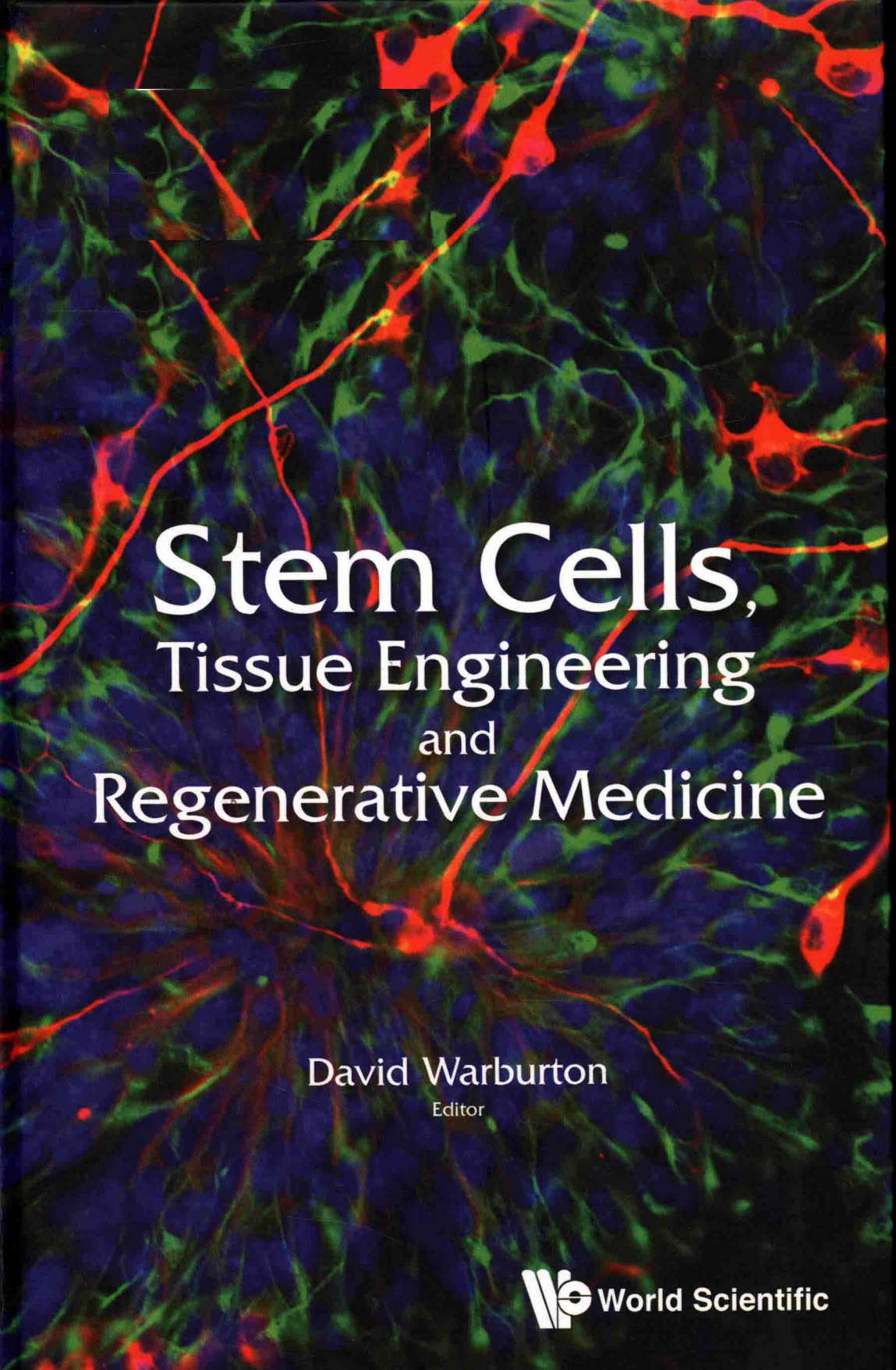
Stem Cells,
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and
Regenerative Medicine
David Warburton
Editor
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Editor

David Warburton

OBE, DSc, MD, MMM, FRCP, FRCS, FRCPCH

The Saban Research Institute

Children's Hospital Los Angeles

Keck School of Medicine and Ostrow School of Dentistry

University of Southern California, USA

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Stem Cells, Tissue Engineering and Regenerative Medicine

Introduction

Developmental Biology, Regenerative Medicine and Stem Cells: The Hope Machine is Justified

David Warburton

Developmental Biology and Regenerative Medicine Program, Saban Research Institute, Children's Hospital Los Angeles, Keck School of Medicine and Ostrow School of Dentistry, University of Southern California

All human beings begin as one cell, when the sperm enters an ovum to become a fertilized egg. As the unique information encoded within the 2N DNA in a person's genome unfolds, this egg begins to divide in a highly organized and orientated fashion until it comprises many billions of stem, progenitor and lineage specific cells, within a self-assembling, self-repairing corporeal machine called a human being. The scientific discipline that studies this process is called developmental biology. As a baby doctor, who participated actively in the emergence of the specialty of neonatal-perinatal medicine, it became obvious to me that if we can understand and harness these normal processes of developmental biology, then correcting congenital abnormalities, repairing tissue injury and even generating or regenerating whole organs should be theoretically possible.

Several Nobel prizes have already been awarded in what is by now the wide-open field of stem cell biology. By now, the first *in vitro* fertilized human baby girl has children of her own. Dolly the sheep, the first cloned

large mammal is stuffed and on display, in a glass case, in the science museum in London.

Embryonic stem cells, first discovered in mice and since isolated from humans, and the cause of much initial ethical angst, have become almost routine in the face of more recent advances. Now it is possible to drive differentiated cells backwards towards a more embryonic like state of induced pluripotency by means of as few as four factors and the Nobel has already been awarded in record time for this discovery. Additionally, many classes of stem-like cells originating from the various mesenchymal compartments of the body (marrow, adipose, amnion, amniotic fluid, etc.) have been shown to exert promising healing properties in certain inflammatory and fibrotic diseases. Moreover neural stem cells can be programmed to act as "Trojan horses" to attack otherwise inaccessible brain tumors. Moreover, identification of treatment resistant endophenotypes within certain aggressive cancers may suggest new alternative approaches to complete extirpation.

Tissue engineering is the discipline that takes advantage of the developmental programming of stem, and progenitor cells to self-assemble within natural or artificial matrixes facsimiles of natural organ structure and function.

Applying these discoveries to the betterment of human diseases has brought forth much hope but continues to present many challenges. The hope for cures prompted the people of California to strongly mandate an amendment to the State Constitution establishing the California Institute for Regenerative Medicine. This has proven to be a wise investment in scientific infrastructure, personnel and processes that is already a major wealth engine for the state, which now enjoys a worldwide leadership position in the regenerative medicine field. The pipeline in California is in fact bulging with potential regenerative medical applications, but it is proving to be challenging in moving many of them past all of the necessarily stringent FDA regulatory milestones into routine use in clinics before 2016, which is the date by which CIRM funding will need to be revitalized.

This book contains a global collection of monograph essays from collaborating investigators in Australia, Brazil, California, Connecticut,

Illinois, Iran, Minnesota, Pennsylvania, Taiwan and the United Kingdom. They describe exciting progress in basic stem cell biology, tissue engineering technology as well as diverse regenerative solutions for airways, cancers, craniofacial structures, intestine, heart, kidney, liver, lung, and nervous system.

Foreword

Towards Broader Approaches to Stem Cell Signaling and Therapeutics

Edwin Jesudason

Reader in Paediatric Surgery, University of Liverpool, UK,
E-mail: ejesudason@caltech.edu

Introduction

The recent award of the Nobel Prize for Medicine and Physiology illustrates salient points about the science behind how cells can be returned to a stem cell state. First, the contribution by John Gurdon, shows that this is an issue that has been of long interest to biologists and where significant understanding of the controls could be achieved without delving into the depths of molecular biology. Conversely, the award to Yamanaka tells of the capacity to reprogram cells using a few fairly simple manipulations of cell signaling. This chapter will argue that the attractions of reducing this reprogaming task to a few key signaling pathways ought to be resisted to prevent the field overlooking many under-explored layers of control for pluripotency. In addition, in the lungs and airways, where this author's work have focused on, the attempts to identify and control tissue specific stem cells have been dogged with some controversy. Underpinning this view is the sense that for therapeutic applications in people, we need a far more holistic understanding of stem cell regulation than is currently available via reductionist approaches alone.

Ontological Enquiry in Stem Cell and Progenitor Biology

The field of stem cells can be seen as a particular case of a much broader ontological problem, namely how does one thing change to become another, whilst retaining a continued identity. Aristotle wrote of this metaphysical problem, which philosophers distinguish as the 'problem of identity.' For example, one challenge is to explain how any individual is the same person as their previous, even childhood self, when their physique, experience and memory all differ. By analogy, developmental biology in general and the stem cell field in particular is concerned with how certain cells become several other cell types in order to construct the whole organism. However, Aristole's early insight into this problem is a reminder both of how old this is, and also how widely we may need to search for the best answers.

How Molecular Biology Does Not Suffice to Describe Stem Cell Regulation

The central dogma of molecular biology has governed much of the discourse in this area, and hence dominates the highly cited discoveries in this field.[1] Powerful though these observations may be, it is sobering to reflect that the limited reproducibility of so many scientific findings severely limits the understanding that they convey, and any therapeutic goal to which they may be directed.[2] Moreover, the limited tools with which to interrogate the full richness of biology also leads us to overestimate our understanding by forgetting what we never assay. In this regard, the wealth of information contained in the glycome is still only just being accessed.[3] Similarly, the older cell signaling systems based around transport and sequestration of particular ions is still revealing unexpected roles. Furthermore, the mechanical controls of development and cell fate specification remain stubbornly resistant to illumination.[4]

Alternate Regulation of Stem and Progenitor Cells: The Lungs as a Case Study

To illustrate these points, this chapter will look at (1) how new tools have revealed a role for the glycome in cell fate specification within a

developing organ, the lungs; (2) how ion-regulated mechanical phenomena sculpt the developmental programs of progenitor cells within the lung. These areas of enquiry have grown out of a specific and highly refractory clinical problem, hypoplastic lung development, which will therefore be described briefly below.[5–8]

Like many clinical concepts, lung hypoplasia is a messy one, with fuzzy definitions and frustratingly limited therapeutic options. As the name suggests, the set is defined by lungs that underperform at birth due to inadequately developed surface area for gas exchange. Such a state can be associated with a primary problem of lung development and/or mechanical factors, such as space-occupying lesions within the developing thorax or failure of routine fetal breathing excursions or loss of the distending pressure afforded by fetal lung secretion.[9,10] Clearly, given the range of potential antecedents it is no surprise to learn that the impacts on stem and progenitor cells can be varied, whether proposed as cause and/or effect of the observed lung hypoplasia.[11,12] As a consequence, connecting changes in stem/progenitor cell function to these pathologies has been a challenge, and thus far few therapeutic cell based therapies have reached the point where full translation is feasible.[13–16]

Two clear illustrations of this challenge to the stem cell field follow below. First, a study in the *New England Journal* suggested that all lineages necessary to repopulate the damaged lung could be and were derived from c-kit +ve progenitors.[17] This has been a controversial finding, not least as it seems to be so out of kilter with the developmental studies where a range of stem and progenitor cells seem to be necessary to furnish the final organ.[18–21] Indeed in the gut, where such analyses may be arguably at a more advanced stage, a picture is emerging of a few cell types that have the necessary propensities.[22] A second challenge is that the developing fetus in general and the developing lungs and gut in particular have spectacular access to native stem and progenitor cells, whether circulating in the blood, or living transiently within the amniotic fluid. Nevertheless, inborn errors in development of these organs are well-recognized in all human populations where these anomalies have been studied.[23] So, the question for the translational scientist becomes: how can isolation of putative stem cells for this organ prevent or treat problems like lung hypoplasia, when the intrauterine environment has failed? In paediatric specialties, this is particularly pertinent given evidence that stem cells

which are aberrantly located in either time or space may lead directly to lethal tumor formation.[24,25]

Moving closer to the therapeutic realm does not make these issues any easier. The recent studies looking at the use of decellularised organ scaffolds have been greeted simultaneously with enthusiasm and skepticism.[14,15] The studies show that recellularisation can permit, for a transient period, gas exchange in the lung context. The findings also suggest that much of the glycome, which nature elaborates at significant cost, is dispensable for at least this early rescue.[26] Finally, the recellularisation studies emphasize the need for mechanical stimuli during this process. Together these highlight the danger of reducing pluripotency to a few transcription factors. The recellularisation studies show that the behavior of progenitor cells can depend on both the presence of a suitable scaffold as well as of mechanical motion. Neither of these factors is readily understood in terms of cell-autonomous transcription factor dependent regulation.

Glycomic Regulation of Stem/Progenitor Cells in Development and Regeneration

The recellularisation studies were particularly surprising in their suggestion that, in the short term at least, the glycome was not so important as previously believed.[26] Several studies from fruitfly to mammals had been clear that the glycome is essential for proper regulation of airway morphogenesis, and the appropriate allocation of progenitors within that system.[27–29] So, loss of the biosynthetic enzymes required for synthesis of certain glycans leads to substantial disruption of the behavior of airway progenitors in *Drosophila*.[30] These mutants, *sulphateless* and *sugarless* are both associated with major malformation of the *Drosophila* tracheal network. The latter is a genetic homologue of the developing mammalian airway, where again studies have shown a requirement for glycan synthesis to support proper development. For example, interrogation of hypoplastic lungs for expression of glycan epitopes using phage display antibodies shows significant divergence between normal and hypoplastic lung in not only the airway compartment, but also the pulmonary vasculature.[31–33] Lethal pulmonary hypertension is associated with lung

hypoplasia.[34,35] These studies support the concept that the glycome is essential for normal airway development. Therefore the contrasting findings in the recellularisation studies suggest this tissue engineering process needs to be approached with some caution, with a view to first understand how the glycome really contributes to behavior of stem and progenitor cells. However, the glycome is elaborated by biosynthetic enzymes rather than the nucleic acid template of molecular biology and this makes it hard to test their role as one might with proteins, via gene knockout studies.[3] Instead, use of the phage display antibodies showed how glycans with various epitopes were distributed unevenly between normal lung and its hypoplastic counterpart.[31–33] This provides circumstantial evidence that the glycan profile may be critical for this lesion of development, and is supported in further studies.[36–38] Together this emphasizes the need for stem and progenitor biology to pay attention to non-template driven processes in order to obtain a fuller and hence more useful view of their regulation.[3]

Mechanobiology of Stem/Progenitor Cells: a View from a Mechanical Organ

If the above is true of glycomic biology, the mechanobiology of stem and progenitor cells is another arena ripe for exploration and likely to be essential for any meaningful understanding of stem cell use in major organ regeneration. Again, the limitation in terms of technology has hampered progress in this area. However, increasing interest in this subject has led to small breakthroughs that allow glimpses into how the mechanics govern all aspects of development. At their core these concepts are not new, in so far as they were well-annunciated in D'Arcy Thompson's *On Growth And Form*. Writing prior to the revolution in molecular biology, these enquiries found interest with academics and their students alike. It is arguable that the advent of the central dogma of molecular biology was detrimental to the investigation of a range of epigenetic influences on development.[39] At a clinical level, it can be similarly argued that the focus on genes and disease has distracted from many healthcare problems whose solutions are at the societal and policy level.

In this context, the stem and progenitor biology of the lung is highly illustrative. From a functional perspective it may well be argued that just as the heart, the lungs' primary purpose is mechanical-induced motion of inhaled and exhaled respiratory gases. Viewed from this perspective, it ought then to be no surprise that the lungs, its development and its maintenance are all critically dependent on mechanical influences.[4,40] Yet, this realm of regulation seems to be regularly overlooked in favor of the latest set of transcription factor pathways and/or the hottest new mediators of inflammation and immunity. One might go so far as to contend that some of the impasse in major lung disorders like asthma has arisen as a result of this gulf between biochemical and mechanical portraits of the lungs. In fact, from its earliest stages the developing lung is an intrinsically mechanical organ, both in terms of the distending pressure induced by epithelial secretion of lung liquid, and the regular peristaltic contractility of the airway smooth muscle.[10,41,42] The latter is a conserved phenomenon that is seen in avian and mammalian species, including humans. Its precise purpose remains the subject of inquiry. However, the activity is responsible for the rhythmic propulsion of lung liquid throughout the developing airway tree. It has therefore been argued that this is helpful in distributing hydraulic pressure throughout the developing structure, and indeed maintaining that pressure locally.[7,43] Airway peristalsis begins shortly after the onset of lung development (equivalent to a few weeks of human gestation) and continues toward term. So despite radical changes that occur in lung epithelia over this time, the contractility persists and indeed increases in frequency as birth approaches. An open question remains whether this activity persists at any major level after birth. One proposal is that this rhythmic pacemaker driven regulation of airway smooth muscle contractility is a foundation on which dysrhythmic contractions arise in the context of asthma. In this paradigm then, asthma is an airway dysrhythmia in which other more 'traditional' triggers, like allergens, then supervene.[43]

Given this evidence that the lung is in constant motion throughout life, it is sobering to consider how little is still known of the mechanobiology of stem and progenitor cell biology in the lungs. Recent studies have shown that airway motion is underpinned by propagating calcium waves, and there is evidence also that this is conserved. Gradually, a picture is

emerging in which ion channels regulate not only the immediate state of the airways, such as their calibre, but also the broader realms of epithelial development and homeostasis.[44] Most exciting perhaps has been the recognition of mechano-sensitive ion channels which are again conserved from fly to mammals and which seem to play a key role in connecting mechanical and developmental processes in the airway.[45,46] It is to be hoped that these recent advances portend an expansion of research in this area. However, to realize this potential information about stem and progenitor cell regulation in complex organ environments requires recognition of the role of 4D imaging techniques. Advance in this area has reminded us that the dynamic nature of biology is neglected at our peril. Again, it may be contentious to state, but it can be argued that some of the failure of biological research to translate can be traced to inadequate attention to the dynamic nature of the processes being studied. Only with this view can one hope to understand the often highly divergent results achieved via static assays. With regard to the airway, dynamic imaging has revealed a wealth of mechanical activity during development at multiple scales, from whole organ to the cellular level too.[4,47] Returning to the lessons from the experience of lung recellularisation, it seems that the mechanical excursions imposed on the lung being treated are critical to enabling the infused stem and progenitor cells to repopulate the denuded scaffold en.[14,15] Similarly, during normal development, the lung is variously shaken by mechanical activity, from airway peristalsis, lung liquid fluctuations and fetal breathing movements.[7,10] Yet, the importance of these stimuli for lung progenitor cells is really only just being glimpsed.

Real-Time Functions of Stem/Progenitor Cells: The Role of Multi-Scale Imaging

Looking forward, proper analysis of stem cell biology and its application to inherently mechanical organs like the lung requires not only biological tools to interrogate mechanics, or the imaging tools to examine temporal change, but also advances in imaging that allow scrutiny of the mesoscale. In other words, dynamic imaging of discrete cell biology is of limited value to understanding a major multicellular organ that is itself undergoing major mechanical shifts. Significant advances have been made in this

area, from windowing techniques that allow visualization of cell biological events even in the whole animal.[48] As one increases the scale of visualization the tendency has been to resort to cross-sectional imaging like CT to examine the lung and its function. This has the advantage of providing high spatial resolution and can be extended into a pseudo 4D technique. However, this comes at the expense of high X-ray doses and the reality that the lung is often 'switched-off' by breath holding at the point of image capture. Recent advances have provided a new solution to this imaging problem, using phase contrast X-ray, independently of synchrotron sources, to deliver real time imaging at diverse points within the airway throughout the breathing cycle.[49] Already this approach has revealed that lung function testing by spirometry is insensitive to highly informative and important loco-regional changes in ventilation and perfusion that are otherwise obscure. This observation has direct consequences for any

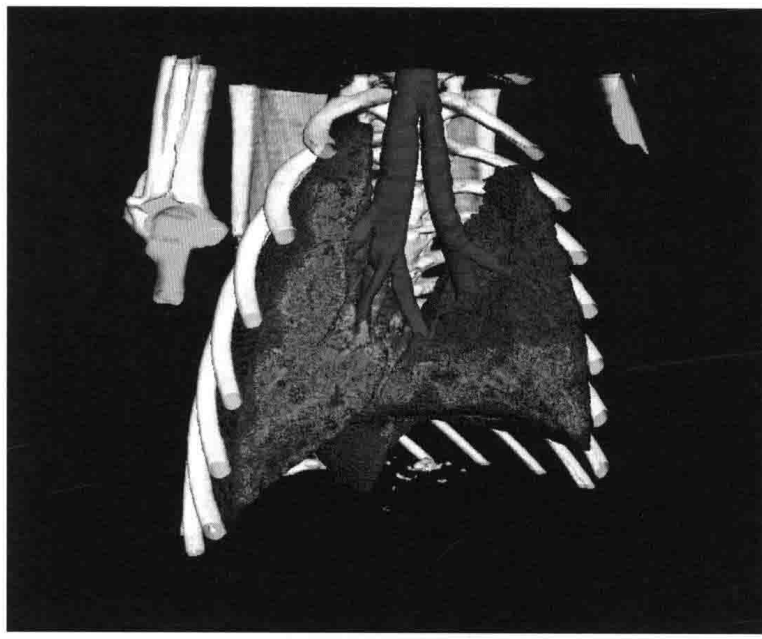

Figure 1. Phase contrast imaging of ventilated rodent lungs provides false-colored quantification of loco-regional flows across the lungs throughout the breath cycle. This contrasts with lung function testing at the mouth, which is blind to the heterogeneity inherent across the organ, and cross-sectional static imaging, which yields structure without function.

Image used courtesy of Dr. Andreas Fouras and lab.

group interested in lung regeneration strategies using stem cells. Such experimental approaches tend to depend on the induction of lung injury and the attempt to then ameliorate this via introduction of stem or progenitor cells.[13] A critical part of this enterprise is proper assay of the original injury, its extent and the degree of recovery that then ensues. Most of all, it is important to know about the functional rather than histological recovery. Using present techniques, there is substantial variation in the degree of initial injury, but this is impossible to assay in the individual animal without its sacrifice and removal from assays at subsequent time points. At the other end of the study, the use of traditional lung function testing is the norm, and yet it is revealed to be insensitive to loco-regional changes. This is troubling given the evidence that key lung diseases are in fact loco-regional at stages where early intervention might be most effective in averting later catastrophe. Asthma falls squarely into this category.[50] Four dimensional phase contrast imaging of the lung in such experiments allows the measurement of airflow at all points in the airway right down to the level of the terminal bronchi. This represents a breakthrough in so far as it provides researchers with access to an informative and important lung physiome (Fig. 1). If the primary purpose of the lung is efficient gas exchange, then these measurements are central to any concept of injury and recovery, and more important than histological surrogates.

Conclusions

The advance of stem cell biology in major mechanical organs like the lung requires a broader approach to signaling that captures the biochemistry of motion and an approach that is dynamic and multiscale in order to properly assess the frequent claims made for stem cell rescue of experimentally-induced pathologies. These capabilities do not lie routinely within the ambit of a single traditional lab, or indeed within the spectrum of organ specific or stem cell institutes. Some of the expertise is within the engineering realm, beyond the biomedical campus altogether. Therefore, meeting these needs to arrive at a more integrated approach to stem cell regulation is going to take more than lip service to interdisciplinary research. One way forward will be the establishment of institutes that

weave biology and engineering technology together in a seamless manner. In a few institutes, this promise is being realized. In others, the traditional molecule focused approaches look set to continue. It is the contention of this chapter that the application of stem cell signalling to organ regeneration requires more of the former than the latter.

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