem cells: paracrine signaling and differentiation during cutaneous wound repair. *Exp Cell Res*. 2010;316:2213–9.
26. Kamimura K, Inoue R, Nagoya T, et al. Autonomic nervous system network and liver regeneration. *World J Gastroenterol*. 2018;24(15):1616–21.
27. Kiba T, Tanaka K, Numata K, Hoshino M, Inoue S. Facilitation of liver regeneration after partial hepatectomy by ventromedial hypothalamic lesions in rats. *Pflugers Arch*. 1994;428:26–9.
28. Hendricks MT, Thuluvath PJ, Triger DR. Natural history of autonomic neuropathy in chronic liver disease. *Lancet*. 1992;339:1462–4.
29. Luster AD, Alon R, von Andrian U. Immune cell migration in inflammation: present and future therapeutic targets. *Nat Immunol*. 2005;6:1182–90.
30. Oliver T, Lee J, Jacobson K. Forces exerted by locomoting cells. *Semin Cell Biol*. 1994;5:139–47.
31. Kuo S, Lauffenburger DA. Relationship between receptor/ligand binding affinity and adhesion strength. *Biophys J*. 1993;65:2191–200.
32. Ma L, Li Y, Peng J, Wu D, Zhao W, et al. Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. *Cell Res*. 2015;25:24–38.
33. Kriebel PW, Barr VA, Rericha EC, Zhang GF, Parent CA. Collective cell migration requires vesicular trafficking for chemoattractant delivery at the trailing edge. *J Cell Biol*. 2008;183:949–61.
34. Burtcher V, Hotka M, Sandtner W. Detection of ligand-binding to membrane proteins by capacitance measurements. *Bio Protoc*. 2019;9(1):e3138.
35. Basset C, Pawluk R, Pilla A. Augmentation of bone repair by inductively coupled electromagnetic fields. *Science*. 1974;184:575–7.
36. Brighton C, Wang W, Seldes R, et al. Signal transduction in electrically stimulated bone cells. *J Bone Joint Surg*. 2001;83-A:1514–23.
37. Fitzsimmons RJ, Gordon S, Kronberg J, Ganey TM, Pilla AA. A pulsing electric field (PEF) increases human chondrocyte proliferation through a transduction pathway involving nitric oxide signaling. *J Orthop Res*. 2008;6:854–9.
38. Ek B, Westermark B, Wasteson A, Heldin CH. Stimulation of tyrosine-specific phosphorylation by platelet-derived growth factor. *Nature*. 1982;295(5848):419–20.
39. Keating MT, Williams LT. Processing of the platelet-derived growth factor receptor. biosynthetic and degradation studies using anti-receptor antibodies. *J Biol Chem*. 1987;262:7932–7.
40. Shulman T, Sauer FG, Jackman RM, Chang CN, Landolfi NF. An antibody reactive with domain 4 of the platelet-derived growth factor beta receptor allows BB binding while inhibiting proliferation by impairing receptor dimerization. *J Biol Chem*. July 1997;272(28):17400–4.
41. Elangovan S, D'Mello SR, Hong L, Ross RD, Allamargot C, Dawson DV, Stanford CM, Johnson GK, Sumner DR, Salem AK. The enhancement of bone regeneration by gene activated matrix encoding for platelet derived growth factor. *Biomaterials*. 2014;35(2):737–47.
42. Le Douarin N. Particularités du noyau interphasique chez la Caille japonaise (*Coturnix japonica*). Utilisation de ces particularités comme "marquage biologique" dans des recherches sur les interactions tissulaires et les migrations cellulaires au cours de l'ontogenèse. *Bull Biol Fr Belg*. 1969;103:435–52.
43. Funahashi J, Okafuji T, Ohuchi H, Noji S, Tanaka H, Nakamura H. Role of Pax-5 in the regulation of a mid-hindbrain organizer's activity. *Develop Growth Differ*. 1999;41:59–72.
44. Nakamura H, Watanabe Y, Funahashi J. Misexpression of genes in brain vesicles by *in ovo* electroporation. *Develop Growth Differ*. 2000;42:199–201.
45. Creuzet S, Martinez S, Le Douarin NM. The cephalic neural crest exerts a critical effect on forebrain and midbrain development. *Proc Natl Acad Sci U S A*. 2006;103:14033–8.
46. Le Douarin NM, Teillet M-A. Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neurectodermal mesenchymal derivatives, using a biological cell marking technique. *Dev Biol*. 1974;41:162–84.
47. Chekhov A. Collection of the association "Knowledge" for 1903. Book Two. SPb, 1904, pp. 29–105.
48. Le Douarin NM. The avian embryo as a model to study the development of the neural crest: a long and still ongoing story. *Mech Dev*. 2004;121(9):1089–102.