1

Stem Cell Tissue Engineering and Regenerative Medicine: Role of Imaging

*Bo Chen, Caleb Liebman, Parisa Rabbani, and Michael Cho**

Department of Bioengineering, University of Texas, Arlington, TX, USA
**Corresponding author email: michael.cho@uta.edu*

1.1 Introduction

There has been steady progress of the development of methodologies or techniques to identify stem cells. While the cell surface markers [1, 2] and gene upregulation [3] provide strong evidence for the identity of stem cells, these unique and potent cells are primarily characterized by their ability to self-replicate without differentiation and in response to various cues initiate and execute intracellular processes that lead to specialized cell types and tissues [4]. Stem cells have been classified into several categories including totipotent, pluripotent, multipotent, and unipotent stem cells. The so-called plasticity of stem cells varies from one type of stem cells to another. Totipotent stem cells are formed by fertilizing an egg and considered the most versatile [5]. By definition, the totipotent stem cells give rise to all cell types but are not widely used in tissue engineering. At the early stage of embryonic development (~4 days), the cells become more specialized, and they are referred to as pluripotent or embryonic stem cells (Figure 1.1). Pluripotent stem cells (PSCs) still provide the potential for formation of all tissue types but not necessarily an entire organism [6]. Multipotent stem cells (sometimes referred to as adult stem cells) are considered less plastic and can give rise to limited phenotypes. Found in various tissues, the multipotent stem cells can remain in a quiescent state until they are activated by external or internal cues to repair and replace the damaged tissue [7, 8]. Among the adult stem cells, mesenchymal stem cells (MSCs) have been shown to be capable of promising cell-based therapies. The factors contributing to the potential MSC therapeutics include relatively easy isolation, proliferative capacity to produce a large population of MSCs, and

Magnetic Resonance Imaging in Tissue Engineering, First Edition. Edited by Mrignayani Kotecha, Richard L. Magin, and Jeremy J. Mao.

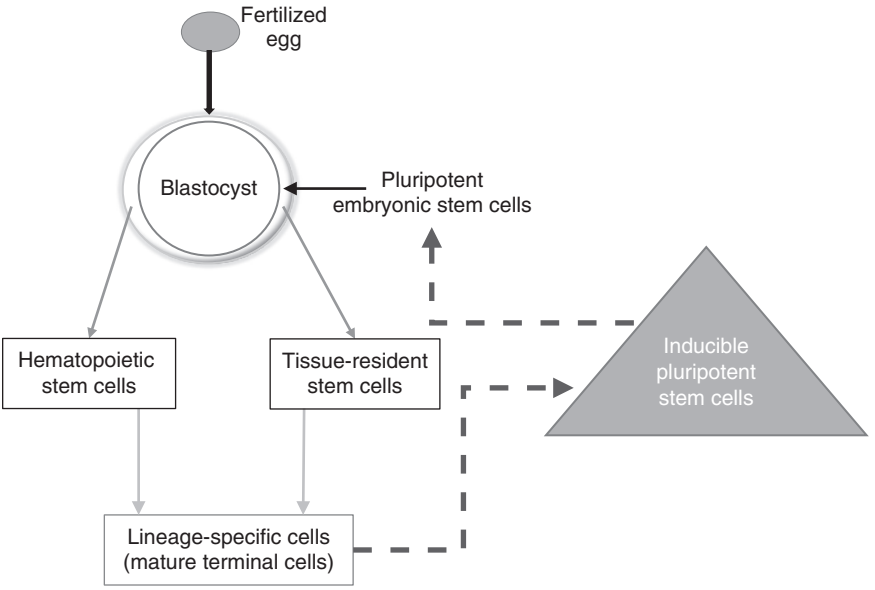

Figure 1.1 A schematic of stem cell hierarchy. Harvested from the inner lining of a blastocyst, pluripotent embryonic stem cells have been shown to differentiate to all three germ layers. Adult stem cells, including hematopoietic and mesenchymal stem cells (MSCs) as well as tissue-resident stem cells, may be differentiated to multiple phenotypes. Recent discoveries indicate that, when genetically reprogrammed, inducible pluripotent stem cells (iPSCs) can be derived from somatic cells that behave similar to embryonic stem cells (indicated by the dotted lines). If proven, the ethical issues associated with the use of embryonic stem cells may no longer be hindrance.

multipotency. Unipotent stem cells (sometimes interchangeably referred to as progenitor cells) are typically tissue specific and give rise to one cell type [9].

A new class of stem cell has recently been introduced to tissue engineering: inducible pluripotent stem cells (iPSCs). iPSCs are derived from terminally differentiated adult cells by genetic reprogramming to behave like pluripotent stem cells (Figure 1.1; Ref. [10]). This class of stem cells is rapidly gaining popularity in tissue engineering for the potential that the use of autologous cells may bring us one step closer to achieving regenerative and personalized medicine. iPSCs demonstrate the properties similar to embryonic stem cells such as formation of teratomas, indicating the pluripotency could be comparable. However, iPSCs are not considered identical, nor is it fully established whether this class of reprogrammed stem cells is as clinically viable. One significant challenge still remains in that reprogramming somatic cells rely on transfection with selective genes and factors using viruses as the delivery vehicle [11]. Development of nonviral deliveries would no doubt enhance significantly the

goal of applying and manipulating dedifferentiated cells for restoration and/or regeneration of lost and damaged tissues. It should be noted, however, that the reprogramming efficiency remains very low. More reliable methodologies would have to be developed and refined before the iPSC potential can be fully realized for tissue engineering and regenerative medicine.

1.2 3D Biomimetics

One of the critical components that will advance the goal of regenerative medicine is the development of appropriate 3D scaffolds. This is rather a daunting task because there is no one scaffold design and type that may be suitable for every tissue engineering application. It is more likely that various scaffolds would have to be developed, tested, and optimized for specific tissue phenotype. For example, it is intuitively clear that while the use of mechanically and structurally hard scaffold is acceptable to repair damaged bone, the same biomaterial is not suited to repair/regenerative soft tissues such as skin or brain. A better understanding of the role of biomimetics will be essential in advancing the field of stem cell tissue engineering. Successful design of biomimetics that targets a specific tissue type must be based on recapitulating the biological, mechanical, and topological factors applicable to that natural tissue and thus guiding stem cells to the intended tissue development. Indeed the scaffold design and engineering may be key to unleashing the full potential of stem cells as they are capable of regenerating tissues *in vivo*. The paradigm shift from replacing to regenerating the damaged/lost tissue will likely require continuous development of biocompatible and bioactive 3D scaffolds and availability of monitoring methodologies (e.g., imaging modalities) to determine the efficacy of the 3D biomimetic scaffold.

The tissue development with proper functionality typically requires bidirectional interactions between the cells and the microenvironment (e.g., extracellular matrix or ECM) they are embedded in. Regenerative tissue engineering using stem cells is no exception, and biological and physical signals are to be provided by the appropriately designed scaffold. Extensive research effort is continuing across the globe to engineer and optimize the biomimetic scaffolds suitable for stem cell tissue engineering. Several examples include bioresorbable and biodegradable materials for experimental and clinical purposes. Classified into two categories of natural and synthetic biomaterials, these materials are typically polymerized to form fibrous substrates. For example, natural polymers can be made from proteins (collagen, gelatin, fibrinogen, and silk) [12], polysaccharides (chitosan and glycosaminoglycans) [13], or polynucleotides (DNA and RNA) [14]. It is no surprise that natural materials are biocompatible and viable for cell attachment and growth. However, these natural scaffolds are mechanically weak and may not be the ideal choice for load-bearing tissue engineering applications.

Synthetic polymers have been extensively used to create 3D scaffolds. Materials such as polyethylene glycol (PEG), poly-lactic acid (PLA), poly-lactic-co-glycolic acid (PLGA), polycaprolactone (PCL), polyvinyl alcohol (PVA), and polyvinyl chloride (PVC), and nylon offer not only reproducibility but also the potential to modify them to be tailored for the intended application. Introduction of the desired mechanical properties, scaffold geometry, and topographical features into the scaffold can be contemplated to emulate both soft and hard tissue microenvironments. However, the lack of cell attachment and proliferation poses a major difficulty for the synthetic scaffolds. For example, cells embedded in a PEG scaffold demonstrate behaviors that are much different if the same cells were seeded in a natural collagen gel (Figure 1.2a and b). Cells in the PEG gel form clusters and show round cell morphology, and may not be viable. In contrast, the same cells seeded in a collagen gel demonstrate spread cellular morphology and are likely suitable for tissue development. A potent strategy that had been proven successful is to chemically modify the surface of the biocompatible and yet cell-resistant materials to promote and facilitate cell adhesion and growth [15, 16]. PDMS surface, for example, can be treated with ion plasma and create the OH group on the surface. The peptides (e.g., RGD) or ECM proteins (e.g., collagen) readily bind to the OH group and cells now can attach to the modified PDMS substrate (Figure 1.2c and d). Once polymer surface conjugation is understood and applied, multiple biological factors (e.g., growth factors) can be introduced to the scaffold to promote stem cell differentiation [17]. Recognizing the advantages and disadvantages of the natural and synthetic materials to engineer 3D scaffolds, a significant effort has been devoted to create composite scaffolds [18] that utilize both categories of biomaterials. In principle, composite scaffolds allow control and manipulation of the scaffold characteristics (e.g., mechanical properties and degradation kinetics) while improving the biocompatibility.

It is worth noting a subset of 3D biomimetic scaffolds referred to as hydrogels. Hydrogels are capable of accommodating and retaining a large amount of water [19] and thus are an excellent choice for various tissue engineering applications. Hydrogels can be made for *in vitro* cell seeding and subsequent *in vivo* implantation [20, 21] or injectable and therefore readily form 3D structures *in situ* within the site of damaged tissue [22, 23]. Hydrogels are chemically conjugated to increase their bioactivities and enhance the stem cell differentiation. The mechanical and architectural design of hydrogels is modulated by the selection of polymers, its concentration, and even polymerization kinetics. Because hydrogels can be tailored by optimizing the biochemical and biophysical microenvironment, numerous laboratories have taken advantage of the hydrogels to create an *in vivo*-like 3D scaffold for stem cell tissue engineering, particularly for chondrogenesis and cartilage tissue engineering [24, 25].

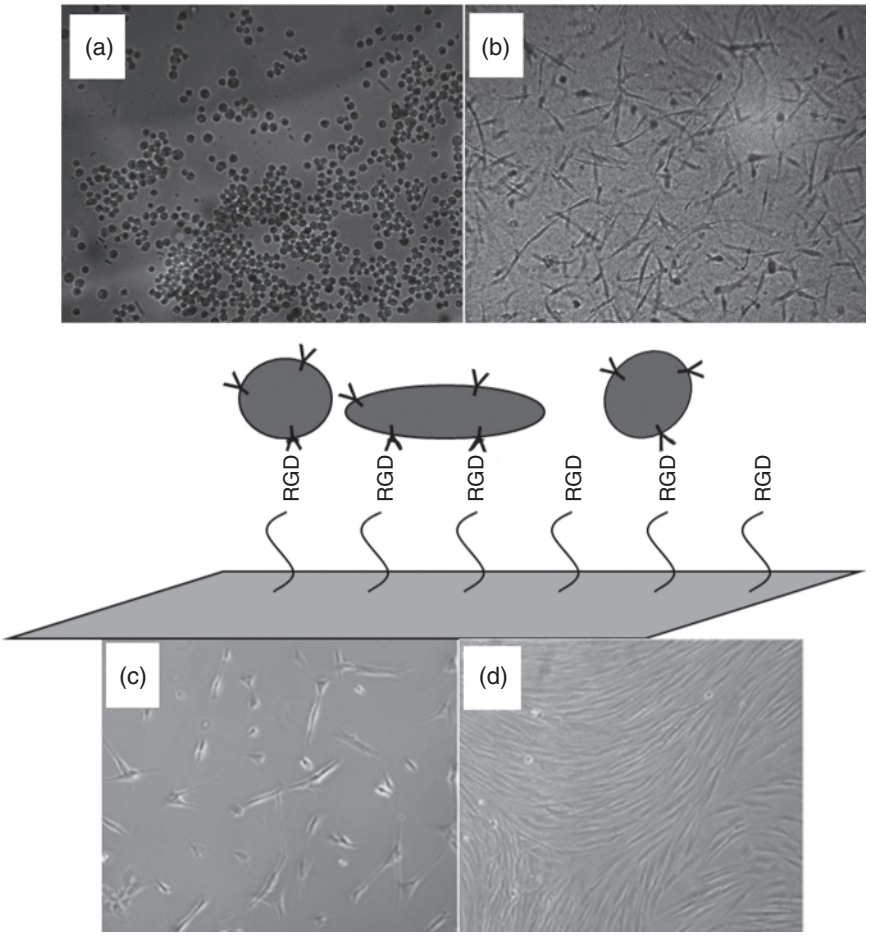

Figure 1.2 Cell attachment to engineered scaffolds. Fibroblasts embedded in a polyethylene (glycol) diacrylate (PEGDA) scaffold exhibited cell clustering and round morphology, indicating potential apoptosis (a). Immunolabeling of viculin failed to visualize focal adhesions. The same cells seeded in a collagen gel appeared elongated, assumed spread morphology, and exhibited integrin-dependent cell adhesion (b). Another cell-resistant biomaterial, polydimethylsiloxane (PDMS), is widely used in tissue engineering, particularly in the field of microfabrication. Cells were seeded on a PDMS substrate without surface conjugation (c). When treated with ion plasma and conjugated with the RGD tripeptide, the cells readily attached to the modified PDMS surface and grew confluent with the spindle-like morphology (d).

1.3 Assessment of Stem Cell Differentiation and Tissue Development

Regenerative medicine that is based on stem cell therapy offers hope for treating many diseases that the current therapies are deemed inadequate. Continuous development of molecular imaging to probe stem cell behavior and intended differentiation will play key roles for facilitating the clinical strategies of translational stem cell therapy. However, challenges still remain to monitor the tissue development of stem cells implanted into a living host. For example, *in vivo* differentiation of stem cells is poor and not as reproducible and predicable. To circumvent this difficulty, stem cells have been differentiated *in vitro* prior to implantation [26, 27]. Incomplete stem cell differentiation coupled with an uncontrolled mixture of differentiated and undifferentiated cells renders such an implantation procedure inadequate and insufficient for clinical translation [28]. Further complication is compounded by the necessity to label individual stem cells to track them and assess the intended differentiation. Because the ultimate goal of stem cell tissue engineering is to regenerate the damaged tissue, the implanted scaffold that is seeded with stem cells must be monitored and imaged over time for safety and efficacy. While there are technically feasible methods and assays available to provide some insights, no definitive standards have been determined, particularly relating to the evolution progress of implanted stem cell therapies.

In vitro assessment of the tissue development is plausible because there are multiple molecular and genetic assay techniques and several imaging modalities that can be readily used. The cell surface or intracellular markers, secretion of extracellular matrix molecules, and gene profiling are routinely carried out to assess, quantify, and determine the extent of stem cell differentiation into a specified lineage. Both *in vitro* 2D and 3D scaffolds are easy to handle, manipulate, and image for tissue-specific markers. For example, our laboratory quantified the chondrogenesis of human MSCs by staining sulfated glycosaminoglycan (sGAG; ECM molecule), collagen type II expression (ECM molecule), and upregulation of collagen type II genes [29]. Similar quantification of chondrogenesis can be achieved in 3D biomimetic scaffolds typically created using hydrogels [30]. Advanced imaging modalities such as confocal microscopy and multiphoton microscopy enable the researcher to probe intact 2D or 3D scaffolds with high lateral resolution and penetration depths of ~150 μm or >1 mm, respectively [31, 32].

1.4 Description of Imaging Modalities for Tissue Engineering

There are numerous imaging modalities that have been applied to characterize the engineered tissue constructs. It is not our aim here to discuss exhaustively each of the imaging techniques. Rather, the goal is to outline several different

modalities that offer advantages and disadvantages and therefore provide some pertinent information to the investigator to select imaging modalities that are most appropriate for particular tissue constructs. It is important to note that the researcher is not limited to using only one imaging modality but instead has the option of incorporating multiple different modalities (e.g., multimodal imaging) to determine, for example, the viability and functionality of the engineered tissue constructs, both 2D and 3D scaffolds.

1.4.1 Optical Microscopy

This simple imaging modality requires no complicated instrumentation, and compound lenses along with a light source are sufficient to form images of small-scale objects such as living cells and tissues. The living objects, sometimes referred to as phase objects, tend not to alter the amplitude of the incident light because they do not typically absorb light, but rather diffract light and cause phase shift in the light rays passing through them. Based on this principle, phase microscopy was invented by Zernike and he won the Nobel Prize in 1953 for this work [33, 34]. Phase microscopy has been used for more than 50 years and yet provides a useful imaging tool to visually inspect, examine, and determine some of the cellular behaviors and response in intact specimen. This noninvasive and labeling-free imaging modality nonetheless suffers from a number of disadvantages. Optics physics dictates that the resolution is inherently restricted to $\sim\lambda/2$, where λ is the wavelength of the illuminating light source. For example, if one were to apply a green light source ($\lambda = 500\,\text{nm}$), the theoretical resolution would be limited to 250 nm. While cells and internal organelles of microns in size are identified and visualized using phase microscopy, the resolution is typically much lower due to imperfect lenses. Moreover, the phase images do not provide accurate assessment along the vertical direction (e.g., Z-axis). When applied with polarized light source and detection, differential interference contrast (DIC) microscopy can produce images with 3D appearance [35]. The Nomarski optics is now widely available to generate 3D-like images but do not offer the capability to quantify the images in the vertical direction. Generally, phase microscopy is useful for visual examination and some quantitative image analyses but is not sufficient to elucidate with molecular details.

1.4.2 Fluorescence Microscopy

One of the most useful imaging modalities that is still heavily applied in biomedical research today is referred to as fluorescence microscopy. It does not rely on the phase image formation but rather uses molecular compounds (e.g., fluorescent dyes) that, upon excitation by an external light, emit light signals in the visible wavelength range. This imaging technique can be applied to elucidate cellular and molecular mechanisms. Because the fluorescent dyes themselves act as a light source of the objects being imaged, the $\lambda/2$ theoretical

resolution limit is no longer applicable [36], but instead the resolution is primarily determined by the microscope objective used to collect the light signals (e.g., magnification and numerical aperture) and the characteristics of light detecting devices (e.g., charge-coupled device or CCD camera). This imaging modality offers various applications relevant to tissue engineering. Ion specific dyes (e.g., Fluo-4 to bind to free Ca^{2+}) are routinely used to characterize and determine the functionality of excitable cells such as neurons and cardiomyocytes. Novel fluorescent dyes are being continuously developed that allow conjugation of antibodies with fluorophores, and therefore antibody binding can be visualized. Because the fluorescent signal is proportional to the extent of molecular interactions in the liner regime, it can be calibrated to quantify, for example, the free Ca^{2+} concentration, protein expressions, or protein–protein association [37, 38]. Unlike phase microscopy, fluorescence imaging requires molecular/chemical compounds to be introduced to the specimen. While there are thousands of different fluorophores already developed and readily available, validated, and extensively applied, they are nonetheless chemical species that might interfere with the intended observations. Phototoxicity [39], fluorophore bleaching [40], and quenching due to excess fluorophore concentration [41] could affect the specimen and generate artifacts.

Conservation of energy demands that the emitted fluorescence signals have longer wavelengths than the excitation lights (i.e., known as Stoke's shift); therefore, well-designed bandpass optical filters are crucial for proper use of fluorescence microscopy. Similar to phase microscopy, the conventional fluorescence imaging suffers from the lack of resolution in the *Z*-axis. Signals detected by the pixels in a camera would have typically been integrated in the vertical direction; therefore, the accumulated signals are reported to the researcher without spatially resolved information in the vertical direction. This challenging technical difficulty was mitigated by the invention of confocal fluorescence microscopy. It utilizes a physical pinhole and a stepping motor to discriminate emitted light signals that originate from the out-of-focus planes [42], allowing a stack of 2D images acquired at different depths of the specimen. Many algorithms have been developed to combine stacked 2D images and generate reconstructed 3D images. Since the area of fluorescence signal collection is greatly diminished by the size of the pinhole, many confocal imaging systems employ photomultiplier tubes (PMTs) instead of CCD cameras. With easy access to lasers, laser scanning confocal instruments are preferred that can precisely control the spatial movement and increase the signal-to-noise ratio for better quality imaging. Spatial scanning demands time and image acquisition is slowed. We have applied confocal fluorescence microscopy to determine the cell penetration depth into a hybrid composite scaffold of PMMA–PHEMA. Migration of corneal fibroblasts into the acellular composite scaffold over a 2-week period of time demonstrates that the cells can penetrate into the scaffold ~70 μm within the first week and a longer incubation time did not increase the cell penetration depth (Figure 1.3).

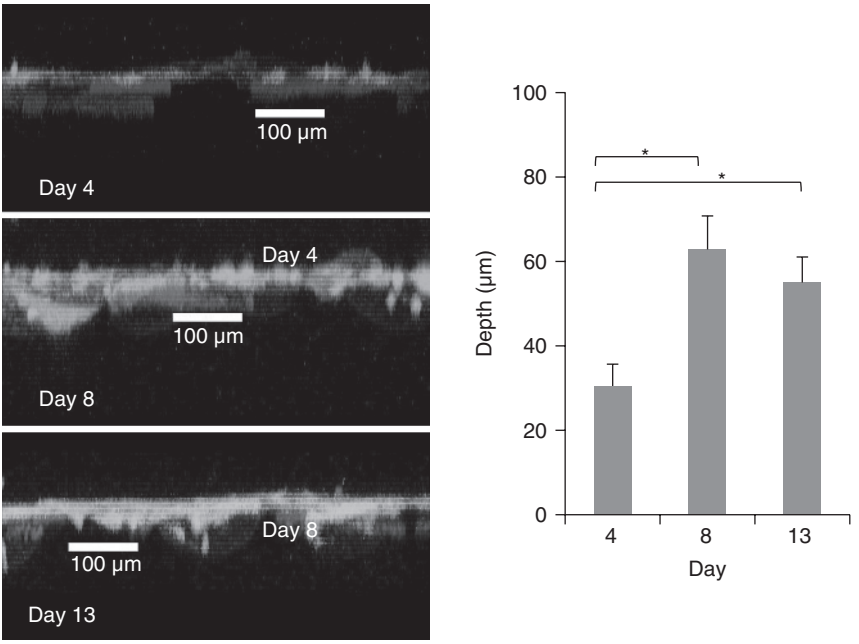

Figure 1.3 Cell migration into a synthetic three-dimensional (3D) scaffold. A composite 3D scaffold composed of poly(methacrylic acid) (PMMA) and poly(hydroxyethyl methacrylate) (PHEMA) was developed for cornea tissue engineering. Confocal microscopy was used to monitor migration of corneal fibroblasts into the acellular scaffold. Using a viability assay, the live or dead cells fluoresce different in different wavelengths. Because the confocal images were collected at different focal depths, reconstructed 3D images can be produced with detailed information in the direction of cell migration into the scaffold. * indicates $p < 0.05$. (*See insert for color representation of this figure.*)

1.4.3 Multiphoton Microscopy

Understanding biological processes in living tissues is often hindered by the limited capabilities to observe and monitor 3D molecular interactions in real time. Conventional imaging techniques such as light microscopy are not able to image and resolve structures above and below the plane of focus. While confocal microscopy allows direct visualization of optical sections within a specimen and provides high-quality images, there are several technical problems that limit its effectiveness. Because a series of images throughout the thickness of a sample is typically collected, the fluorophores are often exposed to the exciting light source for an extended duration of time. The subsequent effects of this exposure include the loss of fluorophore's ability to emit detectable light signal (e.g., photobleaching) and the production of toxic byproducts. A recently developed optical sectioning technique that is more appropriately suited to study living systems is multiphoton microscopy. The essential idea of

this technique is to excite the fluorophore by exposing it to multiple photons of weaker energy. Absorption of more than one photon would be required for the fluorophore to emit a signal. This process is inherently nonlinear and therefore is beyond the conventional optics physics. A fast-pulsed (e.g., 10^{-15} s) laser is used to create a sufficient photon density only within the focal plane of the laser. At this position, the fluorophore is more likely to absorb multiple photons, but those fluorophores located outside the focal point have a very low probability of absorbing more than one photon simultaneously. Therefore, signals are emitted from the focus of the laser only and virtually none are emitted from the out-of-focus planes. There are several significant advantages for using the multiphoton microscopy. First, the fluorophore photobleaching problem is eliminated, allowing longer visualization of the specimen. Second, light-induced cytotoxicity for the living specimen is substantially reduced. This is particularly important for *in vivo* imaging applications. Third, because the wavelength of the laser is at least twice that of the confocal microscopy, the penetration depth in the optical axis is considerably increased. For example, the penetration depth using a typical confocal microscope alone is limited to approximately ~150 μm. In comparison, excellent quality images from deep sectioning of the specimen beyond 1000 μm are routinely obtained by using multiphoton microscopy.

The nonlinear multiphoton imaging is capable of achieving high 3D spatial resolution. Such systems greatly enhance interdisciplinary research among engineering, physical, and biological sciences, which has been proven to be powerful for innovation and development of tissue engineering and regenerative medicine, and providing advanced imaging detection instrumentation capable of high resolution from the subcellular to clinical level. Elucidation of the fundamentals regulating biological responses both *in vitro* and *in vivo* is plausible and should further advance our understanding and knowledge in the life and measurement sciences, and in medical applications. Utilizing multiphoton microscopy, the individual integrin dynamics on the cell surface [43–45], cell attachment in 3D collagen hydrogel [46, 47], role of microfilament regulating the cellular biomechanics [48–52], cell migration into porous 3D biopolymeric scaffolds [53–56], and morphological modulation and calcium dynamics in stem cells undergoing differentiation [57–60] have all been examined and elucidated. As shown in Figure 1.4, a combination of suitably chosen fluorophores was applied to monitor the extent of osteogenesis by visualizing simultaneously nuclei (blue), osteocalcin expression (green), microtubule (yellow), and microfilament (red) organization; four-color imaging. Conventional fluorescence microscopy alone would be difficult to achieve this kind of multicolor images with high resolution.

A label-free nonlinear imaging modality that has proven useful in tissue engineering is second harmonic generation or SHG (see Ref. [61] for review). Rather than relying on fluorophores, the technique exploits nonlinear polarization for

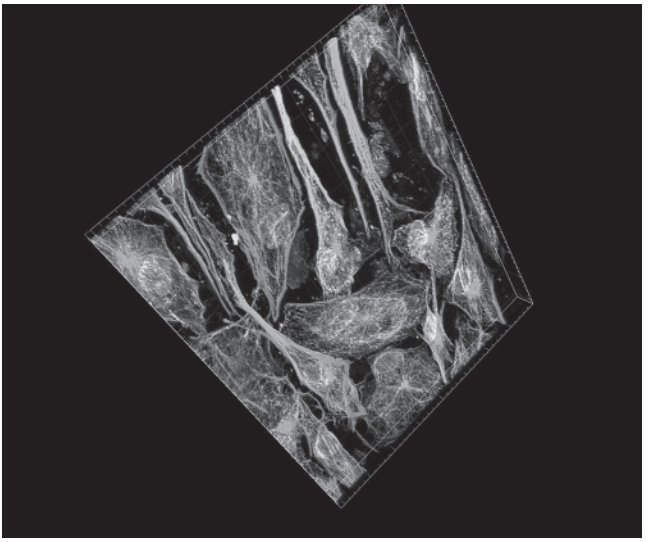

Figure 1.4 Four-color imaging of osteogenesis. Human MSCs were induced to differentiate to osteoblasts. At day 14, the cells were labeled and visualized to quantify the extent of osteogenesis. Using a multiphotom microscope (Bio-Rad, Radiance 2000), a set of four fluorophores was selected to label and image simultaneously the nuclei (blue), osteocalcin expression (green), microtubule (yellow), and microfilament (red) organization. These four fluorophores were carefully chosen to minimize potential spectroscopic overlaps. (*See insert for color representation of this figure*.)

a material under excitation of strong external electrical field created by a high-density pulse laser beam. The material's ability to generate signals proportional to the square of the electric field allows the frequency doubling and emission of light signals that are exactly twice as energetic as the input frequency, thus the term "second harmonic generation". Unlike multiphoton microscopy, SHG does not involve excitation of fluorophore therefore eliminates phototoxicity and photobleaching. Excitation with near-infrared wavelength allows significant depth penetration, and thus SHG is well suited for studying thick tissue samples. While the number of materials that can create nonlinear polarization is limited, several intrinsic structures of cell or tissue (collagen or microtubules) are known to produce SHG. Notably, as one of the most important ECM components, collagen produces and emits detectable SHG. This particular material property of the collagen is of great value to image the collagen fibers in a 3D collagen gel without using fluorophores or relying on immunolabeling (Figure 1.5). The high photon density pulse laser applied to induce SHG is also directed as a light source to photoablate collagen molecules (Figure 1.5), providing a means to introduce spatial patterns (e.g., subtractive manufacturing) in the 3D scaffolds. Disadvantages of SHG imaging include (i) generally small

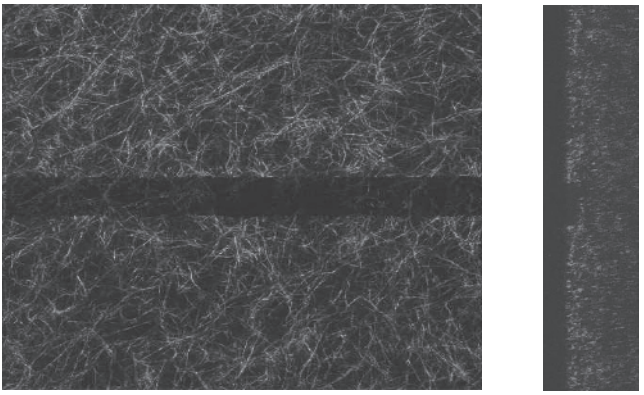

Figure 1.5 Second harmonic generation (SHG) image. An 80-fs pulse laser (Spectra Physics, Mai Tai) was used to excite the label-free collagen molecules in a collagen gel. The collagen undergoes SHG (no fluorophore required) and emits detectable signals. The 3D distribution and organization of the collagen fibers may be visualized with high resolution. The high intensity laser was also directed to ablate a channel in the middle of the collagen gel (appears as a dark strip). This laser ablation technique has been employed by a number of investigators to create spatial patterns for tissue engineering applications.

SHG signal requiring the excitation intensity be large and (ii) the complexity of SHG which makes instrumentation challenging. As the demand for tissue replacement grows rapidly, the proper assessment for functionality and biocompatibility of artificially engineered tissues represents one of the most critical steps toward successful tissue repair and regeneration. Such assessment and product testing can be contemplated with availability of state-of-the-art 3D nonlinear imaging systems.

1.4.4 Magnetic Resonance Imaging

The development and application of 3D imaging techniques must be an important consideration to explore and advance currently unforeseen technologies in biomedical and health-related sciences, including stem cell-based regenerative medicine. While the spatial and temporal resolution is high, nonlinear imaging modalities of multiphoton microscopy and even SHG are still limited by the penetration and detection depth necessary for *in vivo* deep tissue imaging. MRI offers perhaps the best plausible alternative imaging modality to overcome such limitations and shows promise for *in vivo* imaging due to its penetration depth and 3D spatial resolution. Any tissue constructs implanted *in vivo* must be followed and monitored over time for tissue development and functionality. The ability to properly and quantitatively determine the development of implanted tissue constructs is indeed critical to advance the field of

stem cell-based regenerative tissue engineering. To demonstrate the feasibility of monitoring tissue development, MRI has been applied to characterize, just to name a few, chondrogenesis [62, 63], angiogenesis [64, 65] and cell-based neurogenesis [66, 67]. An additional interesting MRI application is to inject stem cells labeled with contrast reagents to accurately track the stem cell movements. The direct tracking of stem cells has been shown clinically feasible in animal models of cardiovascular diseases [68–70].

While there are potentially other modalities such as computed tomography scanning that may also be applicable for *in vivo* imaging, the MRI may offer practical advantages including no radiation and generally painless imaging procedure. The major disadvantage is, of course, the high magnetic field (~10 T) required to align magnetically inducible molecules and long period of time it takes to acquire and average the signals attributed to the relaxation of the molecules. Nonetheless, MRI has become an invaluable imaging modality that will continue to be further improved and likely lead us to the optimal design and implementation of tissue engineering approaches to achieve the ultimate goal of stem cell regenerative medicine. It is indeed timely to dedicate a book to the use of MRI for regenerative stem cell engineering and potentially standardize tissue development following implantation of tissue engineered constructs.

Acknowledgments

This work was supported by a grant from the Office of Naval Research (N00014-13-1-0404) and by a NIH grant (HL083298).

References

1 Lv, F., Tuan, R., Cheung, K., Leung, V. (2014). Concise review: The surface markers and identity of human mesenchymal stem cells. Stem Cells 32: 1408–1419.
2 Lanza, R., Atala, A. (2014). Essentials of Stem Cell Biology. 3rd Ed. Amsterdam: Elsevier.
3 Daley, G. (2015). Stem cells and the evolving notion of cellular identity. Phil. Trans. R. Soc., B. 370: 20140376.
4 Mokry, J., Pisal, R. (2015). The Basic Principles of Stem Cells. In: Vishwakarma, A., Sharpe, P., Shi, S., Ramalingam, M. (eds.), Stem Cell Biology and Tissue Engineering in Dental Sciences. London: Academic Press, Chap. 17.
5 Thomson, J. (1998). Embryonic stem cell lines derived from human blastocysts. Science 282: 1145–1147.

6 Mitalipov, S., Wolf, D. (2009). Totipotency, pluripotency and nuclear reprogramming. Adv. Biochem. Eng. Biotechnol. 114: 185–199.
7 Cheung, T., Rando, T. (2013). Molecular regulation of stem cell quiescence. Nat. Rev. Mol. Cell Biol. 14: 329–340.
8 Dhawan, J., Rando, T. (2005). Stem cells in postnatal myogenesis: Molecular mechanisms of satellite cell quiescence, activation and replenishment. Trends Cell Biol. 15: 666–673.
9 Blanpain, C., Horsley, V., Fuchs, E. (2007). Epithelial stem cells: Turning over new leaves. Cell 128: 445–458.
10 Yildirim, S. (2012). Induced Pluripotent Stem Cell. New York: Springer.
11 Okita, K., Yamanaka, S. (2011). Induced pluripotent stem cells: Opportunities and challenges. Philos. Trans. R. Soc., B 366: 2198–2207.
12 Sangamesh, K., Laurencin, C., Deng, M. (2014). Natural and Synthetic Biomedical Polymers. Amsterdam: Science.
13 Costa-Pinto, A., Reis, R., Neves, N. (2011). Scaffolds based bone tissue engineering: The role of chitosan. Tissue Eng. Part B Rev. 17: 331–347.
14 Chan, G., Mooney, D. (2008). New materials for tissue engineering: Towards greater control over the biological response. Trends Biotechnol. 26: 382–392.
15 Tallawi, M., Rosellini, E., Barbani, N., Cascone, M., Rai, R., Saint-Pierre, G., et al. (2015). Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering: A review. J. R. Soc. Interface 12: 20150254.
16 Ito, Y. (2007). Covalently immobilized biosignal molecule materials for tissue engineering. Soft Matter 4: 46–56.
17 Burdick, J., Vunjak-Novakovic, G. (2009). Engineered microenvironments for controlled stem cell differentiation. Tissue Eng. Part A 15: 205–219.
18 Gautam, S., Chou, C., Dinda, A., Potdar, P., Mishra, N. (2013). Fabrication and characterization of PCL/gelatin/chitosan ternary nanofibrous composite scaffold for tissue engineering applications. J. Mater. Sci. 49: 1076–1089.
19 Van Vlierberghe, S., Dubruel, P., Schacht, E. (2011). Biopolymer-based hydrogels as scaffolds for tissue engineering applications: A Review. Biomacromolecules 12: 1387–1408.
20 Mahoney, M., Anseth, K. (2006). Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. Biomaterials 27: 2265–2274.
21 Hong, M., Kim, S., Om, J., Kwon, N., Lee, Y. (2013). Seeding cells on calcium phosphate scaffolds using hydrogel enhanced osteoblast proliferation and differentiation. Ann. Biomed. Eng. 42: 1424–1435.
22 Radhakrishnan, J., Krishnan, U., Sethuraman, S. (2014). Hydrogel based injectable scaffolds for cardiac tissue regeneration. Biotechnol. Adv. 32: 449–461.
23 Van Tomme, S., Storm, G., Hennink, W. (2008). In situ gelling hydrogels for pharmaceutical and biomedical applications. Int. J. Pharm. 355: 1–18.

24 Park, S., Choi, B., Park, S., Min, B. (2011). Chondrogenesis of rabbit mesenchymal stem cells in fibrin/hyaluronan composite scaffold in vitro. Tissue Eng. Part A 17: 1277–1286.

25 Fan, J., Ren, L., Liang, R., Gong, Y., Cai, D., Wang, D. (2010). Chondrogenesis of synovium-derived mesenchymal stem cells in photopolymerizing hydrogel scaffolds. J. Biomater. Sci. 21: 1653–1667.

26 Nieponice, A., Soletti, L., Guan, J., Deasy, B., Huard, J., Wagner, W., et al. (2008). Development of a tissue-engineered vascular graft combining a biodegradable scaffold, muscle-derived stem cells and a rotational vacuum seeding technique. Biomaterials 29: 825–833.

27 Liu, H., Lin, J., Roy, K. (2006). Effect of 3D scaffold and dynamic culture condition on the global gene expression profile of mouse embryonic stem cells. Biomaterials 27: 5978–5989.

28 Fong, C., Gauthaman, K., Bongso, A. (2010). Teratomas from pluripotent stem cells: A clinical hurdle. J. Cell. Biochem. 111: 769–781.

29 Wise, J.K., Yarin, A.L., Megaridis, C.M., Cho M. (2009). Chondrogenic differentiation of human mesenchymal stem cells on oriented nanofibrous scaffolds: Engineering the superficial zone of articular cartilage. Tissue Eng. 15:913–921.

30 Eyrich, D., Wiese, H., Maier, G., Skodacek, D., Appel, B., Sarhan, H., et al. (2007). In vitro and in vivo cartilage engineering using a combination of chondrocyte-seeded long-term stable fibrin gels and polycaprolactone-based polyurethane scaffolds. Tissue Eng. 13: 2207–2218.

31 Appel, A., Anastasio, M., Larson, J., Brey, E. (2013). Imaging challenges in biomaterials and tissue engineering. Biomaterials 34: 6615–6630.

32 Allan, I., Shevchenko, R., Rowshanravan, B., Kara, B., Jahoda, C., James, S. (2009). The use of confocal laser scanning microscopy to assess the potential suitability of 3D scaffolds in tissue regeneration, by monitoring extracellular matrix deposition and by quantifying cellular infiltration and proliferation. Soft Mater 7: 319–341.

33 Zernike, F. (1955). How I discovered phase contrast. Science 121: 345–349.

34 Frits Zernike - Biographical. Nobelprize.org. Nobel Media AB (2014). http://www.nobelprize.org/nobel_prizes/physics/laureates/1953/zernike-bio.html (accessed on October 24, 2016).

35 Tsunoda, M., Isailovic, D., Yeung, E. (2008). Real-time three-dimensional imaging of cell division by differential interference contrast microscopy. J. Microsc. 232: 207–211.

36 Chan, W., Hart, I. (1991). Potential use of a charge-coupled device camera for imaging fluorescent dyes in vivo. J. Photochem. Photobiol. B 8: 440.

37 Lock, J., Parker, I., Smith, I. (2015). A comparison of fluorescent Ca^{2+} indicators for imaging local Ca^{2+} signals in cultured cells. Cell Calcium 58: 638–648.

38 Kenworthy, A. (2001). Imaging protein-protein interactions using fluorescence resonance energy transfer microscopy. Methods 24: 289–296.

39 Hoebe, R., Van Oven, C., Gadella, T., Dhonukshe, P., Van Noorden, C., Manders, E. (2007). Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging. Nat. Biotechnol. 25: 249–253.

40 Pampaloni, F., Chang, B., Stelzer, E. (2015). Light sheet-based fluorescence microscopy (LSFM) for the quantitative imaging of cells and tissues. Cell Tissue Res. 360: 129–141.

41 Piston, D., Kremers, G. (2007). Fluorescent protein FRET: The good, the bad and the ugly. Trends Biochem. Sci. 32: 407–414.

42 Paddock, S. (2000). Principles and practices of laser scanning confocal microscopy. Mol. Biotechnol. 16: 127–150.

43 Wiseman, P. (2004). Spatial mapping of integrin interactions and dynamics during cell migration by image correlation microscopy. J. Cell Sci. 117: 5521–5534.

44 Li, S., Tachiki, L., Luo, J., Dethlefs, B., Chen, Z., Loudon, W. (2010). A biological global positioning system: Considerations for tracking stem cell behaviors in the whole body. Stem Cell Rep. 6: 317–333.

45 Chen, H.F., Titushkin, I., Stroscio, M.A., Cho, M. (2007). Altered protein dynamics of quantum dot-conjugated integrins during osteogenic differentiation of human bone marrow derived progenitor cells. Biophys. J. 92: 1399–1408.

46 Chen, X., Su, Y., Ajeti, V., Chen, S., Campagnola, P. (2012). Cell adhesion on micro-structured fibronectin gradients fabricated by multiphoton excited photochemistry. Cell. Mol. Bioeng. 5: 307–319.

47 Lang, X., Spousta, M., Lyubovitsky, J. (2015). Detecting the collagen-based hydrogels degradation by multiphoton microscopy (MPM). Proceedings of the SPIE 9329, Multiphoton Microscopy in the Biomedical Sciences, February 7, 2015, San Francisco, CA.

48 Hwang, Y., Kolettis, N., Yang, M., Gillard, E., Sanchez, E., Sun, C., et al. (2011). Multiphoton imaging of actin filament formation and mitochondrial energetics of human ACBT gliomas. Photochem. Photobiol. 87: 408–417.

49 Vielreicher, M., Schurmann, S., Detsch, R., Schmidt, M., Buttgereit, A., Boccaccini, A., et al. (2013). Taking a deep look: Modern microscopy technologies to optimize the design and functionality of biocompatible scaffolds for tissue engineering in regenerative medicine. J. R. Soc. Interface 10: 20130263.

50 Titushkin, I., Cho, M. (2007). Modulation of cellular mechanics during osteogenic differentiation of human mesenchymal stem cells. Biophys. J. 93: 3693–3702.

51 Titushkin, I., Sun, S., Paul, A., Cho, M. (2013). Control of adipogenesis by Ezrin, Radixin and Moesin-dependent biomechanics remodeling. J. Biomech. 46: 521–526.

52 Sun, S., Song, Z.Y., Cotler, S.J., Cho, M. (2013). Biomechanics and functionality of hepatocytes in liver cirrhosis. J. Biomech. 47: 2205–2210.

53 Liu, E., Treiser, M., Johnson, P., Patel, P., Rege, A., Kohn, J., et al. (2007). Quantitative biorelevant profiling of material microstructure within 3D porous scaffolds via multiphoton fluorescence microscopy. J. Biomed. Mater. Res., Part B. 82B: 284–297.

54 Zellander, A., Gemeinhart, R., Djalilian, A., Makhsous, M., Sun, S., Cho, M. (2013). Designing a gas foamed scaffold for keratoprothesis. Mater. Sci. Eng. C 36: 3396–3403.

55 Zellander, A., Wardlow, M., Djalilian, A., Zhao, C., Abiade, J., Cho, M. (2013). Engineering copolymeric artificial cornea with salt porogen. J. Biomed. Mater. Res., Part A 102 (6): 1799–1808. doi: 10.1002/jbm.a.34852.

56 Zellander, A., Zhao, C., Kotecha, M., Gemeinhart, R.A., Wardlow, M., Abiade, J., et al. (2014). Characterization of pore structure in biologically functional poly(2-hydroxyethyl methacrylate)-poly(ethylene glycol) diacrylate (PHEMA-PEGDA). PLoS One 9 (5): e96709:1-8. doi: 10.1371/journal.pone.0096709.

57 Hackl, M., Burford, J., Villanueva, K., Lam, L., Suszták, K., Schermer, B., et al. (2013). Tracking the fate of glomerular epithelial cells in vivo using serial multiphoton imaging in new mouse models with fluorescent lineage tags. Nat. Med. 19: 1661–1666.

58 Calle, E., Vesuna, S., Dimitrievska, S., Zhou, K., Huang, A., Zhao, L., et al. (2014). The use of optical clearing and multiphoton microscopy for investigation of three-dimensional tissue-engineered constructs. Tissue Eng. Part C 20: 570–577.

59 König, K., Uchugonova, A., Gorjup, E. (2010). Multiphoton fluorescence lifetime imaging of 3D-stem cell spheroids during differentiation. Microsc. Res. Tech. 74: 9–17.

60 Sun, S., Liu, Y.M., Lipsky, S.B., Cho, M.R. (2007). Physical manipulation of calcium oscillations facilitates osteodifferentiation of human mesenchymal stem cells. FASEB J. 21: 1472–1480.

61 Perry, S.W., Burke, R.M., Brown, E.B. (2012). Two-photon and second harmonic microscopy in clinical and translational cancer research. Ann. Biomed. Eng. 40: 277–291.

62 Pothirajan, P., Dorcemus, D., Nukavarapu, S., Kotecha, M. (2014). True MRI assessment of stem cell chondrogenesis in a tissue engineered matrix. Conference Proceedings of the IEEE Engineering in Medicine and Biology Society, pp. 3933–3936.

63 Majumdar, S., Pothirajan, P., Dorcemus, D., Nukavarapu, S., Kotecha, M. (2016). High field sodium MRI assessment of stem cell chondrogenesis in a tissue-engineered matrix. Ann. Biomed. Eng. 44: 1120–1127.

64 Li, L., Chopp, M., Ding, G., Qu, C., Li, Q., Lu, M., et al. (2012). MRI measurement of angiogenesis and the therapeutic effect of acute marrow

stromal cell administration on traumatic brain injury. J. Cereb. Blood Flow Metab. 32: 2023–2032.

65 Seevinck, P., Deddens, L., Dijkhuizen, R. (2010). Magnetic resonance imaging of brain angiogenesis after stroke. Angiogenesis 13: 101–111.

66 Guzman, R., Uchida, N., Bliss, T., He, D., Christopherson, K., Stellwagen, D., et al. (2007). Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. Proc. Natl. Acad. Sci. USA 104: 10211–10216.

67 Aswendt, M., Gianolio, E., Pariani, G., Napolitano, R., Fedeli, F., Himmelreich, U., et al. (2012). In vivo imaging of inhibitory, GABAergic neurons by MRI. Neuroimage 62: 1685–1693.

68 Murphy, S., Hale, A., Reid, T., Olson, J., Kidiyoor, A., Tan, J., et al. (2015). Use of trimetasphere metallofullerene MRI contrast agent for the non-invasive longitudinal tracking of stem cells in the lung. Methods 99: 99–111. doi. 10.1016/j.ymeth.2015.11.004.

69 Skelton, R., Khoja, S., Almeida, S., Rapacchi, S., Han, F., Engel, J., et al. (2016). Magnetic resonance imaging of iron oxide-labeled human embryonic stem cell-derived cardiac progenitors. Stem Cells Trans. Med. 6 (1): 67–74.

70 Li, L., Jiang, W., Luo, K., Song, H., Lan, F., Wu, Y., et al. (2013). Superparamagnetic iron oxide nanoparticles as MRI contrast agents for non-invasive stem cell labeling and tracking. Theranostics 3: 595–615.