# Noninvasive optical imaging of stem cell differentiation in biomaterials using photonic crystal surfaces

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## 10.1 Introduction

In this chapter, we describe recent advances in non-invasive optical imaging of stem cells using photonic crystal label-free biosensor surfaces. Technological progress in bioengineering approaches now enables the sophisticated control of live cells in vitro. In such applications, stem cells are often selected as the cell source as they have the capacity to expand to provide a large pool of cells with varying levels of self-renewal and differentiation potential (stem cells, progenitor cells, terminally differentiated cells) necessary to meet desired therapeutic goals. Precisely engineering stem cell fate decisions, however, has remained a challenge due to the lack of analytical tools that allow dynamic monitoring of heterogeneous stem cell populations in situ in real time at the single cell level. Currently available technologies such as fluorescence labeling or clonal expansion assays are almost always end-point analyses of ensemble populations of cells that are expensive, labor intensive, and time delayed. New optical imaging tools may increasingly permit label-free imaging of live cells for subcellular-resolution quantification of cellular activities in real time. Non-invasive optical imaging using a nanostructured photonic crystal surface in place of an ordinary glass or plastic substrate for cell growth can be easily integrated into existing bioengineering platforms such as microfluidics or microarrays and can provide a novel, alternative approach to monitor single stem cell activities in vitro. Here, we first detail the principles of photonic crystal enhanced microscopy (PCEM) then offer a detailed rationale for developing new tools that enable monitoring and screening of increasingly small populations of stem cells in vitro. Finally, we close by describing efforts to apply PCEM to characterize stem cell differentiation events via adhesion-based metrics of stem cell differentiation.

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## 10.2 Motivation for noninvasive optical imaging of stem cells in vitro: adhesion phenotyping of stem cell differentiation

### *10.2.1 Material-based approaches to regulate stem cell fate decisions in vitro*

Stem cell function is carefully orchestrated by an array of intrinsic and extrinsic factors [1–3]. A large number of cellular, biophysical, and chemical cues have been found to directly and indirectly impact stem cell fate decisions at different stages during development, at homeostasis, and following injuries [3]. These findings present opportunities to design and develop material-based approaches to allow better understanding of the underlying mechanisms of stem cell regulation as well as the realization of currently unmet clinical and therapeutic needs [3–5]. Combined with advances in fabrication approaches, sophisticated constructions of micro- and nanoscale features with varying material properties are now possible to selectively decouple or integrate experimental parameters that maximize the intended outcome of bioengineering applications [2,3,5].

Material-based approaches using stem cells often take advantage of the material properties to regulate stem cell function or fate decisions [4,6]. It has been reported that bulk material properties (e.g., pore size, pore alignment, stiffness, permeability) as well as surface properties (e.g., chemistry, topography, surface-bound ligand presentation, and orientation) at the cell–material interface serve as important regulators of stem cell fate: quiescence, self-renewal, differentiation, mobilization, homing, and senescence for several types of stem cells, such as mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), hematopoietic stem cells (HSCs), and neuron stem cells (NSCs) [2,3].

### *10.2.2 Challenges associated with in vitro control of stem cell fate decisions*

Advances in bioengineering approaches now allow sophisticated manipulation of live cells in vitro for a range of applications. For these applications, stem cells are often the cell source of choice as they offer several advantages [7,8]. First, stem cells can be expanded to supply a large number of cells with varying levels of self-renewal and differentiation potential. For instance, there are now well-established protocols for stem cells such as MSCs that enable precise expansion or differentiation into multiple cell lineages [9]. Second, the use of stem cells facilitates temporal control of cellular activities as they can be maintained as undifferentiated cells until appropriate extrinsic cues are presented. Third, advances in stem cell technology permit the use of patient-derived somatic cells as induced pluripotent stem cells, opening up new possibilities for patient-specific applications that may significantly enhance therapeutic outcome.

However, using stem cells is often challenging due to difficulties associated with precisely controlling stem cell fate decisions in vitro [2,3]. Despite technological advances in engineering stem cell fate decisions, underlying mechanisms of stem cell renewal and differentiation remain unclear as the critical factors required to maintain many types of stem and progenitor cells in vitro have yet to be uncovered [2]. This is particularly

challenging with the use of adult stem cells as they quickly lose their stem potential in vitro [2,3]. The heterogeneity of stem and progenitor cell populations poses another challenge [2,3]. Because most stem cell populations are not homogeneous and therefore contain cells with varying functional capacity (e.g., progenitor cells, differentiating cells, terminally differentiated cells, other types of cells), stem cell populations need to be screened routinely at the single-cell level to exclude any unwanted cell populations [3]. Currently available tools (e.g., fluorescence labeling, clonal expansion assays, repopulation assays, in vivo models) rarely allow in situ screening of individual live cells for dynamic monitoring of stem cell populations because they are almost always end-point analyses of ensemble populations of cells that are expensive, labor intensive, and time delayed [2,3]. For more detailed information on stem cell engineering and currently available tools of stem cell characterization, please refer to recent reviews [2,3]. Naturally, the lack of suitable analytical approaches for quantitation of stem cell fate decisions in vitro also hampers efforts to identify factors that are essential for stem cell maintenance. These shortcomings in stem cell fate control efforts in vitro call for a new analytical approach that could spatiotemporally resolve single stem cell activities in real time.

### 10.2.3 Adhesion phenotyping of stem cells

Cells attach and adhere to the surrounding material surfaces to obtain material-mediated extrinsic signals that impact downstream signaling pathways [10]. As such, cell adhesion has been found to be critical in maintaining cellular activities such as growth, division, migration, and apoptosis [11,12]. Similarly, adhesion has been found to play a critical role in the regulation of stem cell activities, from proliferation and expansion to differentiation, mobilization, homing, and senescence [2,3].

As different cell populations exhibit different patterns of adhesion, a concept known as “adhesion phenotyping” has emerged [13,14]. It refers to the comprehensive and quantitative profiling of individual cells based on their adhesion characteristics to an underlying surface and it has shown promise as a new metric for cell function. For instance, adhesive profiles of individual cells have been linked to the aggressiveness of lung cancer, suggesting that adhesive properties of cell populations could be a predictive marker of pathologies [14]. Likewise, it was found that adhesive signatures of individual pluripotent stem cells could be used to isolate stem cell populations from differentiated cells [15]. These findings suggest adhesion-associated cell–material interactions contain rich information that correlates with cell function and that while still in its nascent stage, adhesion phenotyping of single cells or cell populations can provide a new functional metric for cell identification, isolation, and screening applications.

### 10.2.4 Noninvasive optical imaging as a potential new tool of stem cell characterization

Noninvasive optical imaging approaches may provide a novel platform that can dynamically quantify single stem cell activities in real time, in situ, by providing a means to probe individual cells in a label-free fashion [16–20]. In particular, noninvasive optical imaging allows adhesion phenotyping of live cells to dynamically quantify adhesion

patterns of single cells for extended periods (up to days or weeks), which is difficult or impossible to achieve with currently available analytical techniques [16,17].

Specifically, photonic crystal enhanced microscopy (PCEM), a noninvasive optical imaging approach, enables spatially and temporally resolved quantification of single cell adhesion at the cell–surface interface by using photonic crystal (PC) surfaces as the optical transducer for biosensing, along with a modified optical microscope [16,17]. PCEM offers several advantages as a noninvasive optical imaging tool [16,17]. First, as it builds on a conventional inverted light microscope and as PC sensors are inexpensively produced via nanoreplica molding, PCEM can be set up and operated at affordable costs. Second, PCEM offers high sensitivity and high spatiotemporal resolution (submicron resolution at imaging intervals of only several seconds) that allows subcellular details of adhesion-mediated cell attachment and movement events to be imaged and quantitatively visualized. Third, PCEM can be easily integrated into many existing cell-based assay platforms including microfluidics and microarrays by simply introducing the PC element as the material interface.

These capabilities of PCEM enable the collection of adhesion footprints for a range of cell types, from adherent cells to only weakly adherent cells and even suspension culture cells traditionally regarded as nonadherent cells, to build a library of adhesion phenotypes, which may be highly useful for cell identification, isolation, and screening applications [16,17]. As evidence suggests that stem cells at different states may exhibit varying and/or distinct adhesion patterns [2,3], adhesion phenotyping of stem cells may also provide new ways to elucidate the underlying mechanisms of stem cell maintenance as well as enable dynamic screening of individual stem cells at varying stages of differentiation hierarchy to characterize stem cell fate decisions in vitro [2,3].

## 10.3 History: optical imaging of cells using photonic crystal enhanced microscopy (PCEM)

### *10.3.1 Basic principles of PCEM*

The principles of PC label-free biosensor surfaces and the PCEM instrument have been described in recent publications and are briefly summarized here (Fig. 10.1) [16,17,21–24]. PCEM utilizes the sensing function of PC surfaces and is built upon a conventional benchtop inverted light microscope (e.g., Olympus BX51WI or Carl Zeiss Axio Observer Z1) by integrating it with a line-profiled and polarized light illumination source (e.g., laser or LED), a CCD camera, and a spectrometer as the detection instrument. PCEM provides a novel optical platform that enables label-free imaging of a broad range of surface-bound analytes (e.g., nanoparticles, proteins, antibodies, biomolecules). This capacity of PCEM extends to the analysis of surface-attached cells to enable sensing and imaging of whole, live cells at subcellular resolution at the cell–PC surface interface to temporally and spatially resolve adhesion-mediated cellular activities in situ in real time for life science research and engineering applications (Figs. 10.1–10.2).

The key component in PCEM is the PC biosensor, which is comprised of a periodic-modulated dielectric nanostructure as the optical transducer surface (Fig. 10.1(a) and (b))

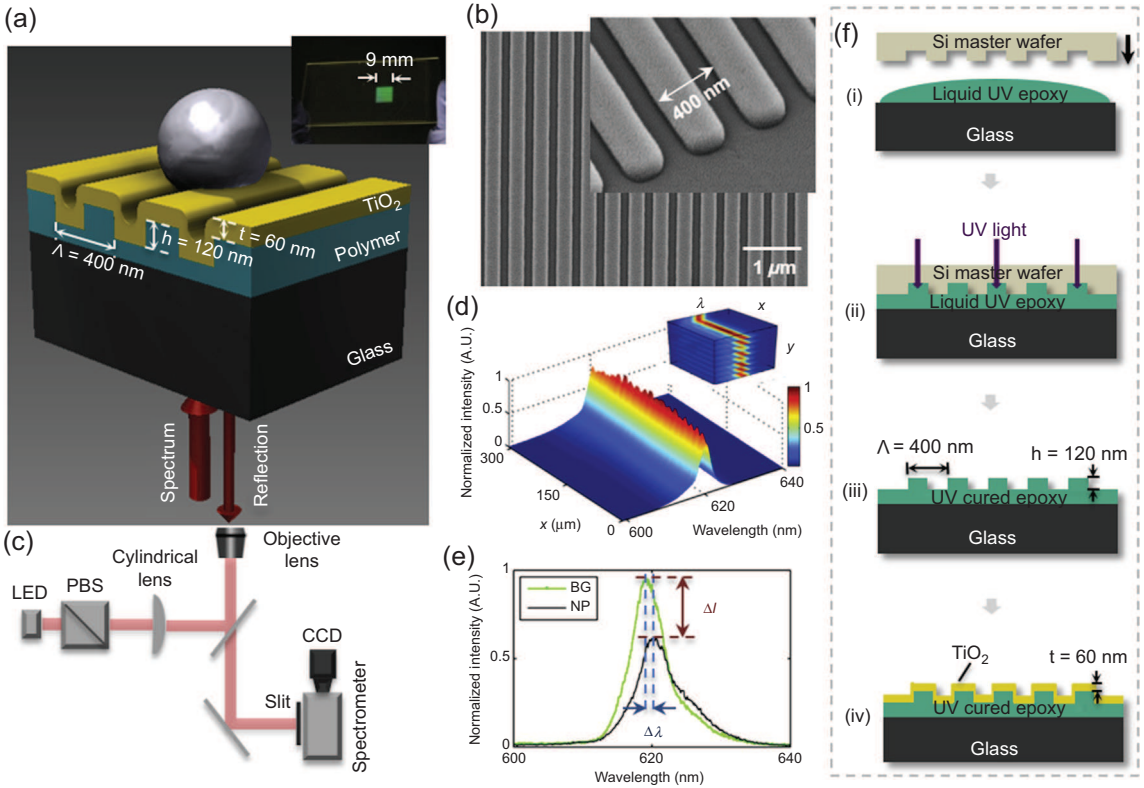


**Figure 10.1** Schematic of photonic crystal enhanced microscopy. (a) A schematic of a nanoparticle attached to a photonic crystals (PC) surface. Inset: photo of a PC fabricated on a glass microscope slide. (b) SEM image of the PC surface. Inset: zoomed in to show the nanograting surface structures. (c) A schematic of the PCEM setup. (d) Normalized spectrum image (surface plot) obtained from PCEM imaging. Inset: PCEM-acquired 3-D spectrum data. (e) Example spectrum from PCEM with a peak wavelength value (PWV) shift and a peak intensity value (PIV) change with or without a nanoparticle attached on the PC surface (*BG*, background; *NP*, nanoparticle). (f) A schematic of nanoreplica molding to fabricate PCs: (i) deposition of a thin layer of liquid UV epoxy polymer between a Si wafer template and a glass substrate, (ii) hardening of the epoxy layer via exposure to UV light, (iii) removal of the Si wafer template, (iv) sputter deposition of a thin layer of $TiO_2$ film on top of the nanograting structure. Reprinted in part with permission from Zhuo Y, et al. Single nanoparticle detection using photonic crystal enhanced microscopy. Analyst 2014;139(5):1007–15, © 2013 RSC Publishing.

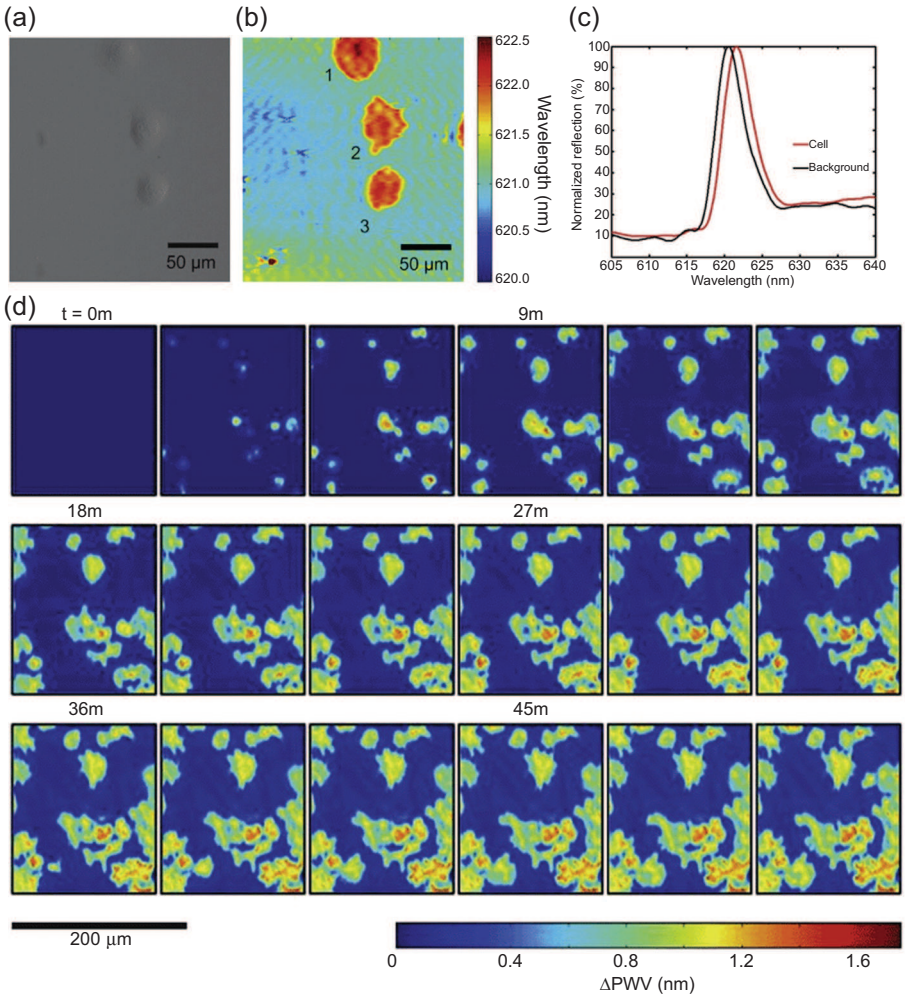


**Figure 10.2** PCEM for in vitro studies of adhesion-mediated cellular behaviors. (a–b) Bright-field and the corresponding PWV images of Panc-1 cells adhered on the PC surface. PWV images clearly detail cellular protrusions at cell edges and regions of higher or lower mass distribution, noted by the differing magnitudes of the wavelength shift across the attached cell area. (c) Representative PWV spectra of the background (PC portion, nothing attached on top) versus the region where a cell is attached. (d) Time series PWS images of dental epithelial stem cells (mHAT9a) to demonstrate cellular attachment, adhesion, and movement.
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[16,17]. The structural features of PC surfaces provide photonic band gaps, where light propagation is prohibited for specific wavelengths [25–27]. When cells attach to a PC surface, the local refractive index of the PC changes. By detecting these changes in the local refractive index of PC surfaces, it is possible to quantify the average response from the entire sensing area (biosensing) or spatially resolve localized responses that

can be differentiated from neighboring locations (bioimaging) to achieve highly sensitive label-free sensing and imaging of surface-attached analytes (Fig. 10.1(a)–(e)).

Photonic Crystal (PC) biosensors have recently been demonstrated as a highly versatile technology for a variety of label-free assays including high-throughput screening of small molecule – protein interactions, characterization of protein–protein interactions, and measurement of cell attachment modulation by drugs [16,17,21–24]. A PC is a sub-wavelength grating structure consisting of a periodic arrangement of a low refractive index material coated with a high refractive index layer (Fig. 10.1(a)) [16]. When the PC is illuminated with a broadband light source, high order diffraction modes couple light into and out of the high index layer, destructively interfering with the zeroth-order transmitted light [17]. At a particular resonant wavelength and incident angle, complete interference occurs and no light is transmitted, resulting in 100% reflection efficiency. The resonant wavelength is modulated by the addition of biomaterial upon the PC surface, resulting in a shift to a higher wavelength. The electromagnetic standing wave that is generated at the PC surface during resonant light coupling inhibits lateral propagation, thus enabling neighboring regions on the PC surface to display a distinct resonant wavelength that is determined only by the density of biomaterial attached at that precise location. By measuring the resonant peak wavelength value (PWV) on a pixel-by-pixel basis over a PC surface, an image of cell attachment density may be recorded. PWV images of the PC may be gathered by illuminating the structure with collimated white light through the transparent substrate, while the front surface of the PC is immersed in aqueous media.

A schematic diagram of the PCEM instrument is shown in Fig. 10.1. The system is built upon the body of a standard microscope (Carl Zeiss Axio Observer Z1), but in addition to ordinary brightfield imaging, a second illumination path is provided from a fiber-coupled broadband LED (Thorlabs M617F1, $600 < \lambda < 650$ nm) [16, 28]. The fiber output is collimated and filtered by a polarizing beamsplitter cube to illuminate the PC with light that is polarized with its electric field vector oriented perpendicular to the grating lines. The polarized beam is focused by a cylindrical lens (f = 200 mm) to form a linear beam at the back focal plane of the objective lens (10x, Zeiss). After passing through the objective lens, the orientation of the line-shaped beam is rotated to illuminate the PC from below at normal incidence. The reflected light is projected, via a side port of the inverted microscope and a zoom lens, onto a narrow slit aperture at the input of an imaging spectrometer. The width of the adjustable slit can be adjusted according to the need of specific applications (e.g., 30 μm). The reflected light is collected from a linear region of the PC surface, where the width of the imaged line, 1.2 μm, is determined by the width of the entrance slit of the imaging spectrometer and the magnification power of the objective lens. The system incorporates a grating-based spectrometer (Acton Research) with a 512 x 512 pixel CCD camera (Photometrics Cascade 512). The line of reflected light, containing the resonant biosensor signal, is diffracted by the grating within the spectrometer (300 lines per mm-1) to produce a spatially resolved spectrum for each point along the line. Therefore, each pixel across the line is converted to a resonant reflection spectrum, containing a narrow bandwidth ($\Delta\lambda \approx 4$ nm) reflectance peak from the PC. The PWV of each peak is determined by fitting the spectrum to a 2nd-order polynomial function, and then mathematically determining the maximum wavelength of the function. By fitting all 512 spectra, in a

process that takes 20 ms, a line comprised of 512 pixels is generated that represents one line of a PWV image of the PC surface. With an effective magnification of 26x, each pixel in the line represents a ≈0.6 μm region of the PC surface and 512 such pixels cover a total width of ≈300 μm. To generate a two-dimensional PWV image of the PC surface, a motorized stage (Applied Scientific Instruments, MS2000) translates the sensor along the axis perpendicular to the imaged line in increments of 0.6 μm per step. Using this technique, a series of lines are assembled into an image at a rate of 0.1s per line and the same area on the PC surface can be scanned repeatedly. Each image is comprised of 512 by n pixels, where n can be selected during each scan session, and each pixel represents a 0.6 x 0.6 μm region of the PC surface (Fig. 10.1(d)). A biosensor experiment involves measuring shifts in PWV. A baseline PWV image is gathered before the introduction of cells, when the PC is uniformly covered by cell media, which is aligned and mathematically subtracted from subsequent PWV images gathered during and after cell attachment (Fig. 10.1(e)).

Using nanoreplica molding, 1-D PC nanostructures can be mass produced at low cost with high reproducibility (Fig. 10.1(f)) [16,17,21–24]. The molding template is made on silicon wafers or quarts substrates via lithography. To being the process of fabricating a PC, a thin layer of ultraviolet-curable polymer (UVCP; $n_{UVCP}=1.5$) with a low refractive index (e.g., grating period of $\Lambda=400$ nm, grating depth of $d=120$ nm, duty cycle of $f=50\%$) is first cured on a solid support substrate (e.g., microscope slide) then a thin layer of dielectric material with a higher refractive index (e.g., $TiO_2$; $n_{TiO_2}=2.4$) is deposited on top (Fig. 10.1(a) and (f)). The thickness of the higher refractive index layer is selected (e.g., thickness of 60 nm) to generate a resonant reflection at a specific wavelength (e.g., resonant wavelength of $\lambda_0=620$ nm) [21–24]. The sensitivity and the spatial resolution of the PC biosensor can be optimized by the choice of the dielectric materials and the geometry of the nanostructure features. For sensing and imaging of biological analytes, the PC surface can be further modified to immobilize application-specific biomolecules (e.g., antibodies, proteins, peptides) to enhance selectivity [17]. This nanoreplica-molding approach allows simple, rapid, and reliable fabrication of PC surfaces to offer benefits of single-use disposable detection for high-throughput screening applications. Additionally, the PC surface may be made planar by filling the nanograting structure with a polymer using a horizontal dipping process to eliminate potentially unwanted effects arising from analytes reacting to the structured surface topography [28].

### 10.3.2 Optical imaging of live cells using PCEM (Cunningham group publications)

PCEM provides the opportunity for noninvasive optical imaging of live cells at the cell–PC surface interface along with the potential to directly visualize and quantify adhesive contact between cells and the surface below at submicron resolution [16,17]. This is challenging for most conventional techniques such as fluorescence labeling, cytotoxic end-point analyses, or other forms of microscopy due to photobleaching [16]. PCEM is therefore particularly suitable for 2-D surface-attached cell studies aiming to

characterize adhesion-mediated cellular behaviors at the cell–surface interface, including cell attachment, detachment, adhesion, chemotaxis, and migration [17].

Several configurations of PCEM instrumentation have been described for applications that include low spatial resolution imaging of biomolecular assays on a dry PC surface and high spatial resolution of cells and nanoparticles on PC surfaces covered with liquid media [21,23,28]. As a proof-of-concept, Lidstone et al. showed in 2011 that PCEM could be used to track the attachment footprint of four types of cells using collimated laser beam at $\lambda = 637$ nm from a 300-mW AlGaInP diode laser as the excitation source and 1-D PC slabs with $SiO_2$ (200 nm thick) and $TiO_2$ (~60 nm thick) layers deposited on top of a UV curable polymer layer as the sensing surface [23]. The PC surface was further coated with collagen, fibronectin, or poly-L-lysine to facilitate cell attachment. Here, PCEM imaging of cancer cells (HepG2/C3 hepatic carcinoma cells, Panc-1 pancreatic cancer cells), primary cardiomyocytes from rats, and porcine adipose-derived stem cells (ASC) showed PWS at cell attachment sites that was distinct from the spectra collected from the cell-free regions of the sensing area (Fig. 10.2(a)–(c)). Additionally, PCEM could dynamically assess when morphological changes were induced within Panc-1 pancreatic cancer cells by introducing staurosporine, an apoptosis-inducing agent. PCEM was also used to assess ASCs by replacing the cell medium with a neuronal induction medium that caused the retraction of the cell body and development of dendritic projections. These findings demonstrated the capacity of PCEM to capture transient shifts in the PC refractive index caused by cellular materials adhering and dissociating from the PC surface, which also indirectly provided a means to qualitatively assess changes in cell adhesion strength as greater PWS indicated stronger attachment to the PC surface.

In 2013, Chen et al. established an improved PCEM setup using a collimated LED light from fiber-coupled broadband LED (Thorlabs M617F1, $600 < \lambda < 650$ nm) as the light source and 1-D PC slabs with a single layer of $TiO_2$ (~60 nm thick) deposited on top of a UV curable polymer layer as the sensing surface [21]. This system provided improved spatial resolution that enabled optical imaging of live cells at subcellular resolution, where each pixel of the acquired image ($512 \times 512$) corresponded to roughly 0.6 $\mu m^2$ of the PC surface. This work examined dental epithelial stem cells (mHAT9a cells) at time intervals of 3 min to monitor their attachment profiles [29]. Attached cells showed highest PWS near cell edges (Fig. 10.2(d)), consistent with the findings from other cell adhesion-based assays that show accumulation of cytoskeletal and focal adhesion-associated proteins at the cell boundary during cell adhesion events [11,30,31]. This PCEM approach also allowed label-free imaging of chemotactic migration of mHAT9a cells to agarose beads soaked in SDF1-α, a chemokine that binds CXCR4 receptors expressed on mHAT9a cells, as well as their detachment profiles in response to the administration of staurosporine, which caused apoptosis-induced detachment of cells.

More recently, Chen et al. showed the use of planar 1-D PC slabs as the sensing surface for PCEM [28]. Although the use of 1-D PC slabs with a grating surface topology allows highly sensitive noninvasive optical imaging of live cells, the structured topography introduces an additional variable that biases cell adhesion in the direction of the PC grating lines. To overcome this, Chen et al. filled $TiO_2$-coated PC slabs with SU-8

epoxy by a horizontal dipping process to planarize the PC surface. When 3T3 fibroblasts were imaged on fibronectin-coated planar PCs, their attachment profiles were similar to those from nonplanar counterparts, with comparable subcellular resolution.

As these publications highlight, progress in the development of PCEM imaging instrumentation and PC surfaces have enabled label-free imaging of adherent types of cells of varying size and morphology from primary and commercially available sources to spatially and temporally resolve cell–surface interactions. Such "adhesive phenotyping" of single cells using PCEM provides adhesion-associated data in real time that may be useful for basic biological research to uncover poorly understood biological mechanisms as well as for designing novel biosensing applications from identification and characterization of individual cells to high-throughput screening of cells to evaluate the efficacy of drugs.

## 10.4 PCEM imaging of stem cell differentiation

With PCEM established as an optical platform capable of noninvasively imaging single cell attachment and adhesion events at the cell–material interface [16,17,21–24], it is now possible to examine how such information may be used as a characterization tool. PCEM provides fast data acquisition and processing time (on the order of several seconds between images) and high spatial resolution (submicron) that enable quantitative, real-time imaging of single cells. These advances make it possible to examine dense adhesion-associated data gathered via PCEM, with the eventual goal of linking changes in cell mass distribution with adhesion strength and cell motility to dynamically trace cell attachment and adhesion kinetics [32].

Ongoing efforts are employing PCEM to establish adhesion phenotypes of single stem cells at discrete stages of differentiation and assess how they relate to their functional capacity. As a first step, we are imaging model stem cell–dental epithelial stem cells (mHAT cell line), IL-3 dependent myeloid progenitor cells (32D cell line), and porcine adipose-derived stem cells, whose differentiation protocols are well-established [20,33,34]. Here, differentiation is induced by replacing cell growth media with the defined induction media at a specified time point, providing the opportunity to use PCEM to resolve changes in cell adhesion in and around differentiation events.

PCEM allows time-lapse imaging of stem cells over the course of differentiation, which typically takes up to several weeks to complete. Preliminary findings suggest that stem cells exhibit dynamic changes in their adhesive properties during differentiation, and that PCEM can effectively image and quantify these changes to generate a large amount of adhesion-associated data. Image analysis software facilitates the processing of PCEM-generated data to extract adhesion-related parameters in spatiotemporal dimensions of interest, including cell contact/spread area, mass density/redistribution, migration speed, and migration distance. Initial analyses of adhesion and motility parameters of stem cell differentiation indicate that adhesion strength and motility of individual stem cells go through significant cell-type specific changes during the course of differentiation, suggesting that adhesion phenotyping of stem cells may provide functional metrics that would enable in situ screening of single stem

cells in a high-throughput manner in vitro as well as enhance our understanding of the physiological regulation mechanisms of stem cells [32].

## 10.5 Conclusions and future outlook

In this chapter, we describe recent advances in noninvasive optical imaging of stem cells using photonic crystal surfaces. In particular, we detailed a type of optical imaging platform termed photonic crystal enhanced microscopy (PCEM). PCEM enables dynamic monitoring of heterogeneous stem cell populations in situ in real time at the single cell level with subcellular spatial resolution, which is difficult to achieve with currently available analytical tools and techniques (e.g., fluorescence labeling, clonal expansion assays), as they are almost always end-point ensemble analyses. PCEM employs photonic crystals as the biosensing element, whose surface properties can be easily modified to facilitate cell–surface interactions. PCEM also has the potential to be integrated into existing bioengineering platforms such as microfluidics or microarrays to provide a novel, alternative approach to monitor single stem cell activity in vitro. As PCEM noninvasively images interactions at the cell–surface interface, it is highly suitable for the characterization of cell adhesion activities as a tool for "adhesion phenotyping," a comprehensive profiling of cell attachment and adhesion-associated events. Ongoing efforts are imaging stem cells during the course of differentiation to specific lineages using several cell lines and primary cells. Here, analyses of adhesion and motility parameters suggest adhesion phenotyping during stem cell differentiation may provide important new functional metrics to enable in situ screening of single stem cells in a high-throughput manner. Such technologies would provide important new insight regarding our understanding of the physiological regulation mechanisms of stem cells.

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