

Biosensors in Tissue and Organ Fabrication

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What You Will Learn in This Chapter

We begin this chapter by looking at the role of sensors during tissue and organ fabrication. We provide a working model for sensor technology to support different parts of the tissue fabrication pathway. We next look at biological sensors in nature, with IGF-1 signaling as an example and study the intracellular signaling events which take place in response to IGF-1 signaling. Next, we look at the design requirements for sensors to support the culture and fabrication of 3D artificial tissue. The next three sections are dedicated to three specific sensing mechanisms, which include acoustic sensors, magnetic sensors and optical sensors. For each of the three sensor mechanism, we provide an overview of the theory, along with some applications to illustrate the principles of operation. We then look at flexible sensors and how they have been used for various applications and the potential role of flexible sensors in tissue engineering. We conclude this chapter by providing a case study from the Artificial Heart Laboratory; the case study is focused on the development of novel sensors to record the EKG properties of 3D artificial heart muscle.

Learning Objectives

After completing this chapter, students should be able to:

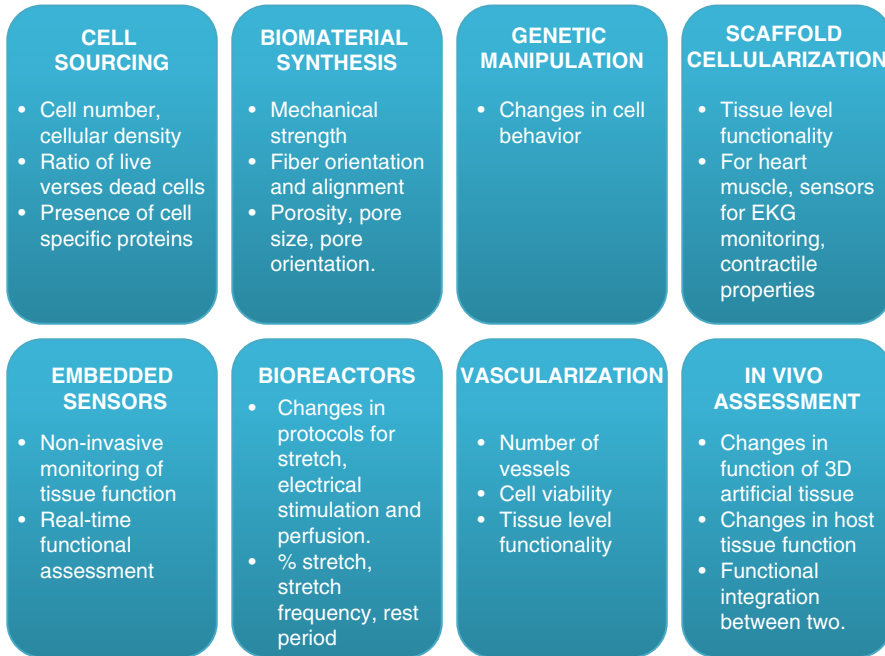
1. Describe the need for sensors at every stage of the tissue fabrication pathway.
2. Explain sensors in biological systems.
3. Describe design requirements for sensors in tissue and organ fabrication.
4. Discuss the use of magnetic biosensors for biological systems.
5. Discuss the use of acoustic sensors for biological systems.
6. Describe the principles of ellipsometry and potential applications as biological sensors.
7. Explain the concept of flexible sensors as applied to biological systems.
8. Discuss the development of sensors to monitor the EKG properties of 3D artificial cardiovascular tissue constructs.

Important Concepts Discussed in This Chapter

- *Sensors and Tissue Engineering*—sensor technology is critical for every stage of the tissue fabrication process.
- *Sensor Platforms*—biological sensors have been developed using many different platforms, some of which include magnetic fields, acoustic waves and optical properties of light.
- *Flexible Sensors*—biological systems are not rigid, but rather flexible, and a new generation of flexible sensors is being developed to match the properties of biological systems.

2.1 Sensors and Tissue Engineering

Sensors are critical during the tissue fabrication process. At every stage of the process, it is critical to measure changes in cell and tissue properties and based on these measurements, adjust the tissue fabrication protocol. Sensors are one of the critical supporting technologies for tissue fabrication; yet, sensor technology is one of the least developed areas in tissue engineering. It is difficult to separate sensors from tissue engineering, as the two are closely molded together. ■ Figure 2.1 illustrates the close relationship between the tissue fabrication pathway and sensor technology; as can be seen from the figure, sensors are required to support every step of tissue fabrication process. It is important to monitor all



■ **Fig. 2.1** Sensors and tissue engineering. At every stage of the tissue fabrication pathway, sensors are needed to monitor changes in cellular and/or tissue function. The *figure* illustrates a few examples of some of the variables that need to be recorded at every stage of the tissue fabrication pathway

aspects of the 3D tissue and organ fabrication process; novel sensors and sensing mechanisms are necessary to support these efforts. Let us look at examples of test variables that need to be monitored.

Sensors to Monitor Culture Environment—This is not explicitly illustrated in ■ Fig. 2.1, but the cell culture environment is an important determinant of tissue/organ function. Temperature, media pH, humidity, oxygen saturation and glucose concentration are just a few examples of variables that affect 3D tissue formation and function. These variables need to be monitored and adjusted during the tissue fabrication process.

Sensors to Monitor Cell Behavior—During the tissue fabrication process, cell isolation, culture and proliferation are critical; as such it is important to monitor cell number, viability, density and live/dead cells. At a more basic level, cellular function needs to be recorded and monitored. As an example, cardiac myocytes undergo changes in intracellular calcium transients, which in turn affect contractile properties; the ability to accurately measure changes in intracellular calcium transient is critical during heart muscle tissue engineering.

Sensors to Monitor Biomaterial Properties—Biomaterial properties need to be monitored during tissue fabrication. Macroscopic properties like tensile strength are important determinants of tissue function and can change during the tissue fabrication process; loss of mechanical integrity during tissue fabrication will result in catastrophic effects for 3D artificial tissue. It is therefore important to monitor and adjust such changes in material properties. At a more basic level, changes in fiber orientation and alignment impact cell-matrix interactions and also affect 3D tissue function; sensors are needed to record these

changes in fiber orientation and alignment. Another important property of biomaterials is the presence of functional binding sites for cell surface integrins; specific cell–matrix interactions are known to mediate and support cellular function. Biological sensors are required to monitor changes in cell–matrix interactions as a function of tissue growth and development.

Sensors for Genetic Manipulation—The purpose of any genetic engineering protocol is to alter cellular properties to increase functional performance of 3D artificial tissue. Hence, the success of such protocols will be assessed by measuring changes in cell behavior, as described in the previous section.

Sensors for Scaffold Cellularization—Coupling cells with biomaterials to fabricate 3D artificial tissue is at the heart of functional tissue engineering. The objectives of these studies are to design cellularization strategies that maximize tissue function; therefore, it is critical to monitor tissue function and adjust cellularization strategies to maximize tissue and organ functionality. The variables that need to be monitored will depend on the tissue under investigation. In the case of 3D artificial heart muscle, electrical and contractile properties are important determinants of heart muscle function.

Sensors to Monitor Bioreactor Stimulation—Bioreactors are used to guide 3D tissue fabrication and are designed to replicate in vivo physiological conditions during in vitro culture. In the case of 3D heart muscle, bioreactors are used for controlled electrical stimulation, mechanical stretch and continuous media perfusion. In the case of mechanical stretch, there are many parameters that can be varied and have an impact on 3D tissue function, ie % stretch, stretch frequency. Sensors are required to accurately measure these variables.

Sensors for Vascularization—Vascularization strategies are designed to support blood vessel growth within 3D artificial tissue. The most important variable that needs to be monitored is the change in the blood vessel count as a function of time. Novel sensing mechanisms need to be developed to accomplish this.


Sensors for In Vivo Assessment—Once 3D artificial tissue is implanted for in vivo assessment, there are rapid changes in the function and architecture of the implanted tissue and these changes can have a significant impact on in vivo performance. In the case of 3D artificial heart muscle, the bioengineered tissue is sutured on the surface of infarcted hearts to evaluate in vivo efficacy. Changes in the function and architecture of the implanted tissue and changes in host tissue recovery and rescue are important variables that need to be monitored as a function of time. Novel sensors need to be developed to achieve this.


The field of tissue engineering is in its infancy; sensor technology to support the tissue and organ fabrication process is at an even earlier stage of development. Significant and rapid changes in sensor technology are needed to support growth in the field and represent a high priority area of research.

2.2 Overview of Sensor Technology

Current State of the Art—During tissue and organ fabrication, it is important to monitor and record changes in tissue function. *How is this accomplished based on the current state of the art?* During the tissue fabrication process, isolated cells and later, 3D tissue are maintained in culture for several days and often times, for weeks. During this culture period, individual tissue culture plates are removed from the controlled culture

environment at intermittent time points. While the time period for functional assessment varies, a common strategy is to record functional properties every 2–3 days. Once the tissue culture plates are removed, the 3D tissue constructs are attached to external sensors for functional recording. The functional properties are then recorded for a specific time period, say for 1–2 min. During functional assessment, the 3D artificial tissue is exposed to the external environment and is physically handled during functional assessment. As a result, the properties of the 3D tissue are altered during functional assessment. After functional assessment, the 3D tissue is sacrificed. This process is repeated at several time points during the tissue culture process. Let's look at one example. During the fabrication of 3D artificial heart muscle, researchers are often interested in measuring changes in the contractile properties as a function culture time. In order to measure contractile properties, the tissue culture plate is first removed from the cell culture incubator. One end of the 3D heart muscle tissue is then connected to an external force transducer. Changes in contractile properties are then recorded in response to electrical stimulation or in response to changes in calcium or some other chemical compound. There are several limitations of this method. First, attachment of 3D artificial heart muscle to the external force transducers requires physical handling of the 3D tissue and this process inherently changes tissue properties. Second, data collection is only for a short period of time and provides information about 3D artificial heart muscle at a single time point; such data is limited and cannot be used to make any conclusions about the contractile properties as a function of time. Third and less obvious, is the limitation of the current generation of force transducers, which do not have the high level of sensitivity required to measure small changes in tissue functionality. Fourth, any data obtained for the contractile properties of 3D artificial heart muscle cannot be used to change the culture environment in a positive feedback loop that regulates the culture environment. These limitations point to the need for a new generation of sensors that are better equipped to record changes in the functional performance of 3D artificial tissue and provide data that can be used to regulate the tissue and organ fabrication process.

Design Requirements for Sensors—Based on our previous discussion, it should be readily apparent that the current state of the art in sensor technology does not adequately meet the requirements of the tissue and organ fabrication process. *So what needs to be done in order to address this technological gap?* In  Fig. 2.2, we provide an overview of sensor technology as applied to the tissue and organ fabrication process. The figure illustrates sensor technology as applied to 3D artificial heart muscle; this example is designed to illustrate the integration of sensor technology with the fabrication of 3D artificial heart muscle. However, these principles can be applied to any tissue and organ fabrication scenario. So what are the most important variables that need to be monitored during the fabrication and culture of 3D artificial heart muscle? While there are many variables that need to be monitored, the phenotype of the artificial heart muscle can be assessed based on the electrophysiological parameters, which refer to changes in the electrical activity of isolated cells and/or 3D artificial tissue.

There are three important design requirements which must be satisfied for any sensors that are used to monitor changes in the electrical activity of 3D artificial tissue ( Fig. 2.2): (1) non-invasive, real-time functional assessment, (2) wireless data transfer and wireless power transmission and finally (3) functional recording to be used in a positive feedback loop to modulate culture environment. Let us look at these three design requirements a bit more.

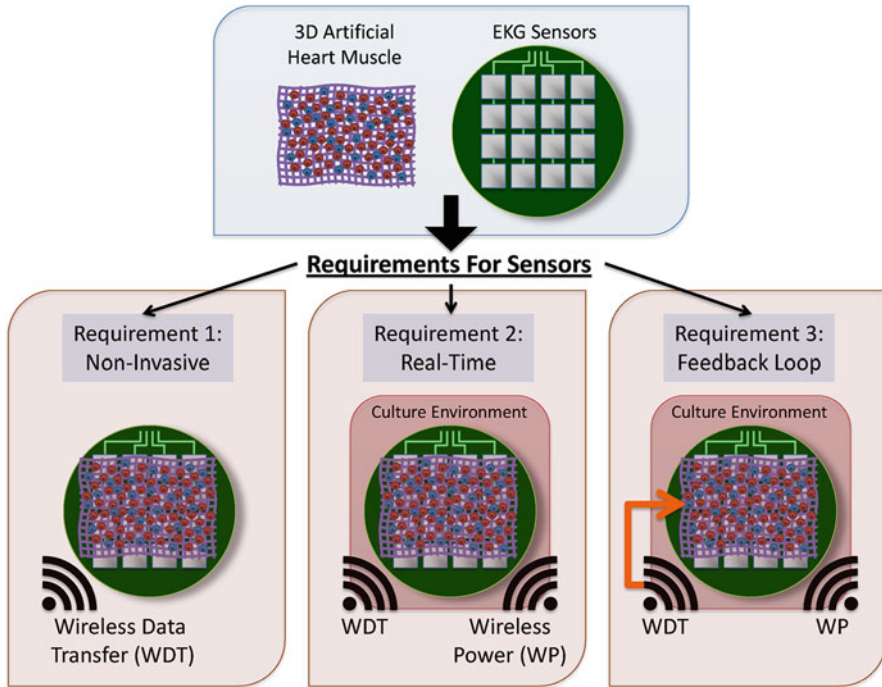


Fig. 2.2 EKG sensors for 3D artificial heart muscle. There are three requirements for EKG sensors: (1) sensors have to be in contact with the 3D heart muscle using non-invasive methods to monitor and record electrical activity. (2) The EKG properties have to be monitored in real-time during the entire culture period. (3) The EKG properties have to be used in a positive feedback loop to regulate electrical stimulation parameters for 3D artificial heart muscle

Non-invasive Real-Time Functional Assessment—In order to monitor electrical properties, stainless steel electrodes are engineered onto the surface of the tissue culture plates. Once isolated cardiac cells are plated on the culture surface, any changes in the electrical activity will be monitored using the surface electrodes. Monitoring of EKG properties will be non-invasive, non-contact and in real-time; this means that the cells and/or 3D artificial tissue, will not be physically handled in any way. Furthermore, real-time assessment of electrical properties will take place during the entire culture period. This is a significant advantage over traditional intermittent EKG measurements as changes in electrical properties can be recorded during the entire tissue fabrication process.

Wireless Technology—It is important to incorporate direction wireless communication with the EKG sensors. Functional data needs to be recovered from the 3D tissue and transferred to a computer for analysis and data processing. At the same time, it is important to have the ability to change electrical stimulation protocols based on the physiological state of the 3D artificial tissue. The ability to have bi-directional communication with the EKG sensors provides an efficient mode for data transfer. Furthermore, it is important to use wireless power in order to drive the EKG sensors and supporting instrumentation. Wireless power is new and is being developed for many commercial applications and is at a stage in development where it is feasible to incorporate with sensors.

Feedback Control—The last component of the EKG sensor system is the development of a positive feedback loop. This means that EKG values that are obtained from 3D artificial

heart muscle are used to program and/or reprogram controlled electrical stimulation protocols. These electrical stimulation protocols are generally fixed and only changed by user intervention and these changes occur infrequently. However, the ability to engineer a positive feedback control loop makes use of real-time electrical properties of 3D artificial heart muscle to regulate the electrical stimulation protocol. Let's look at one simple example of how this is beneficial. During the fabrication of 3D artificial heart muscle, there can be instances where the artificial tissue enters into a state of arrhythmia; this leads to an uncoordinated series of contractions. These changes in electrical activity will be recorded by the EKG monitors in real-time and can be used to relay an increase in electrical stimulation voltage; in essence, the high voltage electrical stimulation protocol will serve as a defibrillator, re-establishing baseline electrical properties.

2.3 Biological Sensors

Biological Sensors in Nature—There are hundreds, if not thousands, examples of biological sensors in nature required to maintain normal mammalian function and homeostasis. Stretch activated receptors (SARs) are abundant in cardiac myocytes and respond to external stretch; in response to stretch, SARs respond by initiating a complex cascade of signaling events that lead to an increase in contractile proteins. Growth factors, hormones and cytokines bind to specific receptors and again, this process triggers intracellular signaling cascades that alter cell function. SARs and growth factor receptors are examples of biological sensors that respond to changes in the local environment by altering cell behavior. In this section, we look at one specific example of biological sensors by studying the insulin-like growth factor 1 (IGF-1) signaling pathway.

IGF-1 Structure and Function—IGF-1 is a protein which plays an important role in the regulation of many cellular functions, including cell proliferation, metabolism, migration, cell survival and suppression of apoptosis [1, 2]. IGF-1 is a small peptide which consists of 70 amino acids that are organized to form a hydrophobic core with three disulfide bonds that serve to stabilize the 3D structure of the protein molecule [1, 2]. IGF-1 is part of a larger family of proteins, which also include insulin, insulin-like growth factor 2 (IGF-2), IGF-binding proteins (IGFBPs) and the IGF-1 receptor (IGF-1R). IGF-1 is predominantly produced in the liver in response to stimulation by growth hormone (GH) and is released into the circulatory system and acts on target cells via endocrine, autocrine and paracrine signaling. The majority of IGF-1 is found in the circulation bound to IGFBPs, which regulates the concentration of the protein molecule in circulation and extends the half-life. IGFBPs also regulate the tissue distribution of IGF-1 at the cellular level by providing a mechanism for controlled release within target tissues. IGF-1 exhibits its cellular response by binding to the IGF-1R, which is described in the next section.

IGF-1R Structure and Function—IGF-1R is a cell surface receptor that is located on the plasma membrane, with an extracellular ligand binding domain and an intracellular activation domain [3–5]. IGF-1 exhibits its cellular response by binding to the IGF-1R. IGF-1, IGF-2 and insulin have similar 3D structures and therefore, can all bind to the IGF-1R; however, IGF-1R has the highest binding affinity for IGF-1. The IGF-1R is a large protein of approximately 400 kDa consisting of two α -subunits, which are located in the extracellular space and two β -subunits found within the intracellular space. There are several disulfide bridges that provide structural stability for the complex IGF-1R molecule. There is a single disulfide bridge between the two α -subunits and two disulfide bridges between

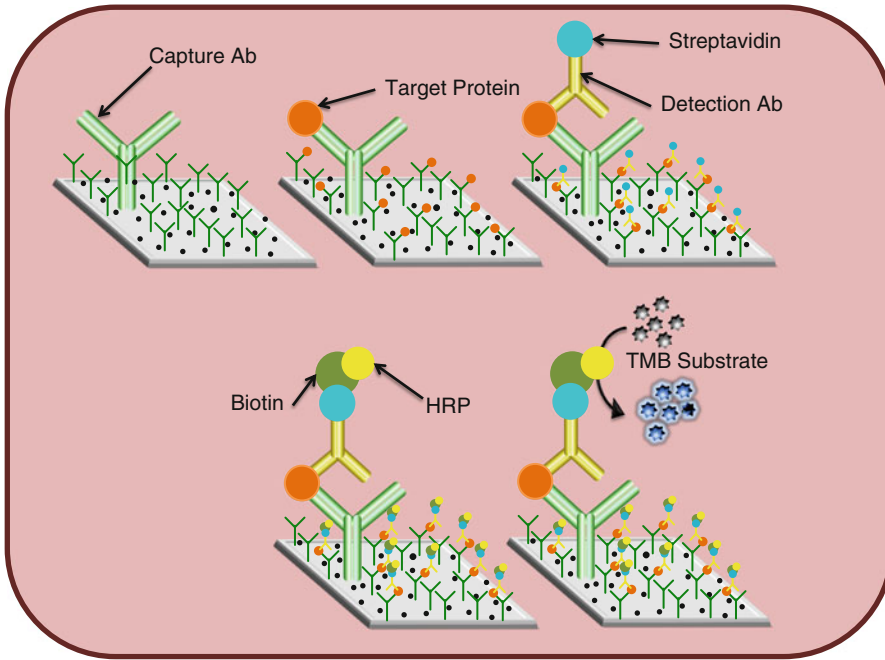
the α - and β -subunits. The extracellular α subunits are the active sites for ligand binding while the intracellular β -subunits contain tyrosine kinase catalytic domains that are activated upon ligand binding. Activation of the intracellular domains of the IGF-1R molecule initiates a complex signaling pathway which regulates cellular function, as described in the next section.

IGF-1 Signaling—Binding of IGF-1 to IGF-1R triggers a complex series of events that affects many cellular functions including growth, differentiation, metabolism and apoptosis [6–20]. Binding of IGF-1 to the extracellular components of the IGF-1R result in activation of the intracellular kinase domains located in the β -subunits of the receptors. Ligand activated kinase activity is responsible for triggering intracellular signaling events; this process requires participation of several proteins along the signal transduction pathway. SHC and insulin receptor substrates (IRSs) are proteins that act as intermediary factors between ligand activated kinase and intracellular signaling cascades. These intermediate proteins are supported by many other proteins, including Grb2, GTP-binding protein RAS, Sos, c-Raf, MEK, ERK1 and ERK2. IGF-1 binding to IGF-1R is known to have an effect on cell proliferation resulting from a prolonged transition through all phases of the cell cycle. IGF-1 signaling acts to regulate cellular apoptosis serving as an anti-apoptotic agent; the signal transduction pathway involved in the anti-apoptotic function of IGF-1 involves the P13K-Akt pathway. In cardiac myocytes, IGF-1 signaling has shown to initiate a complex sequence of signaling events which leads to an increase in intracellular calcium, which in turn increases mitochondrial respiration and metabolism.

Sensors and Tissue Engineering—How does all of this apply to the tissue and organ fabrication pathway? There is one important message to take away from this section—biological sensors respond to changes in the local environment by mediating a graded change in cellular function. IGF-1R not only binds to IGF-1, but also triggers an intracellular signaling pathway leading to an increase in cellular proliferation, decrease in cell apoptosis and in the case of cardiac myocytes, an increase in intracellular calcium. *This is exactly what we need sensors to do during the tissue and organ fabrication process*—the ability to measure and record changes in the local culture environment and trigger events that modulate functional and biological properties of bioengineered tissue constructs.

2.4 Magnetic Sensors in Biological Systems

Introduction—There is a new and evolving area of research that makes use of magnetic fields as sensors for detection in biological systems [21]. Magnetic biosensors in biological applications make use of a property known as giant magnetoresistance (GMR) [22–32], which refers to a significant change in electrical resistance based on the applied magnetic field; a non-magnetic material like copper is placed between two layers of a magnetic material like cobalt. The Nobel Prize in Physics for 2007 was awarded to Dr. Albert Fert and Dr. Peter Grunberg who independently discovered the phenomena of GMR in 1988. Magnetic techniques make use of magnetic nanotags (MNTs) [33, 34], which allow specific molecular recognition for protein moieties; MNTs can be compared to fluorescent probes that are commonly used for protein detection. Fluorescent based sensors in biological applications are very well established and used extensively and therefore, we begin our discussion with a brief overview of fluorescent based techniques. We then present an overview of magnetic based methods, discuss advantages of these techniques and end with specific applications of MNTs for biosensing.

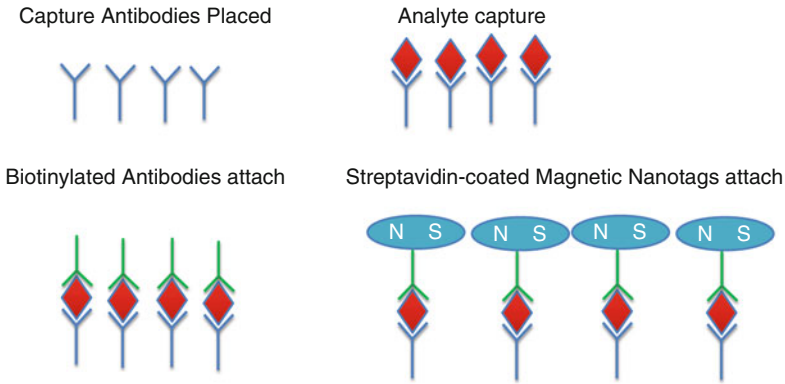


■ **Fig. 2.3** Fluorescent based ELISA. Primary antibody binds to protein of interest, followed by a fluorescently tagged secondary antibody

Fluorescent Based Biosensors—The objective of fluorescent based techniques is the detection and quantification of specific biomarkers in a given sample, which in most cases, is a specific protein. One of the most common methods to quantify the amount of protein present in a given sample is by ELISA—enzyme-linked immunosorbent assay (■ Fig. 2.3). ELISA methods are based on the binding of a primary antibody to the protein of interest; a fluorescently tagged secondary antibody then binds to the primary antibody [35–44]. The fluorescent tag is used to determine the amount of secondary antibody bound to the primary antibody, which in turn reflects the amount of protein of interest in the sample. Therefore, using this method, the fluorescent intensity of the tag on the secondary antibody is used to quantify the amount of protein that is present in the original sample. The fluorescent intensity of the tag is compared with values from a previously determined standard curve correlating the amount of protein with fluorescent intensity.

Magnetic Based Biosensors—The principle of GMR sensors is very similar to ELISA, where the fluorescent tag is substituted with a magnetic probe (■ Fig. 2.4). There are variations to the technique; however, in one configuration, the primary antibody binds to the protein of interest, followed by a secondary antibody that is labelled with a MNT. In an alternative configuration, the magnetic probe can be attached to the primary antibody that has specificity towards the protein of interest. In either case, the magnetic probe replaces the fluorescent tag in ELISA's and is directly related to the amount of protein that is present in the sample. The overall principle of magnetic based biosensors is very similar to fluorescent based techniques, with a magnetic tag being used in place of a fluorescent tag.

Advantages of Magnetic Biosensors—When compared to fluorescent based methods, magnetic biosensors have four advantages. First, magnetic probes are more stable over



■ **Fig. 2.4** Magnetic based biological sensors. Primary antibody binds to protein of interest, followed by a magnetically tagged secondary antibody

time in culture and can be used for long term labeling assays. Fluorescent tags are chemical compounds that can lose integrity as a function of time; this is not the case with MNTs. This property can prove advantageous for long term labeling assays during tissue and organ fabrication. Second, magnetic materials do not lead to background noise effects, which do occur with fluorescently labelled samples. Background fluorescence is a common phenomenon in biological samples and is due to the inherent properties of the tissue. Third, application of controlled magnetic fields on the external surface provides a mechanism for remotely measuring and regulating the biological environment. And finally, the sensitivity of magnetic assays has been shown to be greater than fluorescent assays, as we will see in the examples that we present next. The high sensitivity allows detection at significantly lower protein concentrations, compared with fluorescent based techniques.

Examples of Magnetic Biosensors—There are several examples in the literature that have adopted magnetic biosensors [34, 45–51]. In one such example, the system was used for the detection of several proteins, including tumor necrosis factor- α (TNF- α), interleukin-1 (IF-1) and human chorionic gonadotropin (hCG) [34]. Using hCG, it was demonstrated that magnetic biosensors could measure 10 femtomolar concentrations of hCG, compared with 4 pM for commercially available ELISA kits [34]. In a second example, magnetic biosensors were used for DNA detection in the range of 16 pg/ μ l to 10 ng/ μ l and in all cases (except at the highest concentration tested), the sensitivity of the magnetic biosensors was shown to be greater than fluorescent probes [50]. In a third example, magnetic biosensors were used to develop a Bead Array Counter (BARC), with potential applications in cell sorting and particle counting [49]. The BARC system was shown to have a very high sensitivity and was able to detect 10 micro-beads, each with a diameter of 2.8 μ m [49]. Most of these examples served a proof of concept studies to illustrate the feasibility of MNTs as biosensors and illustrate their relative advantages when compared with fluorescent methods, particularly the increase in sensitivity.

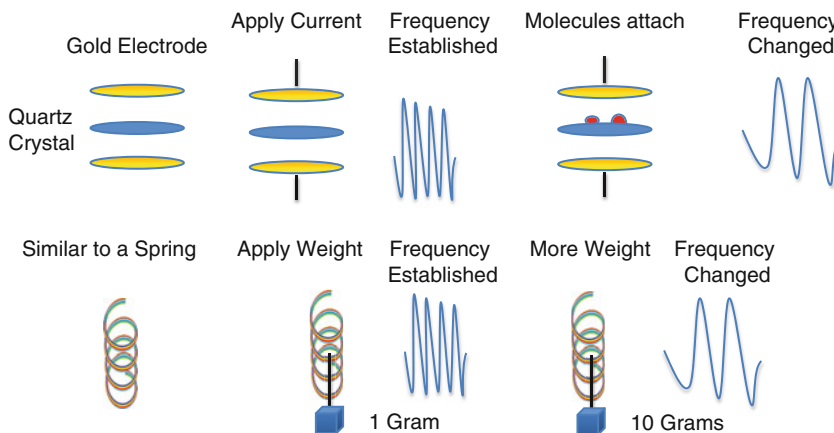
Magnetic Biosensors Applied to Tissue Engineering—MNTs can provide a very powerful tool to support tissue and organ fabrication. The ability to label individual cell types using specific MNTs provides two significant advantages. First, an external magnetic field can be used to regulate the spatial positioning of different cell types, thereby supporting 3D organization of complex mammalian tissue. Using this method, different cell types can be delivered to different regions of complex 3D tissue and organs. Second, the long term

stability of MNTs provides a mechanism to monitor the growth and development of 3D tissue as a function of time; since many of these processes require weeks in culture, MNTs provide an excellent tool to monitor changes in 3D architecture as a function of time.

2.5 Acoustic Sensors in Biological Systems

Introduction—Acoustic sensors have gained widespread applicability for applications in biological systems. These sensors are based on changes in acoustic wave properties in response to changes in metabolite concentration and/or changes in cellular properties (■ Fig 2.5). We begin this section with an overview of the principle of acoustic sensors. We then provide a few examples of acoustic sensors being used to measure glucose concentration and cell proliferation. We end with a discussion of the potential applications of acoustic sensors to support the tissue and organ fabrication process.

Principle of Acoustic Sensors—One of the commonly used types of acoustic sensors is known as quartz crystal microbalance, with dissipation (QCM-D) [52–57]. At the center of QCM-D technology is a sensor that is fabricated using quartz that has been sandwiched between two gold electrodes. The most important property of quartz related to application in biosensors is its piezoelectric properties; this refers to the propagation of acoustic waves in response to an applied electric field. When an electric field is applied to the quartz crystal, the material oscillates and this results in the propagation of acoustic waves. Under baseline conditions, the quartz sensor is subjected to a controlled oscillating potential difference that is applied to the two gold electrodes. In response to this, the quartz sensor oscillates and generates acoustic waves that have specific properties which can be characterized by frequency and amplitude. Under baseline conditions, the relationship between applied electric field and acoustic wave properties is well defined and characterized. At the heart of the QCM-D sensor is the change in the properties of acoustic waves in response



■ **Fig. 2.5** Acoustic sensors for biological systems. Baseline operation of sensor—quartz crystal is positioned between two gold electrodes. An oscillating potential difference is applied, resulting in oscillations of the crystal. Change in surface mass—any change in the mass on the surface of the sensors results in a decrease in the frequency of the acoustic wave. Change in viscoelastic properties—as the material changes from a rigid material to one which is soft and/or possess viscoelastic properties, there is dampening of the acoustic wave

to changes at the surface of the quartz sensor. Cells, proteins and other molecules like glucose can be deposited on the surface of the sensor. In response to changes in the surface properties of the QCM-D sensor, there are measurable changes in acoustic wave properties. There are two important properties of the output acoustic wave that provide information about the cells, proteins or other molecules that are deposited on the surface of the sensor.

The first is the change in the frequency of the acoustic wave which is related to changes in mass of the material deposited on the sensor. This relationship is characterized by Sauerbrey's equation. Sauerbrey's equation states that the change in mass on the surface of a quartz crystal is directly related to the change in oscillating frequency of the crystal. Any increase in mass on the surface of the quartz sensor results in a decrease in the oscillating frequency of the corresponding acoustic wave.

The second property relates to dissipation or dampening of the acoustic wave and is related to changes in the viscoelastic properties of the surface material. As the properties of the surface material change from a rigid configuration towards soft and viscoelastic configurations, there is dampening of the corresponding acoustic wave.

This way, recording and measuring changes in the frequency and amplitude of the acoustic waves can be used to provide information about the properties of the material deposited on the QCM-D sensor.

Applications of QCM-D Sensors—There have been several examples demonstrating the feasibility of QCM-D sensors for the detection of macromolecules like glucose and also to study cellular attachment and spreading on substrates [58–74]. In one example, QCM-D sensors were used for the detection of glucose [74]. The method made use of competitive binding of glucose and dextran on concanavalin A (ConA) binding sites on the surface of grapheme coated QCM-D sensors. Dextran molecules were assembled on the surface of the modified QCM-D sensors and ConA molecules were then immobilized on the dextran. Varying concentrations of glucose were added to the detection chamber, which competed with dextran for ConA binding sites. Glucose has a higher affinity for the binding sites compared with dextran and this resulted in displacement of the dextran from the surface of the QCM-D sensor; this was accompanied by a decrease in acoustic wave frequency. It was demonstrated that an increase in glucose concentration in the range of 0.01–7.5 mM was related to a linear decrease in acoustic wave frequency. As the glucose concentration in human serum is in the range 3.3–6.1 mM, this mechanism demonstrates the feasibility of QCM-D sensors for clinical work relating to glucose monitoring. Furthermore, QCM-M sensors were shown to exhibit high sensitivity in the lower concentration ranges, 0.01–0.1 mM, making these sensors suitable for high sensitivity assays in cell culture studies.

In another example, QCM-D sensors were used to study cell adhesion and proliferation on substrate surfaces [68]. Three different cell types were tested and included MDCK-1 (Madin-Darby Canine-Kidney), MDCK-11 and 3 T3 fibroblast cells. In all cases, the cells were cultured on the surface of the quartz crystal and changes in acoustic wave frequency monitored over time. It was demonstrated that during initial phases of cell proliferation, there was a linear relationship between cell number and frequency; an increase in cell number was shown to correlate with a decrease in acoustic wave frequency. This relationship plateaued as the cells approached confluency, meaning that small increases in cell number did not result in significant changes in the acoustic wave frequency. The linear portion of the curve was dependent on cell type and was in the range

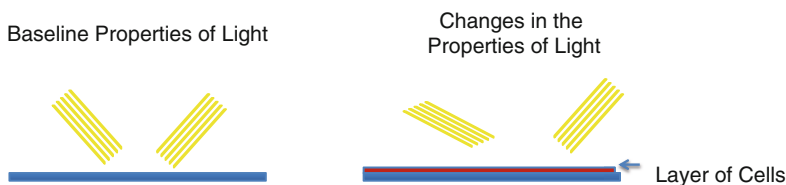
of 0–200,000 and 0–500,000. Using this strategy, QCM-D sensors could be used to characterize cell proliferation as a function of time, particularly in low cell density range.

QCM-D Sensors in Tissue Engineering—The ability to monitor changes in metabolite concentration of glucose, along with the ability to study cell adhesion and proliferation provides an excellent tool for tissue and organ fabrication studies. One mechanism to develop this is to develop compact sensors that are a couple hundred microns in diameter. Such sensors can be embedded within 3D tissue and organ systems and be used to provide real-time non-invasive information about changes in the metabolite (e.g. Glucose) concentration, along with changes in cellular properties like cell number. Such systems have the potential to provide valuable information about the tissue and organ fabrication process and regulate input variables to maximize functional performance.

2.6 Optical Sensors in Biological Systems

Introduction—The properties of light have been used extensively in the development of optical biological sensors [75–91]. The incident wave of polarized light can be reflected, absorbed or transmitted from a solid surface. This way, the properties of light waves are changed based on the properties of the material and these changes are dependent on material properties (■ Fig. 2.6). By analyzing the properties of light waves, one can obtain information about surface properties; the changes in the properties of light waves after interaction with material surfaces provide data about the surface and/or bulk properties of the material. This is the general principle of optical sensors, with ellipsometry being one such example. In this section, we will provide an introduction to the principles of ellipsometry and its use in biosensors for antibody detection and for screening of viruses.

Introduction to Ellipsometry—How exactly does ellipsometry work? Ellipsometry is based on the principle that the properties of polarized light change after being reflected off the surface of any material [92, 93]. The changes in the properties of polarized light are measured by ψ and Δ , where ψ is a measure of the differential change in amplitude and Δ is the differential change in phase [92, 93]. Based on the relative changes in phase (Δ) and amplitude (ψ), surface properties can be obtained [92, 93]. Changes in Δ and ψ are measured in terms of a ratio, known as the complex reflectance ratio, which is based on changes in the vectors of reflected light. The polarization state of incident and reflected light is defined in terms of two component vectors denoted R_s and R_p prior to contact with the surface and r_s and r_p after reflection from the surface. The reflectance ratio is defined



■ **Fig. 2.6** Ellipsometry based sensors for biological systems. (a) Ellipsometry is based on the relationship between the incident and reflected properties of polarized light after contact with the test surface. (b) An increase in thickness of the test surface results in changes in the properties of reflected light. (c) Coating of a culture surface with specific adhesion proteins, followed by attachment of cells, results in changes in thickness of the culture surface, which can be measured using ellipsometry

as r_p/r_s and is an important parameter used in ellipsometry [92, 93]. The ratio r_p/r_s is determined from two measured variables, Δ and ψ using the following relationship: $r_p/r_s = \tan \psi \cdot e^{i\Delta}$. As the surface properties change, the properties of the reflected light change and the ratio r_p/r_s also changes.

What Specific Information Can Be Obtained by Ellipsometry? The most important piece of information that can be obtained by ellipsometry is the thickness of the surface layer. Any changes in surface thickness will be correlated to changes in the properties of the reflected light and computed using the ratio r_p/r_s . There are several cases during the tissue fabrication process where this technique can prove to be valuable. As one example, in the case of biological applications, the culture surface is often coated with adhesion proteins and cells attach to these adhesion properties via specific integrin mediated cell–matrix interactions. At every stage of this process, there are measurable changes in the surface thickness, which can be accurately deducted using ellipsometry. As the cells attach to specific proteins, the changes in the surface thickness can be measured using ellipsometry. A more direct application of ellipsometry during tissue engineering is the calculation of the thickness of thin films, which are often deposited on the culture surface to support cellular adhesion and proliferation. The thickness of the films is an important determinant of cell adhesion and function, which can accurately be measured using optical techniques like ellipsometry.

What Are Some of the Advantages of Ellipsometry? Ellipsometry does not require any special sample preparation and can be applied to a broad range of specimens. Optical techniques like ellipsometry are label-free, which means that no labeling step is required as a part of the experiment. The actual experiment is quick, provides real-time data and is non-destructive and non-contact; this means that the experiment does not require any contact with the specimen, a significant advantage of ellipsometry.

How Can Ellipsometry Be Used to Support Tissue Engineering and Tissue/Organ Fabrication? Biomaterial synthesis is a critical component of the organ and tissue fabrication process. Material properties affect cellular adhesion, which in turn have a significant impact on tissue and organ function. Thin films are used extensively in tissue engineering to support cellular adhesion; changes in the thickness of these films have an impact on cell properties and function. The ability to accurately control and measure the thickness of thin films is important in tissue engineering. Ellipsometry provides a powerful tool to accurately measure the thickness of thin films in tissue engineering.

Applications of Ellipsometry in Biosensors—There have been several examples in the literature that make use of ellipsometry in biological applications for use in the detection of antibodies, the detection of biomarkers for specific diseases and for the detection of viruses [94–108]; let us look at one example of each application.

Ellipsometry for Detection of Viruses—In our first example, ellipsometry was used to screen antibodies against ricin, a toxin found in castor beans [108]. Patients exposed to ricin need to be treated with the correct dose of antibodies to reduce the threat from ricin. It is therefore, of significant interest to develop titration curves for antibodies that are known to react with ricin. In this particular study, two kinds of antibodies, pVHHS1 and 5S1R were used and dose response curves were obtained. It was demonstrated that the limit of detection was 1 $\mu\text{g/ml}$ for pVHHS1 and 5 ng/ml for 5S1R. While the detection limits using ellipsometry was similar to results obtained with ELISA techniques, methods based on ellipsometry offer significant advantages as they are non-contact and non-destructive.

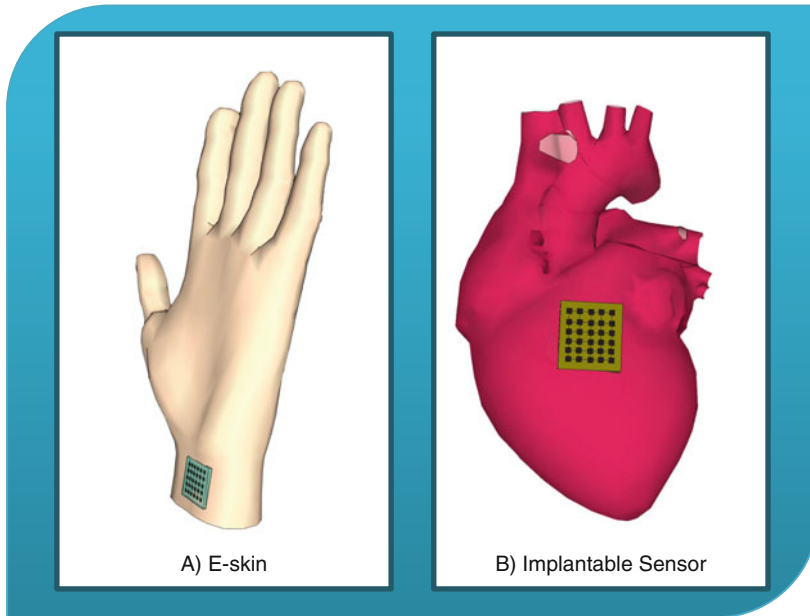
Ellipsometry for Biomarker Detection—In our second example, ellipsometry was used for the quantification of CD146, which is a cell adhesion molecule used to characterize endothelial cells and is also involved in angiogenesis [99]. CD146 is used as a biomarker for the detection of tumors, as the growth of tumors is associated with an increase in angiogenesis and therefore, an increase in the expression of CD146. In this example, ellipsometry was used to quantify CD146 in serum specimens of patients, with a lower detection limit of less than 1 ng/ml. As in the previous example, the results compared closely with those obtained via ELISA, though ellipsometry offered the advantage of non-contact and non-destructive measurement.

Ellipsometry for Detection of Viruses—In our third example, ellipsometry was used for detection of phage M13K07, a virus known to affect *E. coli* [101]. Phage M13K07 does not have any known adverse effects on human health and was selected for this study to demonstrate feasibility of the imaging technology. Furthermore, the size, structure and other properties of M13K07 are comparable to the properties of lethal virus that affect human health; therefore, feasibility studies with phage M13K07 can be translated to other virus systems that are known to impact human health. In this study, a solution containing the phage M13K07 was passed over a surface with a specific ligand that binds to the virus; as the solution was passed over the surface, phage M13K07 binds to the ligand. The increase in concentration of phage M13K07 on the test surface resulted in an increase in thickness of the biological layer and this change in thickness was recorded using ellipsometry. The sensitivity of the assay was reported to be 10^9 pfu/ml. Assays based on ellipsometry or other optical methods are particularly useful for the detection and quantification of viruses, as they do not require contact with the pathogens and can be conducted using non-contact methods, thereby reducing any potential health risks to the experimenter.

2.7 Flexible Sensors

Introduction—In the past, measuring biological structures both internally and externally has employed many, often, bulky devices. Currently a new genre of bio-sensing devices that use very thin layers of various sensors can be used for a wide range of applications (▣ Fig. 2.7). This emerging technology can aid in the detection of both electrical and mechanical signals from the body. The flexibility of a sensing system can be adjusted to fit each individual application. This can be done using different fabrication methods such as nanomolding/micromolding [109–113], and low temperature deposition [114]. In addition, to advance a flexible biometric sensing system it is necessary to integrate circuit elements with a variety of malleable substrates [115]. Among the different circuit elements that can be used are, organic/inorganic matrix arrays [113, 116–122], grapheme [123, 124], nanotube or nanowire assemblies [125–127], and hybrid composites [128–131]. To date, flexible sensors are classified into one of four different categories, these include: electronic skins, wearable devices, implantable devices, and sensors with additional advanced features such as transparency, self-power, and the ability to self-heal [115]. With the purpose of illustrating the usefulness of flexible electrodes, we begin our discussion with an overview of the four different categories in this field. We then discuss the advantages of these techniques, an example from each group, and end with applications of flexible sensing systems in the tissue engineering discipline.

Flexibility Based Biosensors—As previously stated there exist four main groups for the classification of flexible biosensors. First, e-skin is a thin electronic material that is



■ **Fig. 2.7** Flexible sensors technology. (a) E-skin is a thin electronic material that is designed to mimic the ability to sense external stimuli such as pressure, temperature, and tension. (b) Implantable devices can be directly attached to internal organs, such as the heart to monitor electrical signals

designed to mimic the ability to sense external stimuli such as pressure, temperature, and tension just as human skin. In previous studies different methods of designing and fabricating e-skin have utilized materials such as organic transistors [116, 117], germanium (Ge)/Si nanowire circuits [125], and microstructured poly(dimethyl siloxane) [113]. The use of these materials allows for the formation of highly flexible and sensitive structures for the development of a wide range of applications. Second, wearable or skin adhering monitoring devices allow the continuous monitoring of physiological signals. Some examples of this methodology being implemented are the use of single walled carbon nanotubes (SWCNTs) in a device fabricated to sense various dynamic motions of humans [126], and detecting various types of mechanical stimuli by using a layered strain-gauge sensor based on nanoscale mechanical interlocking between metal-coated high-aspect ratio nanofibers [110]. The latter method measured and distinguished three mechanical loads: normal pressure, shear, and torsion by interpreting the gauge factor of each case.

Third, implantable devices are those that can be directly attached to internal organs. These devices can be used as alternative to the clinical system for monitoring electrical signals such as electrocorticographic and epicardial electrogram signals. Examples of these devices include but are not limited to, inorganic electrode arrays on a meshed polyimide (PI) substrate (used to measure brain activity) that are laminated with an absorbable silk fibroin substrate that can be dissolved in biofluid in approximately an hour allowing for direct gapless attachment of the device to the brain to obtain accurate mappings of brain signals with high spatial resolution [132]. Another example uses a catheter balloon with high performance multisensory circuits on the surface of the heart to monitor its electrical activity. This type of system allows for the sensing of other properties such as temperature, flow, and tactility at the tissue-balloon area in real-time [133]. Fourth,

advanced sensors with additional features provide innovations to the currently existing sensing platforms. These modifications encompass properties such as transparency, self-energy harnessing and/or self-healing devices. Skin-like pressure sensors fabricated with PDMS elastic films of carbon nanotubes are used as electrode arrays of flexible capacitors, which serve as strain and pressure sensors [134]. Flexible self-powered, self-healing, highly sensitive (as high as 0.4 Pa), transparent nanogenerators were fabricated by assembling a PDMS microstructure and PET/ITO substrate into micropyramidal patterned arrays, the structure of which increased the efficiency of the nanogenerator [109].

Advantages of Flexible Biosensors—Flexible sensors have five main advantages that make them attractive for different applications. First, flexible sensors can be highly sensitive and multifunctional depending on a variety of parameters, which grants the tailoring of the system to the pertinent detection application. Second, flexible type systems have high bendability that allow them to be used in a wide range of biological applications, such as direct contact with organs or as wearable sensors. Third, flexible sensors are highly elastic, which give the ability to move with and grow with the organ or tissue as it develops. Fourth, flexible sensors have been designed to be biocompatible with their target specimens so as to limit rejection and be affixed to the tissue/organ without and immunological response from the host's immune system. And lastly, flexible electrodes can be manufactured at a low cost with a geometrically controlled shape for specific applications. Additionally, the polymers used for the development of these devices do not only play a crucial role in their flexibility, but also act as a layer that provides direct contact and attachment to the target specimen, as well as, allow for the transfer of various electrical and mechanical inputs.

Examples of Flexible Biosensors—There are numerous examples in published studies that show a wide variety of flexible biosensors for several different applications [113, 118, 132, 135]. In one such study, highly sensitive flexible pressure sensors were developed by joining microstructured PDMS dielectric layers that were used in the fabrication of e-skins that mimic the tactile sensing properties of natural skin. It was demonstrated during this study that both the sensitivity and pressure range are adjustable by changing the shape of the PDMS structures and this system exhibited higher-pressure sensitivity than that seen in unstructured elastomeric films of similar thickness [4]. In a second example, flexible biosensors were used to fabricate an epidermal electronic system that served as a wearable device to monitor temperature, strain, and other electrophysiological properties of the skin. This device was fabricated through the integration of active/passive circuit elements, microscale LEDs, wireless power coils, and devices for radio frequency that were integrated on the surface of a $\sim 30\ \mu\text{m}$ elastomeric sheet. The device was mounted on a water-soluble PVA film that dissolved to leave the sensors firmly attached to the skin [8]. In a third example, a flexible sensing device was fabricated with ultrathin ($2.5\ \mu\text{m}$) spin-cast films of polyimide (PI) as a support for arrays of electrodes designed for direct contact passive neural recording in feline brains due to low bending stiffness. The device was laminated with an absorbable fibroin substrate, which dissolved when in contact with biofluid and left the device in direct contact with the brain to provide mapping of physiological signals with appropriate spatial distribution [132]. In a fourth and last example, a composite material composed of a supramolecular organic polymer with embedded nickel nanostructured microparticles was fabricated. This device shows mechanical and electrical self-healing properties at ambient conditions. The electrical conductivity can be tuned by varying the amount of nickel particles and can reach values as high as 40 S/cm. This study demonstrates that natural skin's repeatable self-healing capability can be mimicked

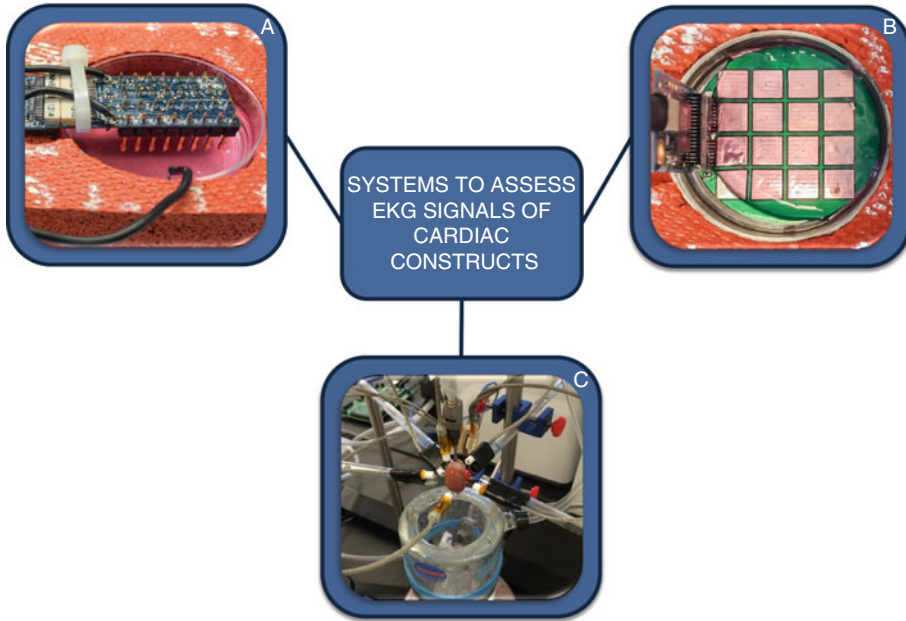
in conductive and piezoresistive materials [135]. All of these examples serve to illustrate the extensive use and ability of flexible biosensors to accurately measure different metric in a wide variety of applications.

Flexible Biosensors Applied to Tissue Engineering—Flexible biosensors can provide a powerful tool for the monitoring of different metrics in a wide range of applications. The ability to develop a highly flexible, sensitive, biocompatible and affordable system that can monitor mechanical and electrical signals allows for the evaluation and enhancement of 3D artificial mammalian tissue. These devices allow for the comparison of mechanical and electrical properties of artificial tissues and native tissues in order to establish what aspects of the 3D tissue must be improved, therefore allowing for the implementation of the necessary stimulation protocols. These devices also allow for the continuous monitoring of changes in the 3D structure of fabricated tissues during the formation process in order to improve existing methodology.

2.8 Case Study 2.1: EKG Sensors for Cardiovascular Tissue Engineering

Introduction—The electrophysiological properties of the heart are crucial. Understanding the electrical properties of the heart can aid researchers in the development of cardiac tissues that can be used to augment, repair and/or replace damaged portions of the heart. During normal mammalian function the electrical activity of the heart plays a central role to support atrial and ventricular contraction. The process of the contraction of the heart starts when the electrical signals arise in the sinoatrial (SA) node, which spontaneously generates propagating action potentials at regular intervals. These electric potential spreads to both the right and left atrium as both atria are electrically coupled. The electrical impulse then travels to the atrioventricular (AV) node through the internodal pathways. Following a delay at the AV node the electrical signal is conducted by the bundle of His and Purkinje fibers to the apex of the heart. The depolarization of cardiac myocytes initiates propagation of the electrical current at the intercellular level through the gap junctions. Excitation of each cardiomyocyte leads to an increase in calcium that subsequently generates heart muscle contraction [136]. Understanding the electrophysiological properties of cardiac constructs developed in laboratories, and replicating values found in vivo is important to move towards implantation of such constructs in a clinical environment. To achieve this goal, systems need to be developed that are capable of evaluating EKG properties in real time for different types of constructs. Two types of system are described here, those used for 3D artificial heart muscle (3D-AHM) and those used for more complex organs such as whole bioengineered artificial hearts (BAHs), bioengineered artificial ventricles (BAVs), and tissue engineered heart pumps (TEHPs).

EKG Sensing Systems for 3D-AHM—It is necessary to produce heart muscle tissues that closely resemble the initial mammalian myocardium from which they were derived. The ability to replicate mammalian electrophysiology is imperative to the success of the artificial tissue. By monitoring changes the tissues undergo during formation, development, and maturation we can further interpret the electrical propagation within each specimen. Prior to in vivo utilization, it is important that 3D-AHM's electrophysiological properties be at a level to remain viable to each specimen's scope of application. To gather these metrics we have designed a 32-channel direct contact system to evaluate the electrical properties of the tissue fabricated in our lab (■ Fig. 2.8a). With this system we are able

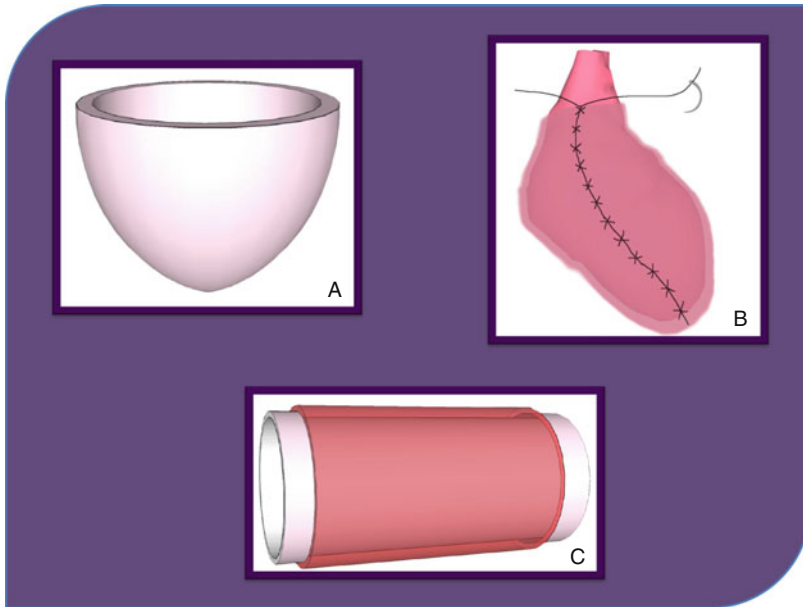


■ **Fig. 2.8** Case study 2.1: EKG monitoring of 3D heart muscle. (a) 32 electrode direct contact system. (b) 16 electrode direct contact noninvasive system. (c) 16 electrode flexible system

to acquire conduction velocities, optical maps, and other metrics associated with the electrical properties of our tissues. However, this system is invasive and can lead to damage of the tissue constructs. For this reason we have designed a second system that consists of a 16-channel electrode board that provides direct contact with the tissue, while remaining noninvasive and minimizing damage to the tissues (■ Fig. 2.8b). This system allows us to obtain the same data that could be acquired with the first system, while minimizing damage to the cardiac constructs.

EKG Sensing System for Complex Organs—With organs of a more complex nature, previously developed systems are not capable of gathering all the electrical metrics due to constraints of the size and shape of the acquisition systems. For this reason we designed a 16 electrode flexible system capable of acquiring the electrical signals of such constructs (■ Fig. 2.8c). Within the structure of this system, we are able to place the electrodes in any desired location on the organ and collect data from all the points simultaneously. In addition, this system allows us to obtain the conduction velocities and conduction maps and evaluate the EKG data acquired. This system can be used to assess constructs other than 3D-AHM that we develop in the lab, such as BAHs, BAVs and TEHPs (■ Fig. 2.9).

Future Approaches—To move the designs forward, integration of electrical stimulation to the developed systems is imperative. The incorporation of electrical stimulation will allow for the assessment in real time of its effect during the fabrication, development and maturation process. Additionally, to further improve the systems we will move toward wireless data gathering and transmitting in real time to display onto the user and for ease of data extraction and analysis.



■ **Fig. 2.9** Case study 2.1: EKG monitoring for complex cardiac constructs. (a) Bioartificial ventricle. (b) Bioartificial hearts. (c) Biological cardiac assist devices

Take Home Message

The important elements in this chapter are summarized here:

- Sensor technology is one of the less developed areas in tissue and organ fabrication.
- Much of the current methods used to assess functional performance of 3D artificial tissue/organs during formation and culture are based on existing technology developed for other applications.
- Many of the functional measurements require invasive procedures and lead to tissue/organ damage. Furthermore, functional assessment can only be performed at intermittent time-points.
- In order to move the field of tissue and organ fabrication forward, there is an urgent need to develop new sensors that are specifically designed to meet the demands of the tissue and organ fabrication process.
- Functional and biological metrics need to be acquired in real-time using non-invasive sensor technology; furthermore, output from these sensors needs to be part of a feedback loop that can modulate and adjust input variables for tissue/organ fabrication.
- Sensor technology is at a stage of infancy in the technology development cycle and significant advancements are required to support the tissue and organ fabrication process.

Review Questions for Chapter 2: Sensors in Tissue and Organ Fabrication

1. Discuss the role of sensors in biological systems. How are sensors used in nature and what can we learn and apply towards the tissue and organ fabrication process.
2. Pick any one example of sensors in biological systems and describe how sensors work in this specific application.
3. Discuss and describe the role of sensors during tissue and organ fabrication.
4. There are three requirements for sensors in tissue and organ fabrication: (1) non-invasive, (2) real-time and (3) positive feedback loop. Discuss each of these three.
5. Describe the role of biological sensors in nature. How and why is biological sensing important?
6. Identify and describe any one biological sensing pathway.
7. What can we learn from biological sensors that can be applied to tissue and organ fabrication?
8. Describe the use of magnetic sensors in tissue and organ fabrication. What are some of the relative advantages and disadvantages of magnetic biosensors, as applied to tissue and organ fabrication.
9. Magnetic biosensors are at an early stage of technological development. What are some advancements that need to occur in the development of magnetic biosensors prior to their use in tissue and organ fabrication?
10. Explain the principles of QCM-D sensors.
11. In the text, we described the use of QCM-D sensors for glucose monitoring and to quantify cell proliferation. Research and describe one additional application of QCM-D sensors in biological systems.
12. Discuss the potential use of QCM-D sensors to support the tissue and organ fabrication process. What are some advantages and disadvantages of these sensors as applied to tissue and organ fabrication.
13. Explain the principles of ellipsometry.
14. What are some advantages of ellipsometry as biological sensors?
15. How can ellipsometry be used as sensors to support tissue and organ fabrication? Pick any tissue or organ fabrication process and explain how ellipsometry can be used as to develop sensors for tissue/organ fabrication?
16. Compare acoustic sensors to optical sensing. What are the relative advantages and/or disadvantages of acoustic sensors versus optical sensors?
17. There are many modes of optical sensors that are used in biological applications. Ellipsometry is just one example of such techniques. Research another optical based biosensor technology and describe the principles of operation, along with specific examples in bio-sensing.
18. Explain the concept of flexible sensors. Why are flexible sensors an attractive option for biological options?
19. Describe four classes of flexible sensors and provide one example of each.
20. Explain how flexible sensors can be used to support tissue and organ fabrication.

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