Delivering regeneration

V. Prasad Shastri

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The current status of regenerative medicine or therapies can be traced back to the seminal contributions by many groups in the late 1970s through mid-1990s, most notably Bell [1], Yannas [2], and Langer [3], when the early concepts of tissue engineering began to take shape. Some of the key contributions of this first evolutionary period were cell-directed organization of extracellular matrix (ECM) using cell traction, processing of mechanically defined supports (scaffolds) from biopolymers and synthetic polymers for the association and development of anchorage-dependent cells [4, 5], and the concept of using materials with well-defined composition and ultrastructure to confine and orchestrate the organization of cells *de novo*. An example of the latter, the *de novo* formation of neo-dermal tissue using a polymer sheet scaffold as a template, remains to date one of the most compelling and intriguing attempts to recapitulate, and mimic, the elements associated within a regenerative environment [6]. The idea of using dissociated cells to generate an organized, tissue-like structure, however, dates much earlier and can be attributed to Malcolm Steinberg's papers in the early 1960s, which perhaps influenced some of the early thinking that went into the development of tissue engineering [7, 8]. Three full decades later, tissue engineering has evolved into a robust discipline in its own right, still holds a lot of promise, and is a fine example of the need for

a highly interdisciplinary research enterprise in tackling human health problems.

The development of protocols for the isolation of human embryonic stem cells (hES) [9] and tissue-specific cells derived from hES in the late 1990's [10] provided the much needed impetus for tissue engineering to undergo an evolutionary change that is still in progress. The focus of tissue engineering shifted from simply engineering a mass of tissue to *functional* engineering of tissue that can be transplanted into a living system [11]. This translational transformation has promoted a much more comprehensive thinking not only in both design of systems for cell encapsulation and delivery but also in the strategies for tissue repair and restoration. Tissue engineering therefore, is no longer perceived as an exercise in growth of a tissue mass through the association of cells and scaffolds in a controlled *ex vivo* environment, but as a self-contained system that can recognize and respond to the cellular microenvironment; and in doing so, tip the process away from tissue repair toward regeneration (neo-morphogenesis). Regenerative medicine, which essentially encompasses a broad range of strategies to induce repair and tissue morphogenesis, has benefited immensely from this revised approach to tissue engineering. This new evolving paradigm in regenerative medicine paradigm and its key elements are depicted in Fig. 1.

The primary objective of this themed issue is to present this new emerging face of regenerative medicine and highlight some of the key elements alluded to in Fig. 1 through engaging articles by preeminent scientists in their respective disciplines, and to offer the readership a unique perspective into the amalgamation of interdisciplinary concepts in the evolving regenerative medicine landscape. Furthermore, the critical role of delivery technologies in controlling and altering cellular environments is brought to focus.

The realization of an autonomous, self-regulating system for repairing and restoring tissue function is a work in progress that is being aided by significant developments in

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Dedicated to the late Prof. Malcolm Steinberg, The Henry Fairfield Osborn Professor of Biology Emeritus and Professor of Molecular Biology Emeritus, Princeton University, USA

V. P. Shastri (✉)

Institute for Macromolecular Chemistry and BIOS—Center for Biological Signalling Studies, University of Freiburg, Freiburg 79104, Germany

e-mail: prasad.shastri@gmail.com

e-mail: Prasad.Shastri@makro.uni-freiburg.de

Fig. 1 A schematic of the emerging regenerative medicine paradigm with some of its key functional elements

materials engineering, nanotechnology, drug delivery systems, cell signaling, systems biology, and synthetic biology (biological engineering) [12]. The translation from “bench to bedside” requires significant input from the clinicians with respect to adaptation of prevailing standards in surgical intervention to the development of new biomaterials. A good example of such a collaborative approach is the *in vivo* bioreactor paradigm [13, 14]. In the *in vivo* bioreactor paradigm, the *de novo* formation of a fully functional bone or cartilage is invoked and controlled purely through the minimally invasive placement of a biomaterial with precise physico-chemical characteristics. The approach of inducing neomorphogenesis through endogenous recruitment of cells and signals presents the way forward in regenerative medicine. However, much needs to be done with respect to understanding the precise signals that are activated during the acclimatization of the biomaterial to the body. This presents an opportunity to explore the role of inflammation and related processes in the regulation of cell fate and function.

The lynchpin of the new regenerative medicine paradigm is the recruitment, preservation, and directed development of stem and progenitor cells. A significant development in this field occurred in 2006 when Takahashi and Yamanaka showed that through the induction of four transcription factors (Oct3/4, Sox-2, c-Myc, Klf4), adult cells can be reprogrammed to an embryonic state [15, 16]. With these induced pluripotent stem cells (iPS) at hand, the stage is set for regenerative therapies to make a grand statement. One can envisage combining processing techniques like 3D printing with drug delivery to create intricate, anatomical accurate structures for *in situ* programming of cell fate.

Improved tools for visualizing, quantifying, and modeling the biological environment have fundamentally altered our understanding of how signals inside and outside the cells are triggered, how these signal networks link and temporally evolve to control stem cell fate, function, and organization [17]. The development of methods to visualize cellular processes in real time with high anatomical accuracy [18] can provide critical data to make the mathematical models more predictive. In this context, functional proteomics present a new frontier in the understanding of the signaling microenvironment of a cell. Characterizing of molecules in the cellular environment can be significantly accelerated by the development of simpler tools such as the reagent described by Hwang et al. in this issue, for the recovery of cytosolic proteins. This knowledge gain, through faster analysis, can be further leveraged through the sophisticated tools of synthetic biology and optogenetics [19, 20] which are likely to become commonplace in the design of “smart” biomaterials. A step in this direction is the localized presentation of molecules. The contribution from McCall et al. presents the concept of tethered growth factors and their potential in regulating the environment of human mesenchymal stem cells (MSC) and directing their lineage. A fully synthetic extracellular matrix (ECM) that can replicate every aspect of the cellular microenvironment is a key step in the implementation of a regenerative medicine paradigm wherein the fate of a pluripotent cell is *a priori* determined through local cues. Macromolecules are abundant in nature and are the main constituent of ECM. Many ECM molecules exhibit unique structure property relationships, and their assembly in nature into supramolecular structures is more often than not driven by self-assembly.

In this context, Wnek and Fullana and Sivaraman et al. present in this issue the recent advances in the processing of collagen and elastin into scaffolds for dermal and vascular tissue engineering, and the use of these scaffolds for the spatio-temporal delivery of biomolecules. Since the ECM is essentially a complex matrix composed of polymers, it is not surprising that it possesses both texture and defined mechanical properties. In recent years, substrate stiffness (matrix mechanics) [21] and topography [22, 23] have emerged as a very important exogenous signal which in some situations can dwarf the effects of soluble signals, in the lineage-specific differentiation of pluripotent cells. Mechanobiology [24], the coupling of matrix mechanics with cellular signaling, offers opportunities to identify new paradigms for controlling signal networks and is expected to play an important role in the design of “smart” biomaterials. Interestingly, the organization of collagen into sheets by fibroblasts, and the flow-induced alignment of endothelial cells are some of the earliest examples of mechanobiology at play. Understanding the relationship between mechanics and signalling in such model systems can provide valuable insights for design of cell-responsive biomaterials. In this context, Vonwil et al. present in this issue findings that suggest a role for substrate elasticity in modulating the effect of soluble signals on human cell differentiation. While it is clear that signaling is important in defining cell function, our understanding of how signaling changes with cell-related variables is still being elucidated. The study by Li et al. has identified cell density as an important parameter in limiting replicative senescence in human-synovial-derived stem cells and proposes a role for the mitogen-activated protein kinase signaling pathway in maintaining stemness. So, why is stemness important? Stemness confers plasticity to a cell, a characteristic highly desired in cells participating in tissue repair, as this allows the lineage of the cell to be manipulated by exogenous cues. A predominantly materials engineering approach for driving the osteogenic differentiation of MSCs is presented by Hong et al., in this issue, where a layer-by-layer assembly of polyelectrolytes is employed to incorporate micelles containing dexamethasone, a glucocorticoid that has powerful effects on cell function. Surface engineering is also an important element in the development of materials with good blood compatibility. Irvine et al. present herein a robust review of the state of the art in hemocompatibilization. In addition to delivery of soluble signals, the restoration of tissue function may on occasion require the silencing of an aberrant gene in order to restore signaling homeostasis. Synthetic vectors that can efficiently deliver genetic material into specific cellular compartments are therefore desirable. In this issue, Itaka et al. and Monaghan and coworkers describe strategies for gene silencing and sustained transgene expression using nanomicelles and tailored synthetic polymers.

In conclusion, it is evident that delivering regenerative medicine requires a complex thought process that involves a highly interdisciplinary interface. The convergence of concepts and technologies from materials science, biology, and engineering should enable a more rational design of systems to invoke favorable cellular processes, and this is bound to change the implementation of regenerative therapies and move regenerative medicine one step closer to being a more predictive science.

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