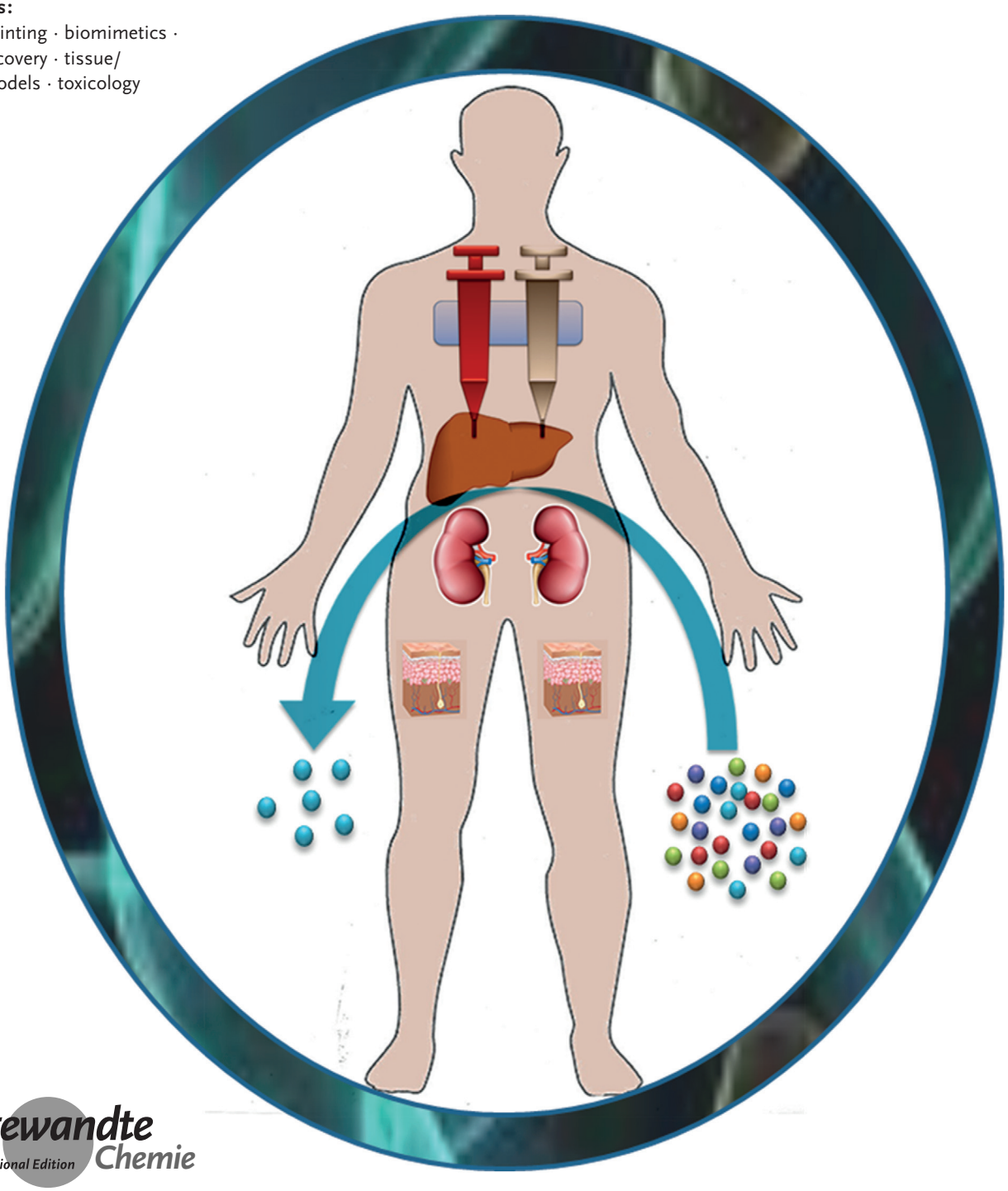
3D Tissue/Organ Models

International Edition: DOI: 10.1002/anie.201505062
German Edition: DOI: 10.1002/ange.201505062

3D Bioprinting of Tissue/Organ Models

*Falguni Pati, Jesper Gantelius, and Helene Andersson Svahn**

Keywords:
3D bioprinting · biomimetics · drug discovery · tissue/organ models · toxicology



In vitro tissue/organ models are useful platforms that can facilitate systematic, repetitive, and quantitative investigations of drugs/chemicals. The primary objective when developing tissue/organ models is to reproduce physiologically relevant functions that typically require complex culture systems. Bioprinting offers exciting prospects for constructing 3D tissue/organ models, as it enables the reproducible, automated production of complex living tissues. Bioprinted tissues/organs may prove useful for screening novel compounds or predicting toxicity, as the spatial and chemical complexity inherent to native tissues/organs can be recreated. In this Review, we highlight the importance of developing 3D in vitro tissue/organ models by 3D bioprinting techniques, characterization of these models for evaluating their resemblance to native tissue, and their application in the prioritization of lead candidates, toxicity testing, and as disease/tumor models.

From the Contents

1. Introduction

Our ability to understand the formation, function, and pathology of tissues/organs often depends on studies performed on two-dimensional (2D) cell-culture or animal models. However, a drawback of 2D cell-culture studies is that cells grown in such culture substrates can differ substantially in their morphology, cell–cell and cell–matrix interactions, and differentiation from those growing in more physiological three-dimensional (3D) environments.[1–4] Although animal models frequently provide definitive tests of the importance of specific molecules and processes, there have been inconsistencies between conclusions drawn from studies related to gene ablation and chemogenomics.[5] The main concern with using animal models for drug discovery is that they are unpredictable.[6] For example, around 50% of the drugs that pass preclinical testing may turn out to be toxic for humans and some drugs may in fact be nontoxic for humans even if they fail in animals.[7] This causes rejection of potentially important drugs even before they reach clinical trials. Furthermore, animal models are often ineffective in reproducing features of human tissues, for example, human tumors, autoimmune diseases, and drug therapeutic/toxic responses.[8,9] The main reason behind this is likely the difference in the evolution of two complex systems, for example, mouse and human, and hence responses to perturbations such as disease and drugs can give rise to substantially different clinical/physiological endpoints.[10,11]

In vitro 3D tissue models provide an excellent alternative to traditional 2D cell cultures and animal testing.[3,12] These models satisfy the need for reductionist approaches to understand in vivo molecular mechanisms.[13] Furthermore, the powerful current cell and molecular biology methods can often be applied to 3D tissue models. An increasing use of 3D models that mimic specific tissues could promote advances in understanding tissue morphogenesis and also facilitate the screening of new therapeutics. In fact, in vitro 3D tissue models can better represent the spatial and chemical complexity of living tissues than their 2D counterparts.[14] Moreover, these models have been shown to be useful for studying the molecular basis of tissue function and better capture signaling pathways and drug responsiveness compared to 2D cultures.[15–17]

However, most of the 3D tissue models lack multiscale architecture and tissue–tissue interfaces, such as the interface between the vascular endothelium and surrounding connective tissue and parenchymal cells,[18] which are vital to the function of nearly all organs. Furthermore, the cells in most current models are generally devoid of any exposure to fluid mechanical cues, such as fluid shear stress, tension, and compression, which are crucial for organ development and function in health and disease.[18–20] Microfluidic organs on chips offer the possibility of overcoming many of these limitations by providing cell-culture devices that contain continuously perfused chambers inhabited by living cells arranged to simulate a part of the tissue or organ functions.[18] However, they are unable to create the complex multiscale architecture because of their planar fabrication technique. Hence, such models mostly concentrate on simulating the basic function of the respective tissue.

3D bioprinting is an approach that can be used for printing millimeter- to centimeter-sized biological constructs, including several cell types, biomolecules, and biomaterials simul-

[*] Dr. F. Pati, Dr. J. Gantelius, Prof. H. A. Svahn
Division of Proteomics and Nanobiotechnology
Science for Life Laboratory
KTH-Royal Institute of Technology
Stockholm (Sweden)
E-mail: helene.andersson.svahn@scilifelab.se

taneously.[21] This technique can be utilized for making realistic 3D tissue or organ models, as it is capable of mimicking the actual cellular arrangement of a tissue or organ while constructing the 3D structure.[22] Cells can be placed with high precision throughout the construct, including simultaneously placing several cell types next to one another at targeted locations.[22]

Creating in vitro 3D tissue models of human cells that mimic specific in vivo behavior can help to enable more accurate prediction of therapeutic/toxic responses as well as decreasing the cost of drug discovery. Furthermore, in vitro disease/cancer models can be developed by fabricating constructs with diseased or dysfunctional human cells for studying tissue pathology or testing new therapeutics.[23]

This Review focuses on general principles, ideas, and methods pertaining to the application of 3D bioprinting technologies for generating realistic in vitro 3D tissue models. We present recent examples that were chosen to illustrate key concepts. We also discuss exciting opportunities of 3D bioprinting technologies for further fundamental and translational research on realistic tissue and cancer models, toxicological studies, and physiological models.

2. Recent In Vitro Tissue/Organ Models

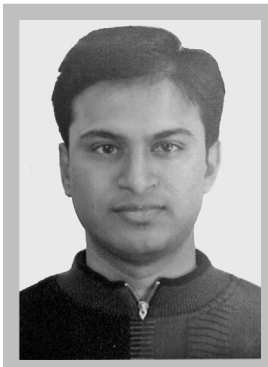
In the last decade, the use of in vitro 3D models has increased significantly as a result of the development of new enabling technologies and tightening controls on the use of animals for scientific experimentation.[24–28] Recent developments in tissue bioengineering can offer great promise for the development of novel 3D tissue models for preclinical drug testing, toxicological studies, and as physiological models.

Several culture systems have been developed over the years, starting from 2D cultures, cultures on hydrogel membranes, sandwich cultures, to 3D cultures for the in vitro evaluation of drugs/chemicals (Figure 1). For example, cultures of primary cells such as hepatocytes on 2D plates have routinely been used for drug screening for many years.[29,30] However, the primary cells undergo changes in cell morphology, structure, polarity, gene expression, and lose their tissue-specific functions during culture on flat surfaces.[31] This process is referred to as de-differentiation, which is a biological phenomenon whereby differentiated cells regress from a specialized function.[32] Hence, they can only be used for a limited time.

To mimic the interaction between cells and extracellular matrix (ECM), mammary epithelial cells have been cultured on floating collagen membranes,[33] which leads to the production of milk protein.[34,35] Primary human breast carcinoma cells are difficult to establish in cultures on 2D surfaces, whereas a reconstituted basement membrane (BM) can be used to culture all normal human breast epithelial cells and a subset of human breast carcinoma cells.[36] The 3D reconstituted BM cultures allow distinction between malignant and normal mammary epithelial cells (MECs) by virtue of the ability of normal cells to re-express a structurally and functionally differentiated phenotype within the BM.[36]

To maintain tissue-specific functionality over relatively long culture periods, a sandwich culture has been explored in which primary cells such as hepatocytes are placed in between two layers of a matrix (traditionally collagen or Matrigel).[37] Despite the many attributes of the sandwich culture technique, the expression of genes responsible for many liver-specific functions decreases over time (90 h).[38]

A number of 3D culture models have been developed that enable the organization of cells, thereby allowing them to polarize and interact with neighboring cells.[39,40] These models offer great potential for studying the basic mechanisms of tissue/organ physiology and pathophysiology. They are principally suitable for studying biological phenomena that depend on tissue microarchitecture and typically need 3D structure.[40] Such 3D models can take many forms, including cells randomly interspersed in a matrix or clustered in self-assembling cellular microstructures known as organoids.[16,17,41] A hydrogel with an inverted colloidal crystal scaffold has also been employed to culture tissue-like 3D structures.[42] Coculture systems have been developed for primary cells with other supporting cell types that result in enhanced viability and functionality of the primary cells under in vitro conditions. For example, hepatocytes have been cultured with fibroblast and endothelial cells to have enhanced viability of the former cell type.[43–47] However, lack of multiscale architecture, missing structural hierarchy, absence of exposure to fluid mechanical cues, and long-term culture conditions are the main drawbacks of these in vitro



Falguni Pati studied at Jadavpur University, India, and completed his PhD in Bioengineering at IIT Kharagpur, India. He was a Postdoctoral Fellow at Intelligent Manufacturing Systems Lab, POSTECH, South Korea, from 2011 to 2014. He is currently working as a Postdoctoral Fellow in the Division of Nanobiotechnology at the Royal Institute of Technology (KTH), where his main focus is to develop tissue/organ models by 3D bioprinting for biomedical and pharmaceutical applications.

Helene Andersson Svahn studied molecular biotechnology at Uppsala University and completed her PhD in Electrical Engineering at the Royal Institute of Technology in 2001. From 2002 to 2005 she worked at Silex Microsystems and from 2005 to 2008 she was professor in Applied BIOMEMS at the MESA+ Research Institute in Holland. She currently heads the Nanobiotechnology division at the Royal Institute of Technology in Stockholm and is on the Editorial board of Lab on a Chip. She was the first President of the Young Academy of Sweden and is Scientific Director at the Science for Life Laboratories.

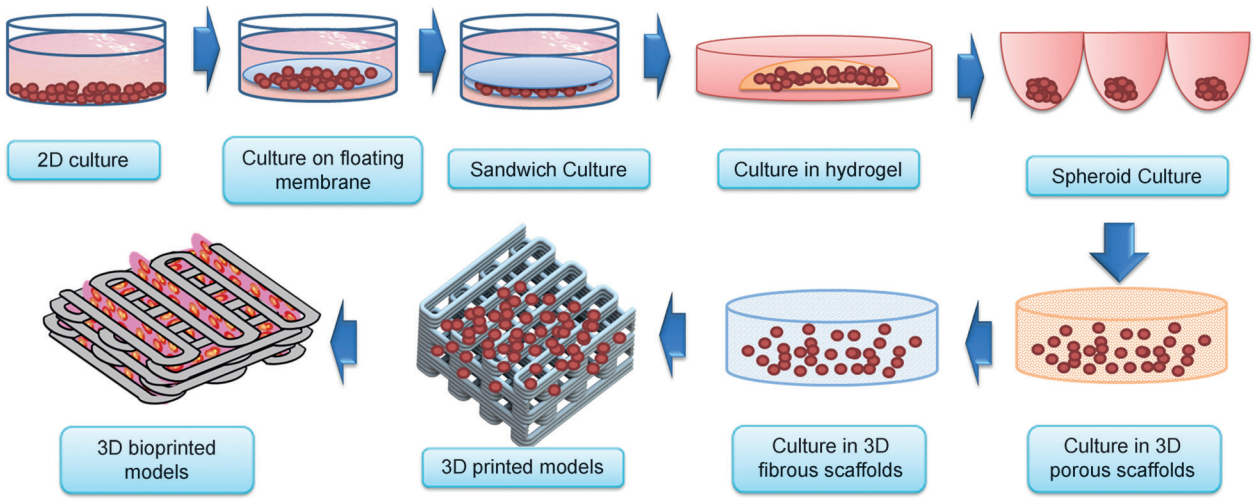


Figure 1. Evolution of cell-culture models. 2D cell cultures have been used for many years and is still being used for simple cell-based assays. Membrane and sandwich cultures were later developed. Hydrogels are usually used for cell encapsulation to culture cells in three dimensions. Spheroid cultures are used as a scaffold-free culture where cell–cell interactions are predominant. 3D scaffolds, either salt-leached or fibrous, are produced by various techniques, and cells are generally seeded on them. The advent of 3D printing encouraged the fabrication of complex structures in a reproducible manner, with control over their architecture and geometry. However, cells are still seeded on the scaffolds after fabrication. 3D bioprinting technology, whereby cells can be included in the printing process, enables the fabrication of cell-laden constructs. Interestingly, the cellular microenvironment can be modulated with these techniques. To date, 3D bioprinting is the most sophisticated technique to make tissue/organ constructs. Some images were reproduced from Refs. [106, 111] with permission.

models. Recent developments in in vitro 3D models are reviewed in Refs. [40, 48].

Drawbacks of recent tissue/organ models

- Multistep fabrication methods
- Limited cell viability and functionality
- Missing complex and hierarchical tissue structure
- Absence of a specific ECM environment
- Missing 3D structure in the case of biochips
- Large variation in results obtained from animal studies
- Incapability of animal models to reproduce features of human tissues and organs
- Unpredictable and false-positive or false-negative results from animal testing
- Tightening controls on use of animals for scientific experimentation

In recent times, microfluidic biochips have rapidly become one of the most popular tools for the in vitro modeling of tissue, as microfabrication provides a level of precision and control that cannot be achieved with other techniques.[49–52] Recent developments in microfluidic biochips have been reviewed in Refs. [18, 53]. However, such biochips mainly focus on the generation of cellular-level architecture, generally mimicking only part of the tissue structure but not that of the whole complex tissue or organ. For example, current liver-on-chip models typically consider the creation of an acinus-mimicking structure for generating a concentration gradient across the acinus,[54] however, a multiple lobule-mimicking 3D microliver would facilitate better creation of liver zonation and prediction of zone-dependent toxicity. The creation of multiple lobule-mimicking 3D microliver structure is challenging with the planar fabrication techniques used for generating microfluidics chips. There is a great need to expand the designed microenvironments produced with planar fabrication into the third dimension to generate realistic tissue/organ models.[55]

3. 3D Bioprinting of Tissue/Organ Constructs

3.1. Fabrication Strategies/Working Principles

The concept of bioprinting is essentially an extension of additive manufacturing techniques used to build complex tissue constructs through a layer-by-layer process.[55] Basically, the process can be divided into three steps:[56,57] A) preprocessing for preparation of the bio-ink, including generation of the computer-aided design (CAD) "blueprint"; B) the processing step, which typically involves the printing of the 3D structure; and C) postprocessing, where the printed construct is cultured in a bioreactor. The postprocessing step is essentially included to induce maturation of the printed construct and transformation into a functional tissue.

Requirements for fabricating biological tissues and organs have been summarized by various groups.[57–62] However, disagreement exists regarding the resolution and organization required on a microscale. Some researchers believe that biological tissues should be generated with the highest resolution possible to mimic the functions and behavior of cells in in vitro 3D environments and control over the 3D architecture and inner composition is essential,[58] whereas others have suggested that merely printing cellular aggregates allows cells to organize themselves to form tissues, as they

have organizational capacities. For example, endothelial cells (ECs) form tubular structures when optimal external conditions are provided.[63]

Based on the working principle/strategy, the 3D bioprinting technologies can primarily be classified into three categories,[64–66] inkjet-based, laser-assisted, and extrusion-based bioprinting (Figure 2).

3.1.1. Laser-Assisted Bioprinting

Laser-assisted bioprinting (LaB) utilizes a pulsed laser source, an absorption layer, and a substrate to directly position multiple cells and biological components onto an arbitrary surface by using laser beams to print living tissues or organs (Figure 2A).[67] Prior to laser exposure, the absorption layer, which is transparent to the laser radiation wavelength, is coated with biological materials (bio-ink) that encapsulate the living cells and/or proteins. A focused laser beam is then exposed on the absorption layer to transfer heat and eject the cell suspension toward the substrate.[55] The absorption layer plays an important role in preventing direct interaction between the laser and biological materials. LaB is capable of printing small volumes of cell suspension with high resolution.[68] The printed droplet volume could be controlled from 10 to 7000 pL by adjusting the viscosity and thickness of the bio-ink layer. Furthermore, printing of high cell densities and highly viscous hydrogels is possible with LaB, whereas this is more challenging in inkjet printing.[69]

Recently, LaB has been used widely to print various tissue constructs.[67,70,71] Bone regeneration was attempted by printing human osteoprogenitors with nanohydroxyapatite, which is an inorganic component of bone.[67] For skin regeneration, keratinocytes and fibroblasts encapsulated in collagen were printed according to the native cellular arrangement of skin.[70] The potential for LaB in adipogenesis has also been demonstrated by printing undifferentiated stem cells derived from human adipose that could differentiate into adipogenic lineages.[71] However, the limited availability of photocurable materials and cytotoxicity from UV exposure are the primary concerns.[22]

3.1.2. Inkjet-Based Bioprinting

Inkjet-based bioprinting is a promising biofabrication approach.[72] This is a noncontact technique that prints the 3D constructs layer by layer by depositing ink drops on successive layers (Figure 2B). This technique provides a useful method for depositing multiple cells[73,74] or proteins[75–77] in very small droplets onto a targeted spatial position. It offers many advantages, including high-throughput capability, high resolution, inexpensiveness, reproducibility, and ease of use.[78] In particular, commercially available inkjet printers can be easily modified for printing cells and biomolecules.[79–81] In fact, Boland and co-workers demonstrated that Chinese hamster ovary and embryonic motor neuron cells can be printed onto various hydrogel substrates by using a modified Hewlett Packard printer.[79] Nakamura and co-workers applied inkjet technology to print simple cell-supporting structures in the shape of fibers, sheets (with one or more layers), and 3D tubes.[82] Researchers at the Wake Forest Institute for Regenerative Medicine have developed modified ink-jet technology to build a variety of tissue and organ prototypes by arranging multiple cell types and other tissue components in predetermined locations with high precision.[83] The effect of the printing conditions, including substrate stiffness,[84] surfactant concentration, and agitation, on printed cells has also been investigated.[85] It has been shown that the substrate onto which the cell droplet is deposited should be soft enough to absorb the kinetic energy of droplets so that the impact force on the cells is reduced substantially.[84] Furthermore, the addition of a surfactant was shown to improve the reliability of droplet formation, and gentle agitation could avoid sedimentation and aggregation of cells in the reservoir.[85]

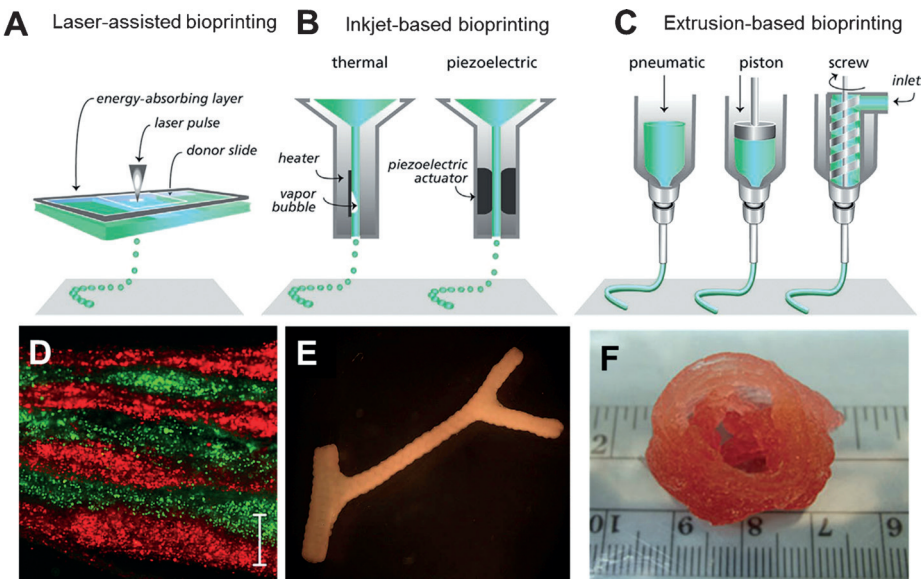


Figure 2. The three approaches to bioprinting: A) laser-assisted, B) inkjet-based, and C) extrusion-based. Adapted from Ref. [86] with permission. The bioprinted tissue types: D) skin, by laser cell printing (seven alternating layers of red and green keratinocytes with each layer consisting of four printed sublayers; scale bar 500 μm), E) branched vasculature, by inkjet printing, and F) heart aortic valve, by extrusion bioprinting. Reproduced from Ref. [70] with permission.

3.1.3. Extrusion-Based Bioprinting

Extrusion-based bioprinting technologies allow the printing of living cells onto target-specific positions while encapsulating them in a hydrogel. To date, extrusion-based bioprinting technologies, comprising a syringe, nozzle, and pressure system, seem to be the most promising approach for generating 3D tissue or organ con-

structs of clinically relevant size and shape (Figure 2C).[55] Prior to printing with this technique, cells or proteins are encapsulated in a hydrogel and loaded into sterilized syringes with a micronozzle. The cell-laden hydrogel or cell spheroids are then dispensed by air pressure or a motorized plunger onto the substrate according to a customized design. There are many reports detailing various extrusion-based 3D tissue-printing systems, for example, the Novogen MMX Bioprinter (Organovo, Inc., San Diego, CA, USA),[87] the Bioplotter (Envision TEC GmbH, Gladbeck, Germany),[21,88,89] a 3D printer (Fab@Home, Cornell University, Ithaca, NY, USA),[90] a direct cell-writing system (laboratory of Prof. Sun, Drexel University, Philadelphia, PA, USA),[91] and a multihead tissue/organ building system (MtoBS; laboratory of Prof. Cho, POSTECH, Pohang, Korea).[92] The printing of various kinds of tissue constructs have been attempted by using these 3D bioprinting systems.[21,92,93]

3.2. Key Attributes

There are three key attributes for 3D bioprinting: biomimicry, tissue liquidity, and modular building blocks. These are essential for achieving realistic 3D tissue models.

3.2.1 Biomimicry

3D bioprinting attempts to reproduce the cellular and extracellular components of a tissue or organ.[94] This can be accomplished by reproducing specific cellular functional components of tissues, for example, by mimicking the lobular structure of the liver or engineering physiologically accurate biomaterials and gradients. Nevertheless, for the successful reproduction of biological tissues on the microscale, an understanding of the microenvironment, including the specific organization and hierarchy of functional and supporting cell types, gradients of soluble or insoluble factors, composition of the ECM, as well as the nature of the biological forces in the microenvironment, is needed.[58] Development of this knowledge base is crucial for the success of this approach and can benefit from basic research in the fields of biomaterials, cell biology, biophysics, imaging, engineering, and medicine.

3.2.2. Tissue Liquidity

Cells have organizational abilities. Thus, after printing cellular aggregates, they can organize themselves to form tissues. Organ development in embryos often serves as a guide for replicating biological tissues by 3D bioprinting. Based on this approach, the 3D printing of self-assembling cellular spheroids was attempted that undergo fusion and cellular organization to mimic developing tissues, for example, epithelium cells (ECs) form tubular structures when optimal external conditions are provided.[56] Autonomous self-assembly, such as that broadly observed throughout embryonic development, relies on the cell as the primary driver of histogenesis, directing the structure, composition, localization, function, and properties of the tissue.[22]

3.2.3. Modular Building Blocks

Organs and tissues comprise smaller, functional building blocks.[63] These can be defined as the smallest structural and functional component of a tissue, such as a liver lobule. These building blocks can be fabricated in modules and assembled into larger constructs by rational design, self-assembly, or a combination of both.[95] 3D bioprinting techniques can be used to print these modules and assemble them into 3D functional living structures; for example, multicellular spheroids have been assembled with the aid of ECM-mimicking hydrogels.[96,97]

3.3. Materials for 3D Bioprinting

The bioprinting of tissue constructs often involves the use of materials with well-defined properties as a vehicle for cell loading. Hence, material engineering can be used to modulate cell–biomaterial interactions. However, the main function of bioprintable materials is not only management of specific interactions with a cell or tissue, but also to provide scaffolding for tissue formation.[98,99] Therefore, two classes of materials are commonly used for 3D bioprinting. The first class is curable polymers, which can be extruded by a thermal process and are often used for scaffolding purposes.[92,100–103] Cells are seeded after printing on these scaffolds so that cells grow within to generate tissue constructs or used as it is for implantation (Figure 3).[104] The second class comprises materials that store a large amount of water (up to $>99\,\%$) and provide a favorable environment for the cells.[55] Hydrogels belong to this class, and are used to encapsulate living cells. Cell-laden hydrogels are typically called "bio-ink" and may also contain other components, such as drugs[105] or biomolecules.[106] Hydrogels that solidify through thermal processes, photo-cross-linking, or ionic/chemical cross-linking may be used to make bio-inks.[89,107–115] Various key properties such as concentration, molecular weight, viscosity, gelation kinetics, and stiffness are important determinants when choosing a hydrogel for 3D bioprinting. For comprehensive reviews on the properties of hydrogels for bioprinting, see Refs. [55,86].

Typically, an excess of hydrogel relative to cells is required to maintain the structural stability of the bioprinted constructs. However, the concentration of cells in the bio-ink can have an impact on the degree of cell–cell interactions in the resultant 3D structure, and a high cell density (10^7–10^8 cells mL^{-1})[116] is needed for that. Hence, a fine balance between the hydrogel concentration and cellular density to serve both purposes is often required.[55] Furthermore, cells produce and deposit the tissue ECM—a process that often depends on the cellular microenvironment.[117] The composition of the bio-ink may play a vital role to boost the production of tissue-specific ECM. Self-assembling cellular spheroids may produce an ECM environment best suited for their own function.[63] However, the bioprinting of cellular spheroids is challenging given the fact that their viability, biosynthetic ability, and nutritional requirement need to be maintained.[62] Besides printing tissue constructs with hydro-

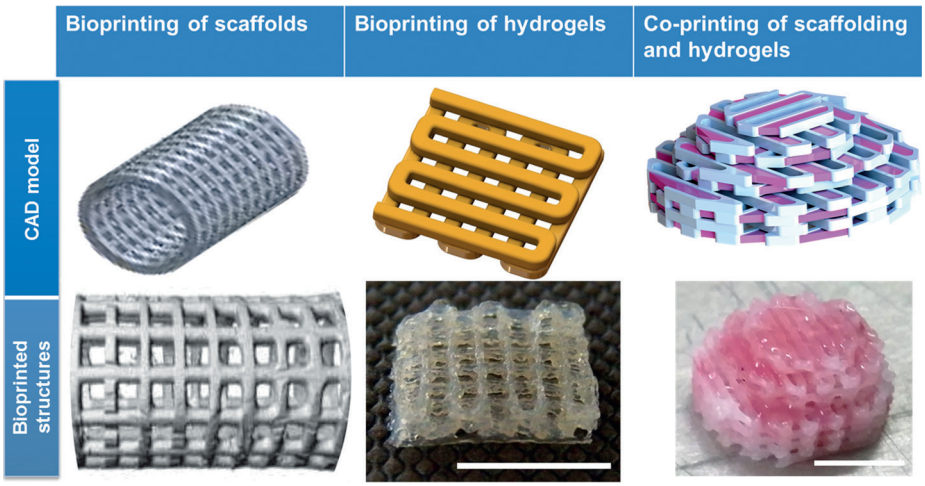


Figure 3. Bioprinting of scaffolds and hydrogels as well as co-printing of the scaffold and hydrogel. The computer-generated models and their corresponding printed structures of poly(propylene fumarate) scaffolds (left column), silk fibroin-gelatin hydrogel constructs (middle column), and polycaprolactone-decellularized extracellular matrix constructs (right column). Scale bar, 5 mm. Reproduced from Refs. [104, 115, 126] with permission.

gels, co-manufacturing solid, curable materials (e.g., thermoplastics such as PCL, PLA, and PLGA) with cell-laden hydrogels has also been used for bioprinting to combine favorable mechanical properties with distribution of cells at defined locations with high cell densities (Figure 3).[118]

Frequently, specific biologically active components have been incorporated into the matrix materials for improved cell adhesion, enhanced cell proliferation, or differentiation.[119,120] For example, ECM proteins bind to soluble growth factors and regulate their distribution, activation, and presentation to cells.[121] The spatial distribution of proteins provides biochemical cues to direct the organized formation of tissue.[122] Proper consideration of the spatial and temporal aspects of the exogenous delivery of such signals are needed to guide tissue remodeling.[121]

To mimic the exact composition, tissue-specific ECM that could foster particular tissue formation can be used for 3D bioprinting.[110] Cell–ECM interactions are extremely complex in nature, and consequently there is a need for a tissue-specific approach to create the native setting for stem cells. Recent studies have validated this tissue-specific approach, with enhanced functions[123] and intricate tissue formation evident[124,125] when site-specific ECM scaffolds were employed. In a recent study, decellularized ECM (dECM) from different tissues has been used to make bio-inks for 3D bioprinting.[110] Increased differentiation of stem cells and tissue formation was observed toward either an adipogenic lineage within dECM from fat tissue or a chondrogenic lineage within cartilage dECM. Furthermore, enhanced structural maturation of myoblasts was observed in constructs prepared with dECM from heart tissue.[110]

3.4. 3D Bioprinted Tissue/Organ Models

3D bioprinting has been applied to the development of several types of tissues and organs by using different cell types and materials (Table 1). While some of the studies have presented only proof-of-concept principles, a few advanced studies clearly demonstrated the potential of this technique for the development of tissue models.

3.4.1. Bioprinted Skin

The potential of inkjet 3D bioprinting for engineering skin tissue has been demonstrated by using keratinocytes and fibroblasts as constituent cells to represent the epidermis and dermis, respectively, and collagen to represent the dermal matrix of the skin.[127] Histology and immunofluorescence characterization showed that the 3D printed skin tissue was morphologically and biologically similar to native human skin. In another study, a laser-assisted bioprinting technique was used to create a fully cellularized skin substitute by positioning fibroblasts and keratinocytes on top of a stabilizing matrix (Matriderm).[128] The printed keratinocytes began to differentiate and formed a multilayered epidermis and a stratum corneum when cultivated at the air–liquid interface. Some of the printed fibroblasts stayed on top of the underlying Matriderm where they produced collagen, whereas the rest migrated into the Matriderm. A laser-induced forward-transfer (LIFT) technique has been used to print skin cell lines (fibroblasts/keratinocytes) and human mesenchymal stem cells (hMSC) for regeneration of human skin without any deleterious effect on cells.[129]

3.4.2. Bioprinted Vasculature

Special attention has been given to the printing of vascular structures because of their unique role in delivering nutrients and oxygen to, and removing metabolic residues from, tissue constructs, which could thus enable the generation of larger and more complex tissues and organs.[130] Cell-loaded gel-based bioprinting has been employed to generate vascular constructs by using extrusion-based bioprinters.[131] More recently, two different gel-based cellular suspensions and one sacrificial (fugitive) gel were printed sequentially on top of each other within a casting chamber subsequently filled with a GelMA gel.[132] After cross-linking of the GelMA by exposure to ultraviolet light, the temperature of the environment was reduced to liquefy the fugitive gel so that it could be removed by aspiration. The resulting channels engraved within the complex gel structure were then perfused with human umbilical vein endothelial cells (HUVECs), which attached to the channel walls, thereby resulting in vascularization of the complex bioprinted construct. This process of generating perfusable channels within tissue-engineered constructs by utilizing 3D-printed sacrificial materials was also employed earlier on to generate a gel containing suspended

Table 1: Examples of 3D bioprinted tissues and organs.

Tissue type	Materials	Cross-linking method	Cells	Bioprinting technology	Experiments performed	Important findings	Ref.
skin	collagen type I for cell encapsulation and Matriderm was used as a stabilization matrix	thermal	fibroblasts and keratinocytes	laser-assisted	in vivo by placing printed constricts into full-thickness skin wounds in nude mice, histology and immunofluorescence characterization	formation of skin tissue, mainly epidermis with sprouting blood vessels from wound bed towards printed cells	[121]
brain microvasculature	collagen type I	thermal	mouse brain endothelial cells (bEnd.3)	extrusion-based for printing frame	transendothelial permeability assay and disruption of barrier function with mannitol, immunofluorescence staining	formation of engineered brain microvasculature with barrier function	[172]
liver	–	–	primary hepatocytes, endothelial and hepatic stellate cells	inkjet-based	biochemical studies including cytochrome P450 activity, tight junction protein expression	multicellular 3D liver constructs in a multi-well format, increased liver specific function of the tissue for up to 135 h	[130]
osteochondral	acrylated peptides and acrylated poly(ethylene glycol) (PEG)	UV-induced	bone marrow derived human mesenchymal stem cells (hMSCs)	inkjet-based	evaluation of cell viability and biochemical analysis of mineral and matrix deposition	mineral and cartilage matrix deposition with the bioprinted bone and cartilage with increased mechanical properties	[132]
cardiac	alginate	ionic	human cardiac derived cardiomyocyte progenitor cells (hCMPCs)	extrusion-based	evaluation of cell viability and expression of cardiac-specific genes	printed cells could migrate from alginate matrix to matrigel layer, indicating feasibility of cell delivery from printed structure	[135]
cornea	recombinant human collagen type III and 2-methacryloyloxyethyl phosphorylcholine (RHCIII-MPC) and fibronectin as ink	chemical cross-linking of RHCIII-MPC	human corneal epithelial cells (HCECs)	microcontact printing to transfer cells onto RHCIII-MPC	cell viability and proliferation, as well as immunohistochemistry	femtosecond laser profiled cross-linked hydrogel fabrication and microcontact printing of viable HCECs according to well-defined pattern	[137]
ear	alginate	ionic	chondrocytes from articular cartilage	extrusion-based	cell viability, biomechanical, and electrical characterization	3D printed bionic ear with intertwined conducting polymer possessing both biologic and nanoelectronic functionalities	[138]
adipose	decellurized adipose tissue matrix	thermal	human adipose derived stem cells	extrusion-based	cell viability, gene expression, immunostaining, in vivo study on mouse model for adipose tissue regeneration	3D bioprinted constructs supported volume stabilized, vascularized adipose tissue regeneration	[142]

cells cast over a 3D-printed carbohydrate glass structure.[133] After cross-linking of the cast gel, the carbohydrate structure was dissolved, thereby leaving behind a channel network that could be further perfused with HUVECs to attach them to the channel walls. The printing of blood vessels was also demonstrated by directly dispensing spheroids of smooth muscle cells and fibroblasts in a supportive agarose gel.[93]

3.4.3. ***Bioprinted Liver***

Bioprinting functional perfusable hepatic constructs is still in its infancy. However, 3D bioprinting techniques have been explored for developing hepatic constructs with limited function.[134,135] Metabolically active 3D hepatic tissue has been printed using the NovoGen MMX Bioprinter (Organovo Holdings, Inc., San Diego, CA, USA).[136] An increased liver-specific function of the tissue (for up to 135 h) compared with matched 2D cell cultures was demonstrated. Furthermore, compartment-specific organization was shown for hepatocytes, hepatic stellate cells, and ECs by rudimentary microanatomy. The extrusion-based bioprinting technique was used to print alginate-encapsulated HepG2 cells, growth factors, and scaffolding materials in an organized 3D architecture.[137] These microlivers were dynamically microper-

fused to mimic an in vivo condition to study drug metabolism. Furthermore, this system was also utilized to perform as a model for a radioprotection study on liver cells.[23]

3.4.4. Bioprinted Bone and Cartilage

Inkjet bioprinting has been used to print bone constructs by the co-printing of acrylated peptides and acrylated poly(ethylene glycol) with marrow-derived human mesenchymal stem cells (hMSCs).[138] The bioprinted and differentiated bone and cartilage tissue demonstrated excellent separation of mineral and cartilage matrix as well as significantly increased mechanical properties. Additionally, the stiffness and structure of 3D-printed hydrogels have been shown to direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages.[139] The bioprinting of bone tissue has been attempted by dispensing bone-marrow stem cells (BMSCs) with various hydrogels (such as alginate, agarose, Matrigel, and Lutrol F127) by a 3D Bioplotter.[21] The printed BMSCs were shown to be viable within the printed constructs. A mechanically enhanced 3D bioprinted construct containing two different cell types has also been fabricated for osteochondral tissue regeneration.[100] In this study, thermoplastic polycaprolactone (PCL) was used as a framework to enhance the mechanical stability of the bioprinted construct. Alginate solutions containing either osteoblasts or chondrocytes were infused into a previously prepared framework consisting of PCL to create the 3D construct for osteochondral printing. A proof-of-concept for printing osteochondral tissue comprising cartilage and bone was developed using MtoBS,[100] which was shown to be especially promising for printing 3D heterogeneous tissue in multicellular arrangements. Recently, a whole porcine cartilage tissue matrix was used as a bio-ink for printing cartilage tissue constructs.[110] The 3D bioprinted constructs fabricated using cartilage-decellularized extracellular matrix (dECM) bio-ink supported chondrogenic differentiation and maturation of encapsulated stem cells as well as formation of neo cartilage tissue.

3.4.5. Bioprinted Cardiac Tissue

A simultaneous 3D bioprinting/photo-cross-linking technique has been utilized to rapidly engineer complex, heterogeneous aortic valve scaffolds with native anatomic and axisymmetric aortic valve geometries (root wall and trileaflets) with inner diameters of 12–22 mm.[140] Scaffolds seeded with porcine aortic valve interstitial cells (PAVICs) and cultured for up to 21 days exhibited an elastic modulus range of 5.3 ± 0.9 to 74.6 ± 1.5 kPa. In another study, a human cardiac derived cardiomyocyte progenitor cell (hCMPC) loaded alginate gel was used to generate an in vitro cardiac patch with 92% cell viability.[141] The bioprinted hCMPCs retained their commitment for the cardiac lineage and expressed early cardiac transcription factors Nkx2.5, Gata-4, and Mef-2c as well as the sarcomeric protein troponin T.

3.4.6. Bioprinted Eye

Computer-aided design has been used to design a schematic model of an eye that can closely simulate the optical performance of the human eye.[142] 3D printing was utilized to fabricate the physical model for use in research on fundus range viewing. In an effort to produce mimics of human corneas in the laboratory, naturally occurring collagen and phospholipids have been bioprinted into robust hydrogels that were laser profiled and patterned to enhance their potential function as artificial substitutes of donor human corneas.[143]

3.4.7. Bioprinted Ear

A bionic ear has been developed by 3D bioprinting of a cell-seeded hydrogel matrix in the anatomic geometry of a human ear, along with an intertwined conducting polymer consisting of infused silver nanoparticles.[144] This study employed in vitro culturing of cartilage tissue around an inductive coil antenna in the ear, which subsequently enabled monitoring of inductively coupled signals from cochlea-shaped electrodes. The printed ear exhibited enhanced auditory sensing for radio-frequency reception, and complementary left and right ears were able to listen to stereo audio music. In another study, 3D bioprinting by a sacrificial layer process was utilized to generate ear-shaped constructs with both auricular cartilage and fat tissue.[145,146] Chondrocytes and adipocytes differentiated from adipose-derived stromal cells were encapsulated in separate hydrogels and then dispensed at particular regions for regeneration of cartilage and fat tissue, respectively.

3.4.8. Bioprinted Adipose Tissue

In an early attempt to produce adipose tissue constructs, adipose-derived stem cells (ASCs) and a gelatin/alginate hydrogel were 3D printed to form cubic 3D constructions ($10 \times 10 \times 10$ mm^3).[147] The ASCs were able to grow, proliferate, and differentiate within these constructions. When basic fibroblast growth factor (bFGF) was added, cells located on the scaffold walls differentiated into endothelial-like cells, whereas cells embedded in the hydrogel differentiated into adipose-like cells. The integrity of the constructions remained intact for more than 60 days. Recently, human adipose tissue matrix has been used as a bio-ink to print adipose tissue constructs.[110] Adipose tissue constructs produced using the adipose dECM bio-ink supported adipogenic differentiation and maturation of encapsulated stem cells as well as the formation of adipose tissue.[126]

The abovementioned studies could potentially prompt the development of pharmaceutical or toxicological models to study the physiology and pathophysiology of particular tissues and organs.

4. Multimodal Characterization of In Vitro Tissue/Organ Models

Characterization of 3D bioprinted tissue/organ constructs is essential for evaluation of their development and function. A variety of characterization techniques have been utilized, such as viability and morphological characterization, biochemical, and biomechanical analysis.

4.1. Viability and Morphology

Cell viability is a critical parameter for the development of tissue models and is the most basic property that needs to be investigated (Figure 4A). Common methods to determine viability involve measurement of the mitochondrial activity by using the MTT assay or its variants.[148] Although these assays are often used as surrogates for cell proliferation in monolayers, they are more suited to establishing viability when dealing with quiescent/senescent cells or adverse conditions.[149] Other methods include measuring levels of calcein AM, which is converted into a fluorescent derivative by intracellular esterases in live cells, or identification of early apoptotic cells by using a combination of annexin V and propidium iodide.[150] However, robust viability assays for 3D environments are still lacking.[151,152]

Morphological analysis of bioprinted and matured tissue constructs are generally carried out through histology and immunohistochemistry.[153] Both have been used for end-point analysis following standard steps such as tissue fixation, drying, slicing, and staining. Various staining methods, such as hematoxylin and eosin (H&E) or Masson's trichrome, are regularly used for histological analysis. Immunohistochemistry (IHC) is more specific and uses antibodies against certain epitopes for the identification and assessment of the distribution of target features within a tissue section.[154] IHC remains a vital component of laboratory testing in the emerging molecular era.[154]

Recently, a map of the human tissue proteome based on quantitative transcriptomics at a tissue and organ level combined with protein profiling using microarray-based IHC was released that showed spatial localization of proteins down to the single-cell level.[155] This resource, known as the human protein atlas (HPA), is particularly useful for the evaluation and validation of tissue and organ models by IHC as it contains over 13 million tissue-based IHC images, each of which have been annotated by pathologists for all sample tissues. Hence, it would be exciting to evaluate the maturation

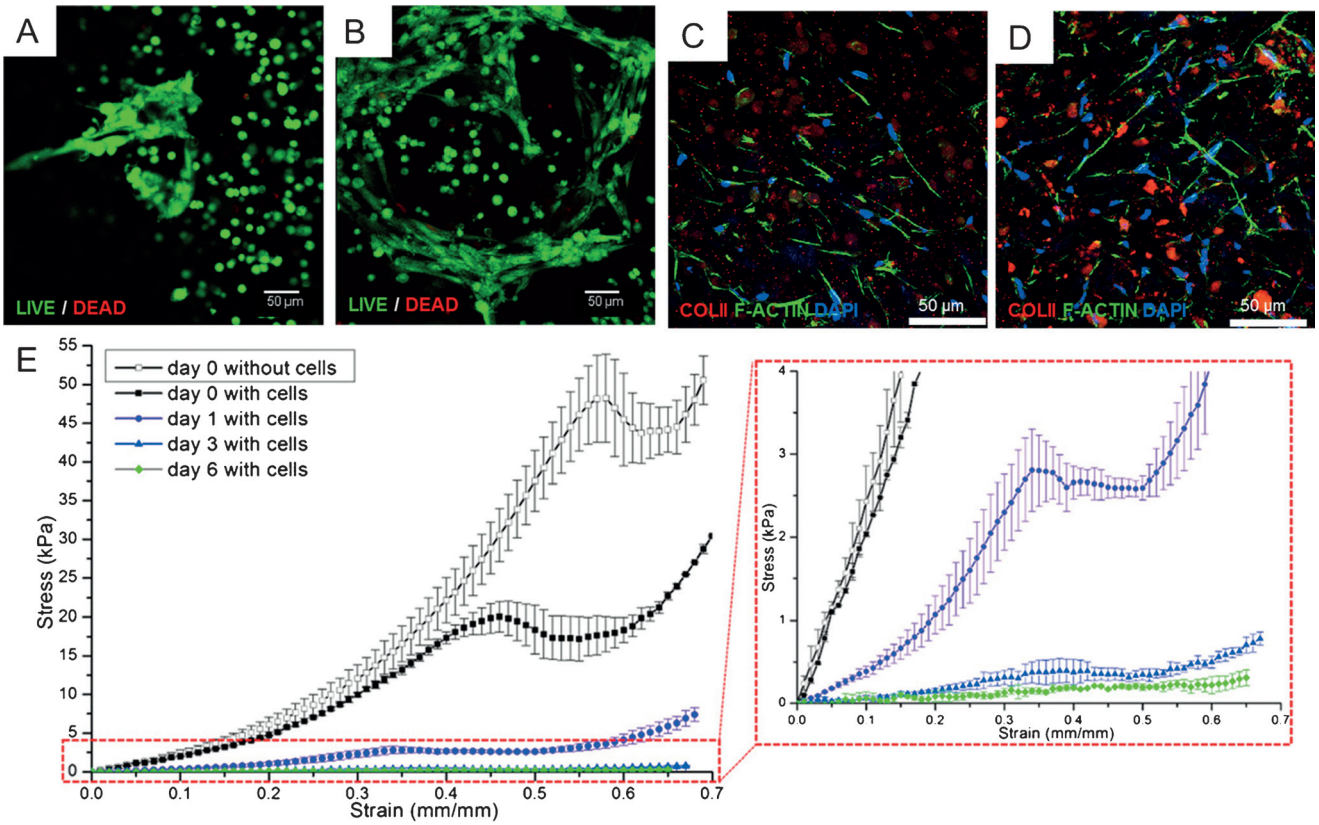


Figure 4. Representative examples of characterization techniques used for the evaluation of the bioprinted tissue constructs. Assessment of the viability of printed human cardiac-derived cardiomyocyte progenitor cells (hCMPCs) in an RGD-modified alginate scaffold after one (A) and two weeks (B) in culture. Evaluation of chondrogenesis within bioprinted cartilage constructs using C) collagen and D) cartilage-derived extracellular matrix bio-ink by immunofluorescence staining, showing collagen type II staining (COLII, red), cell nuclei (DAPI, blue), and F-actin (green). E) Stress–strain curve of bioprinted acellular constructs at day 0 and soft tissue models cultured for a different number of days. Reproduced from Refs. [110, 141, 159] with permission.

of bioprinted tissue/organ models by comparing them with the HPA.

Laser-assisted microdissection (LMD) has been developed to precisely extract cells of interest from a tissue specimen in a rapid and efficient manner.[156] Together with real-time PCR and RT-PCR techniques, it is now feasible to study genetic alterations, gene expression features, and proteins in defined cell populations from complex normal and diseased tissues.[157] Recent progress in automated tissue analysis (tissomics) has allowed reproducible phenotypical characterization of histological specimens. Progress in automated analytics has enabled the acquisition of quantitative data about tissues previously limited to visual histopathology. Such reproducible data sets can be statistically correlated and clustered using bioinformatics. This type of combined approach supports a system-wide view of biology and could potentially facilitate developments toward personalized computational diagnoses.[158]

4.2. Biochemical Analysis

A range of biochemical analyses has routinely been used for the bioprinted tissue models. Among others, genomic and proteomic analyses are mostly performed for the evaluation of tissue/organ models, as they provide an opportunity for hypothesis-free experiments that can yield major insights.[160] Genomic analysis involves the study of the genetic information (DNA, or RNA) of a tissue or organ. Proteomics aims to elucidate protein expression within a tissue/organ.[161,162] Recent genomic and proteomic research has identified many candidate biomarkers, but independent validation of these biomarkers is necessary and reproducibility is still a key concern. Genomic and proteomic screening methods are often used to identify classes of genes or proteins that are differentially expressed in different maturation stages of tissue/organ model development or in vitro disease model progression.[105,110,141,143,163] Differentiation of stem cells into particular lineages has also been evaluated by genomic and proteomic analysis.[110,115]

Evaluation of the function of an engineered tissue can also be carried out by estimating target proteins (Figure 4B). In a liver model that contained a layered coculture of hepatocytes and ECs, functional secretion and uptake was measured using a fluorescent bile acid analogue, which confirmed functional hepatocytes in the cocultures.[164] In the case of a kidney model, enzymes such as cathepsin and alkaline phosphatase were evaluated by measuring the presence of metabolites and cytokines to validate the ability of the model to respond to drug toxicity.[165]

Several other biochemical analyses should be performed for evaluating the function of engineered tissue constructs. For example, estimation of urea, albumin, bilirubin, and transaminases are routinely performed for in vitro liver tissue.[166] Typically, biochemical analysis needs high cell density and often additional steps are required to separate the cells from the matrix.[167]

4.3. Biomechanical Analysis

Biomechanical characterization of tissue/organ models is of paramount importance as the structure and stiffness of bioprinted matrices influence the cell geometry, which in turn affects the differentiation fate and maturation stage.[168–170] The mechanical properties of the bioprinted models can be evaluated through compression tests at various culture times (Figure 4E), and their applicability can be examined by fitting the experimental data with suitable mathematical models such as the Ogden model.[159] The mechanical and physicochemical effects of the chosen 3D hydrogel structure have a vital effect on cell behavior and should be considered before manufacturing functional tissues.[139] Increasing evidence has suggested that mechanical stress, as well as other physical factors, may significantly increase the biosynthetic activity of cells in bio-artificial matrices.[171]

Recent studies have also highlighted the importance of the matrix density as an instructive signal for the differentiation of stem cells.[170] Thus, tissue/organ models should approximate the mechanical properties of the target tissue.[172] Interestingly, osteogenic differentiation was observed preferentially in anisotropic, soft, collagen-rich substrates, whereas adipogenic differentiation was mostly observed in isotropic, stiff, agarose-rich matrices.[139]

Cell stiffness may be a useful biomarker for evaluating the relative metastatic potential of cancer cells. One model has shown that metastatic ovarian cancer cells are less stiff than normal cells, which may indicate more flexibility and invasiveness.[173] Hence, biomechanical analysis of the tissue/organ models could facilitate a better understanding of the importance of mechanobiology in tissue/organ development.

Nevertheless, novel characterization techniques are required for 3D tissue/organ models. There is also a need for the development of standard procedures, new tools, and quantitative analysis methods, including appropriate 3D imaging techniques.

5. 3D Bioprinted Tissue/Organ Models for Drug Discovery and Toxicological Screening

3D matrices with well-defined microarchitectures and tissue-like cellular distribution would allow excellent cell–cell and cell–ECM interactions,[14] and consequently show a similar response to drug compounds as native tissues. Advances in 3D printing have enabled the direct and rapid production of structures that mimic specific tissue morphology and features. Once built, the bioprinted tissue shares many features with the native tissue, including tissue-like cellular density, presence of multiple cell types, and key architectural and functional aspects. Hence, 3D bioprinted tissues can help to accelerate the drug discovery process by allowing the testing of new and promising drugs on functional human tissues during hit-to-lead (H2L) and lead optimization stages of drug development.[3,174,175]

Advantages of 3D bioprinted tissue/organ models

- Automated and reproducible production of tissue constructs
- Creation of a cell-specific microenvironment
- Generation of tissue-specific architecture
- Evaluation of drugs/chemicals on human-specific models
- Integration of microfluidics with 3D structure
- Fabrication of vascularized tissue constructs
- Longer cell viability and functionality
- Possibility of building tissue–tissue interfaces

The feasibility of the 3D bioprinting process has been demonstrated by the construction of a physiologically relevant pharmacokinetic model.[176] In this approach, 3D bioprinted cell-encapsulated hydrogel-based tissue constructs were directly integrated onto a microfluidic device for continuous perfusion drug flow. Characterization of the 3D tissue constructs showed predictable outcomes in terms of cell viability/proliferation and enhanced functionality over traditional culture methods. Organovo Holdings, a biotech firm from San Diego that designs and creates functional 3D human tissues, is currently developing 3D printed tissues for pharmaceutical and toxicological screening. Recently, Organovo delivered its first 3D liver tissue for experimentation, thereby making a breakthrough toward commercial launch of a 3D model of liver tissue.[177] An engineered 3D model of the microvasculature system of the brain was developed by fabricating an array of microchannels comprising collagen I by using microneedles and a 3D printed frame.[178] By culturing mouse brain endothelial cells (bEnd.3) on the luminal surface of cylindrical collagen microchannels, brain microvasculature was reconstructed in vitro with circular cross-sections. This model of the blood–brain barrier could be used for physiological and pathological studies and also for pharmaceutical applications.

Human physiology is tightly regulated by cross-talk between multiple organs including the brain, gut, muscle, liver, and adipose, and this complex control system is of vital importance for preclinical drug testing. For example, there is metabolic cross-talk between the heart and liver which has an impact on familial hypertrophic cardiomyopathy.[179] Cross-talk between adipose tissue and liver through adiponectin is well established.[180] However, the cross-talk that occurs between other organs such as, for example, the liver and muscles, has not been well explored. Currently, these studies are done by using several mouse models.[180] However, 3D bioprinting could present a promising alternative by generating multiple organs connected to each other. This could be particularly beneficial for studying the cross-talk during states of liver diseases, such as fatty liver or steatosis, and could be a target for developing a treatment for liver pathophysiology.

6. In Vitro Disease/Tumor Model

The most effective way of studying tumors and evaluation of antineoplastic agents is clinical trials. However, this method is not commonly used because of ethical and safety concerns. Therefore, preclinical tumor models are being developed that mimic the physiological environments of tumors.[163,181,182] In vitro 3D tumor models based on human cancer cells could be beneficial to accurately reproduce the characteristics of human cancer tissues.[163,183] Various techniques, such as multicellular spheroids, cell-seeding 3D scaffolds, hydrogel embedding, microfluidic chips, and cell patterning, have been explored for the construction of 3D tumor models in vitro.[163] However, simulating the complex 3D physiological tumor microenvironment and developing realistic 3D tumor models is difficult using the abovementioned techniques. Advances in 3D printing have offered an opportunity for the biofabrication of complex structures with simulated pathophysiological microenvironments to construct in vitro disease/tumor models to aid the study of disease pathogenesis.[184] Although there are very few reports to date concerning the 3D printing of tumor models, the following study by Zhao et al. clearly demonstrates the great possibilities of this technique.

3D printing of Hela cells in gelatin/alginate/fibrinogen hydrogels was utilized for the development of in vitro models of cervical tumors. When compared with conventional 2D planar culture models, Hela cells showed a higher proliferation rate in the printed 3D environment and were inclined to form cellular spheroids, whereas only monolayer cell sheets formed in 2D cultures. Hela cells in 3D printed models also showed higher expression of matrix metalloprotease (MMP) protein and chemoresistance than those in 2D cultures.[185] The new biological characteristics of printed 3D tumor models in vitro combined with the novel 3D cell printing technology is likely to considerably advance the 3D study of cancer in the future.

7. Future Directions

3D bioprinting of tissue and organ models is a vibrant research area in which several pioneering results have been obtained over the last few years. The range of available 3D bioprinting techniques has the potential to facilitate the development of realistic tissue/organ models. Researchers have used inkjet printing technology for cell-based gene therapies to show that transfecting genes into cells is possible together with precise delivery of modified cells to a given target, thereby producing genetically modified cells to suit a particular application.[186] Within the next few years, 3D bioprinting is expected to advance to meet the needs of specific applications such as models for pharmaceutical/toxicological screening. Bioprinting has the potential to revolutionize the way drugs are being tested, as well as reducing the cost and time of drug discovery by allowing rapid identification of potential candidates or substances toxic to human tissues.

Most published results were at the early stages of tissue/organ development. Only a few studies have thoroughly investigated the process parameters to derive either predictions or optimization strategies in a systematic way. For the successful application of tissues and organs as in vitro models, standardization and optimization of the printing process with respect to the final demand are necessary in addition to

complying with good manufacturing practice (GMP). Hence, there is a great need for studies targeted toward understanding structure and function relationships of the process parameters of the printed constructs. Moreover, modern fabrication schemes rely on mathematical modeling and computer simulations for optimizing the process design and making predictions.[184,187] The performance and function of tissue constructs can be predicted, and hence improved, before printing using computer simulations. However, this approach needs more attention to be specific to the use of 3D tissues/organs.

Stem cells have an essential role in the construction of 3D tissue because they offer great potential for creating complex constructs, as highlighted by various research groups.[95,184,188] However, various issues need to be resolved before stem cells can be actually used for 3D bioprinting, such as optimization of the cellular microenvironment to combine the advantages of cell attachment, cell stimulation, and mechanical stability to mimic the in vivo environment to the highest degree.[189]

8. Conclusions

The investigation of new methods/techniques to generate realistic 3D tissue/organ models by using complex designs through 3D bioprinting represents an active area of biomedical research. Although the described techniques are still in their infancy, they offer great potential to overcome many challenges associated with the production of complex tissues and organs. These techniques are promising tools for progressively replacing current often misleading, time-consuming, and personnel-dependent, real tissue-based or animal-based pharmaceutical and toxicological assays. However, the interaction and collaboration of researchers from various fields are needed to overcome several hurdles before this technology will have widespread acceptance and impact.

Acknowledgements

The presented research has received funding from the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement No. 615458 and the Swedish Research Council and the KTH Center for Applied Proteomics funded by the Erling-Persson Family Foundation.

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 4650–4665
Angew. Chem. **2016**, *128*, 4728–4743

[1] A. Birgersdotter, R. Sandberg, I. Ernberg, *Semin. Cancer Biol.* **2005**, *15*, 405–412.
[2] E. Cukierman, R. Pankov, K. M. Yamada, *Curr. Opin. Cell Biol.* **2002**, *14*, 633–640.
[3] L. G. Griffith, M. A. Swartz, *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 211–224.
[4] C. M. Nelson, M. J. Bissell, *Annu. Rev. Cell Dev. Biol.* **2006**, *22*, 287–309.
[5] Z. A. Knight, K. M. Shokat, *Cell* **2007**, *128*, 425–430.
[6] T. Gura, *Science* **1997**, *278*, 1041–1042.
[7] Reuters (2011) U.S. to develop chip that tests if a drug is toxic. Reuters, October 6 http://www.msnbc.msn.com/id/44554007/ns/health-health care/-.To5AMnPaixF.
[8] J. Roix, S. Saha, *BMC Nephrol.* **2013**, *14*, 233.
[9] F. J. van der Staay, S. Arndt, R. Nordquist, *Behav. Brain Funct.* **2009**, *5*, 11.
[10] K. Kieburtz, C. W. Olanow, *Mt. Sinai J. Med.* **2007**, *74*, 7–14.
[11] Z. Liu, K. Maas, T. M. Aune, *Clin. Immunol.* **2004**, *112*, 225–230.
[12] A. Rangarajan, S. J. Hong, A. Gifford, R. A. Weinberg, *Cancer Cell* **2004**, *6*, 171–183.
[13] M. H. V. V. Regenmortel, *EMBO Rep.* **2004**, *5*, 1016–1020.
[14] F. Pampaloni, E. G. Reynaud, E. H. K. Stelzer, *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 839–845.
[15] R. Mroue, M. Bissell, in *Epithelial Cell Culture Protocols*, *Vol. 945* (Eds.: S. H. Randell, M. L. Fulcher), Humana Press, Totowa, NJ, **2013**, pp. 221–250.
[16] T. Sato, H. Clevers, *Science* **2013**, *340*, 1190–1194.
[17] M. A. Lancaster, M. Renner, C.-A. Martin, D. Wenzel, L. S. Bicknell, M. E. Hurles, T. Homfray, J. M. Penninger, A. P. Jackson, J. A. Knoblich, *Nature* **2013**, *501*, 373–379.
[18] S. N. Bhatia, D. E. Ingber, *Nat. Biotechnol.* **2014**, *32*, 760–772.
[19] T. Mammoto, A. Mammoto, D. E. Ingber, *Annu. Rev. Cell Dev. Biol.* **2013**, *29*, 27–61.
[20] D. Ingber, *Ann. Med.* **2003**, *35*, 564–577.
[21] N. E. Fedorovich, J. R. De Wijn, A. J. Verbout, J. Alblas, W. J. Dhert, *Tissue Eng. Part A* **2008**, *14*, 127–133.
[22] B. Derby, *Science* **2012**, *338*, 921–926.
[23] J. E. Snyder, Q. Hamid, C. Wang, R. Chang, K. Emami, H. Wu, W. Sun, *Biofabrication* **2011**, *3*, 034112.
[24] T. M. Keenan, A. Folch, *Lab Chip* **2008**, *8*, 34–57.
[25] S. M. Kim, S. H. Lee, K. Y. Suh, *Lab Chip* **2008**, *8*, 1015–1023.
[26] I. Meyvantsson, J. W. Warrick, S. Hayes, A. Skoien, D. J. Beebe, *Lab Chip* **2008**, *8*, 717–724.
[27] H. Park, C. Cannizzaro, G. Vunjak-Novakovic, R. Langer, C. A. Vacanti, O. C. Farokhzad, *Tissue Eng.* **2007**, *13*, 1867–1877.
[28] W. Sun, P. Lal, *Comput. Methods Programs Biomed.* **2002**, *67*, 85–103.
[29] E. L. LeCluyse, *Eur. J. Pharm. Sci.* **2001**, *13*, 343–368.
[30] A. Sivaraman, J. K. Leach, S. Townsend, T. Iida, B. J. Hogan, D. B. Stolz, R. Fry, L. D. Samson, S. R. Tannenbaum, L. G. Griffith, *Curr. Drug Metab.* **2005**, *6*, 569–591.
[31] E. L. LeCluyse, P. L. Bullock, A. Parkinson, *Adv. Drug Delivery Rev.* **1996**, *22*, 133–186.
[32] S. Cai, X. Fu, Z. Sheng, *BioScience* **2007**, *57*, 655–662.
[33] J. T. Emerman, S. J. Burwen, D. R. Pitelka, *Tissue Cell* **1979**, *11*, 109–119.
[34] E. Y. Lee, W. H. Lee, C. S. Kaetzel, G. Parry, M. J. Bissell, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 1419–1423.
[35] C. H. Streuli, M. J. Bissell, *J. Cell Biol.* **1990**, *110*, 1405–1415.
[36] O. W. Petersen, L. Rønnov-Jessen, A. R. Howlett, M. J. Bissell, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9064–9068.
[37] J. C. Dunn, M. L. Yarmush, H. G. Koebe, R. G. Tompkins, *FASEB J.* **1989**, *3*, 174–177.
[38] K. Mathijs, A. S. Kienhuis, K. J. J. Brauers, D. G. J. Jennen, A. Lahoz, J. C. S. Kleinjans, J. H. M. van Delft, *Drug Metab. Dispos.* **2009**, *37*, 1305–1311.
[39] L. G. Griffith, B. E. N. Wu, M. J. Cima, M. J. Powers, B. Chaignaud, J. P. Vacanti, *Ann. N. Y. Acad. Sci.* **1997**, *831*, 382–397.
[40] L. Kimlin, J. Kassis, V. Virador, *Expert Opin. Drug Discovery* **2013**, *8*, 1455–1466.
[41] P. M. Baptista, M. M. Siddiqui, G. Lozier, S. R. Rodriguez, A. Atala, S. Soker, *Hepatology* **2011**, *53*, 604–617.
[42] J. Lee, G. D. Lilly, R. C. Doty, P. Podsiadlo, N. A. Kotov, *Small* **2009**, *5*, 1213–1221.

[43] C. Guguen-Guillouzo, A. Guillouzo, in *Hepatocytes*, *Vol. 640* (Ed.: P. Maurel), Humana Press, Totowa, NJ, **2010**, pp. 1 – 40.
[44] S. Kaihara, S. Kim, B.-S. Kim, D. J. Mooney, K. Tanaka, J. P. Vacanti, *J. Pediatr. Surg.* **2000**, *35*, 1287 – 1290.
[45] A. J. Hwa, R. C. Fry, A. Sivaraman, P. T. So, L. D. Samson, D. B. Stolz, L. G. Griffith, *FASEB J.* **2007**, *21*, 2564 – 2579.
[46] K. Domansky, W. Inman, J. Serdy, A. Dash, M. H. M. Lim, L. G. Griffith, *Lab Chip* **2010**, *10*, 51 – 58.
[47] A. Dash, W. Inman, K. Hoffmaster, S. Sevidal, J. Kelly, R. S. Obach, L. G. Griffith, S. R. Tannenbaum, *Expert Opin. Drug Metab. Toxicol.* **2009**, *5*, 1159 – 1174.
[48] N. T. Elliott, F. Yuan, *J. Pharm. Sci.* **2011**, *100*, 59 – 74.
[49] A. Y. Hsiao, Y.-s. Torisawa, Y.-C. Tung, S. Sud, R. S. Taichman, K. J. Pienta, S. Takayama, *Biomaterials* **2009**, *30*, 3020 – 3027.
[50] A. P. Wong, R. Perez-Castillejos, J. Christopher Love, G. M. Whitesides, *Biomaterials* **2008**, *29*, 1853 – 1861.
[51] P. J. Lee, T. A. Gaige, N. Ghorashian, P. J. Hung, *Biotechnol. Prog.* **2007**, *23*, 946 – 951.
[52] A. Tourovskaia, X. Figueroa-Masot, A. Folch, *Lab Chip* **2005**, *5*, 14 – 19.
[53] E. W. Esch, A. Bahinski, D. Huh, *Nat. Rev. Drug Discovery* **2015**, *14*, 248 – 260.
[54] M.-C. Shih, S.-H. Tseng, Y.-S. Weng, I. M. Chu, C.-H. Liu, *Biomed. Microdevices* **2013**, *15*, 767 – 780.
[55] F. P. W. Melchels, M. A. N. Domingos, T. J. Klein, J. Malda, P. J. Bartolo, D. W. Hutmacher, *Prog. Polym. Sci.* **2012**, *37*, 1079 – 1104.
[56] V. Mironov, G. Prestwich, G. Forgacs, *J. Mater. Chem.* **2007**, *17*, 2054 – 2060.
[57] V. Mironov, N. Reis, B. Derby, *Tissue Eng.* **2006**, *12*, 631 – 634.
[58] M. Nakamura, S. Iwanaga, C. Henmi, K. Arai, Y. Nishiyama, *Biofabrication* **2010**, *2*, 014110.
[59] V. Mironov, R. R. Markwald, G. Forgacs, *Sci. Med.* **2003**, *9*, 69 – 71.
[60] M. G. Li, X. Y. Tian, X. B. Chen, *Biofabrication* **2009**, *1*, 032001.
[61] X. Wang, Y. Yan, R. Zhang, *Tissue Eng. Part B* **2010**, *16*, 189 – 197.
[62] S. V. Murphy, A. Atala, *Nat. Biotechnol.* **2014**, *32*, 773 – 785.
[63] V. Mironov, R. P. Visconti, V. Kasyanov, G. Forgacs, C. J. Drake, R. R. Markwald, *Biomaterials* **2009**, *30*, 2164 – 2174.
[64] T. S. Little, V. Mironov, A. Nagy Mehesz, R. Markwald, Y. Sugi, S. M. Lessner, M. A. Sutton, X. Liu, Q. Wang, X. Yang, J. O. Blancette, M. Skiles, *Biofabrication* **2011**, *3*, 030202.
[65] V. Mironov, V. Kasyanov, R. R. Markwald, *Curr. Opin. Biotechnol.* **2011**, *22*, 667 – 673.
[66] N. E. Fedorovich, J. Alblas, W. E. Hennink, F. C. Oner, W. J. Dhert, *Trends Biotechnol.* **2011**, *29*, 601 – 606.
[67] S. Catros, J. C. Fricain, B. Guillotin, B. Pippenger, R. Bareille, M. Remy, E. Lebraud, B. Desbat, J. Amedee, F. Guillemot, *Biofabrication* **2011**, *3*, 025001.
[68] M. Gruene, C. Unger, L. Koch, A. Deiwick, B. Chichkov, *Biomed. Eng. Online* **2011**, *10*, 19.
[69] B. Guillotin, A. Souquet, S. Catros, M. Duocastella, B. Pippenger, S. Bellance, R. Bareille, M. Remy, L. Bordenave, J. Amedee, F. Guillemot, *Biomaterials* **2010**, *31*, 7250 – 7256.
[70] L. Koch, A. Deiwick, S. Schlie, S. Michael, M. Gruene, V. Coger, D. Zychlinski, A. Schambach, K. Reimers, P. M. Vogt, B. Chichkov, *Biotechnol. Bioeng.* **2012**, *109*, 1855 – 1863.
[71] M. Gruene, M. Pflaum, A. Deiwick, L. Koch, S. Schlie, C. Unger, M. Wilhelmi, A. Haverich, B. N. Chichkov, *Biofabrication* **2011**, *3*, 015005.
[72] T. Boland, T. Xu, B. Damon, X. Cui, *Biotechnol. J.* **2006**, *1*, 910 – 917.
[73] B. R. Ringeisen, C. M. Othon, J. A. Barron, D. Young, B. J. Spargo, *Biotechnol. J.* **2006**, *1*, 930 – 948.
[74] Q. Zheng, J. Lu, H. Chen, L. Huang, J. Cai, Z. Xu, *Anal. Biochem.* **2011**, *410*, 171 – 176.
[75] E. D. Miller, J. A. Phillippi, G. W. Fisher, P. G. Campbell, L. M. Walker, L. E. Weiss, *Comb. Chem. High Throughput Screening* **2009**, *12*, 604 – 618.
[76] S. Ilkhanizadeh, A. I. Teixeira, O. Hermanson, *Biomaterials* **2007**, *28*, 3936 – 3943.
[77] E. D. Miller, G. W. Fisher, L. E. Weiss, L. M. Walker, P. G. Campbell, *Biomaterials* **2006**, *27*, 2213 – 2221.
[78] E. A. Roth, T. Xu, M. Das, C. Gregory, J. J. Hickman, T. Boland, *Biomaterials* **2004**, *25*, 3707 – 3715.
[79] T. Xu, J. Jin, C. Gregory, J. J. Hickman, T. Boland, *Biomaterials* **2005**, *26*, 93 – 99.
[80] M. E. Pepper, C. A. Parzel, T. Burg, T. Boland, K. J. Burg, R. E. Groff, *Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, IEEE Engineering in Medicine and Biology Society, **2009**, 6001 – 6005.
[81] M. E. Pepper, C. A. Cass, J. P. Mattimore, T. Burg, B. W. Booth, K. J. Burg, R. E. Groff, *Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, IEEE Engineering in Medicine and Biology Society, **2010**, 259 – 262.
[82] Y. Nishiyama, M. Nakamura, C. Henmi, K. Yamaguchi, S. Mochizuki, H. Nakagawa, K. Takiura, *J. Biomech. Eng.* **2009**, *131*, 035001.
[83] K. W. Binder, W. Zhao, T. Aboushwareb, D. Dice, A. Atala, J. J. Yoo, *J. Am. Coll. Surg.*, **2010**, *211*, S76 .
[84] A. Tirella, F. Vozzi, C. De Maria, G. Vozzi, T. Sandri, D. Sassano, L. Cognolato, A. Ahluwalia, *J. Biosci. Bioeng.* **2011**, *112*, 79 – 85.
[85] S. Parsa, M. Gupta, F. Loizeau, K. C. Cheung, *Biofabrication* **2010**, *2*, 025003.
[86] J. Malda, J. Visser, F. P. Melchels, T. Jüngst, W. E. Hennink, W. J. A. Dhert, J. Groll, D. W. Hutmacher, *Adv. Mater.* **2013**, *25*, 5011 – 5028.
[87] K. Jakab, C. Norotte, F. Marga, K. Murphy, G. Vunjak-Novakovic, G. Forgacs, *Biofabrication* **2010**, *2*, 022001.
[88] N. E. Fedorovich, I. Swennen, J. Girones, L. Moroni, C. A. van Blitterswijk, E. Schacht, J. Alblas, W. J. A. Dhert, *Biomacromolecules* **2009**, *10*, 1689 – 1696.
[89] L. Pescosolido, W. Schuurman, J. Malda, P. Matricardi, F. Alhaique, T. Coviello, P. R. van Weeren, W. J. A. Dhert, W. E. Hennink, T. Vermonden, *Biomacromolecules* **2011**, *12*, 1831 – 1838.
[90] A. Skardal, J. Zhang, L. McCoard, X. Xu, S. Oottamasathien, G. D. Prestwich, *Tissue Eng. Part A* **2010**, *16*, 2675 – 2685.
[91] K. Buyukhatipoglu, W. Jo, W. Sun, A. M. Clyne, *Biofabrication* **2009**, *1*, 035003.
[92] J. H. Shim, J. Y. Kim, M. Park, J. Park, D. W. Cho, *Biofabrication* **2011**, *3*, 034102.
[93] C. Norotte, F. S. Marga, L. E. Niklason, G. Forgacs, *Biomaterials* **2009**, *30*, 5910 – 5917.
[94] D. E. Ingber, V. C. Mow, D. Butler, L. Niklason, J. Huard, J. Mao, I. Yannas, D. Kaplan, G. Vunjak-Novakovic, *Tissue Eng.* **2006**, *12*, 3265.
[95] J. W. Nichol, A. Khademhosseini, *Soft Matter* **2009**, *5*, 1312 – 1319.
[96] V. Mironov, T. Boland, T. Trusk, G. Forgacs, R. R. Markwald, *Trends Biotechnol.* **2003**, *21*, 157 – 161.
[97] N. E. Fedorovich, J. Alblas, J. R. d. Wijn, W. E. Hennink, A. J. Verbout, W. J. A. Dhert, *Tissue Eng.* **2007**, *13*, 1905 – 1925.
[98] P. X. Ma, *Adv. Drug Delivery Rev.* **2008**, *60*, 184 – 198.
[99] F. Rosso, A. Giordano, M. Barbarisi, A. Barbarisi, *J. Cell. Physiol.* **2004**, *199*, 174 – 180.
[100] J.-H. Shim, J.-S. Lee, J. Y. Kim, D.-W. Cho, *J. Micromech. Microeng.* **2012**, *22*, 085014.
[101] J.-H. Shim, J.-B. Huh, J. Y. Park, Y.-C. Jeon, S. S. Kang, J. Y. Kim, J.-W. Rhie, D.-W. Cho, *Tissue Eng. Part A* **2013**, *19*, 317 – 327.

[102] L. Shor, S. Güçeri, R. Chang, J. Gordon, Q. Kang, L. Hartsock, Y. An, W. Sun, *Biofabrication* **2009**, *1*, 015003.
[103] M. Domingos, F. Intranuovo, A. Gloria, R. Gristina, L. Ambrosio, P. J. Bártolo, P. Favia, *Acta Biomater.* **2013**, *9*, 5997 – 6005.
[104] M. O. Wang, C. E. Vorwald, M. L. Dreher, E. J. Mott, M.-H. Cheng, A. Cinar, H. Mehdizadeh, S. Somo, D. Dean, E. M. Brey, J. P. Fisher, *Adv. Mater.* **2015**, *27*, 138 – 144.
[105] D. Kaigler, E. Silva, D. Mooney, *J. Periodontol.* **2013**, *84*, 230 – 238.
[106] S. Wüst, M. E. Godla, R. Müller, S. Hofmann, *Acta Biomater.* **2014**, *10*, 630 – 640.
[107] N. E. Fedorovich, E. Kuipers, D. Gawlitta, W. J. A. Dhert, J. Alblas, *Tissue Eng A* **2011**, 17, 2473 – 2486.
[108] N. E. Fedorovich, W. Schuurman, H. M. Wijnberg, H. J. Prins, P. R. van Weeren, J. Malda, J. Alblas, W. J. Dhert, *Tissue Eng. Part C* **2012**, *18*, 33 – 44.
[109] J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar, A. Khademhosseini, *Biomaterials* **2010**, *31*, 5536 – 5544.
[110] F. Pati, J. Jang, D.-H. Ha, S. W. Kim, J.-W. Rhie, J.-H. Shim, D.-H. Kim, D.-W. Cho, *Nat. Commun.* **2014**, *5*, 3935.
[111] X. Wang, Y. Yan, Y. Pan, Z. Xiong, H. Liu, J. Cheng, F. Liu, F. Lin, R. Wu, R. Zhang, Q. Lu, *Tissue Eng.* **2006**, *12*, 83 – 90.
[112] R. Chang, J. Nam, W. Sun, *Tissue Eng. Part A* **2008**, *14*, 41 – 48.
[113] Y. Yan, X. Wang, Z. Xiong, H. Liu, F. Liu, F. Lin, R. Wu, R. Zhang, Q. Lu, *J. Bioact. Compat. Polym.* **2005**, *20*, 259 – 269.
[114] W. Xu, X. Wang, Y. Yan, W. Zheng, Z. Xiong, F. Lin, R. Wu, R. Zhang, *J. Bioact. Compat. Polym.* **2007**, *22*, 363 – 377.
[115] S. Das, F. Pati, Y.-J. Choi, G. Rijal, J.-H. Shim, S. W. Kim, A. R. Ray, D.-W. Cho, S. Ghosh, *Acta Biomater.* **2015**, *11*, 233 – 246.
[116] R. Devillard, E. Pagès, M. M. Correa, V. Kériquel, M. Rémy, J. Kalisky, M. Ali, B. Guillotin, F. Guillemot, in *Methods in Cell Biology, Vol. Volume 119* (Eds.: P. Matthieu, T. Manuel), Academic Press, **2014**, pp. 159 – 174..
[117] P. Zorlutuna, J. H. Jeong, H. Kong, R. Bashir, *Adv. Funct. Mater.* **2011**, *21*, 3597 – 3597.
[118] B. Derby, *J. Mater. Chem.* **2008**, *18*, 5717-5721..
[119] S. C. Owen, M. S. Shoichet, *J. Biomed. Mater. Res. Part A* **2010**, *94*, 1321 – 1331.
[120] S. Liao, C. K. Chan, S. Ramakrishna, *Mater. Sci. Eng. C* **2008**, *28*, 1189 – 1202.
[121] K. Lee, E. A. Silva, D. J. Mooney, *J. R. Soc. Interface* **2011**, *8*, 153 – 170.
[122] M. A. Swartz, *Curr. Opin. Biotechnol.* **2003**, *14*, 547 – 550.
[123] T. L. Sellaro, A. Ranade, D. M. Faulk, G. P. McCabe, K. Dorko, S. F. Badylak, S. C. Strom, *Tissue Eng. Part A* **2010**, *16*, 1075 – 1082.
[124] T. H. Petersen, E. A. Calle, L. Zhao, E. J. Lee, L. Gui, M. B. Raredon, K. Gavrilov, T. Yi, Z. W. Zhuang, C. Breuer, E. Herzog, L. E. Niklason, *Science* **2010**, *329*, 538 – 541.
[125] B. E. Uygun, A. Soto-Gutierrez, H. Yagi, M.-L. Izamis, M. A. Guzzardi, C. Shulman, J. Milwid, N. Kobayashi, A. Tilles, F. Berthiaume, M. Hertl, Y. Nahmias, M. L. Yarmush, K. Uygun, *Nat. Med.* **2010**, *16*, 814 – 821.
[126] F. Pati, D.-H. Ha, J. Jang, H. H. Han, J.-W. Rhie, D.-W. Cho, *Biomaterials* **2015**, *62*, 164 – 175.
[127] L. Vivian, S. Gurtej, T. J. P, B. Chris, X. Xiawei, T. T. Nga, Y. Seung-Schik, D. Guohao, K. Pankaj, *Tissue Eng. Part C* **2014**, *20*, 473 – 484.
[128] S. Michael, H. Sorg, C.-T. Peck, L. Koch, A. Deiwick, B. Chichkov, P. M. Vogt, K. Reimers, *PLoS ONE* **2013**, *8*, e57741.
[129] L. Koch, S. Kuhn, H. Sorg, M. Gruene, S. Schlie, R. Gaebel, B. Polchow, K. Reimers, S. Stoelting, N. Ma, P. M. Vogt, G. Steinhoff, B. Chichkov, *Tissue Eng. Part C* **2010**, *16*, 847 – 854.
[130] K. Schmidt-Nielsen, *J. Exp. Zoo.* **1975**, *194*, 287 – 307.
[131] A. Skardal, J. Zhang, G. D. Prestwich, *Biomaterials* **2010**, *31*, 6173 – 6181.
[132] D. B. Kolesky, R. L. Truby, A. S. Gladman, T. A. Busbee, K. A. Homan, J. A. Lewis, *Adv. Mater.* **2014**, *26*, 3124 – 3130.
[133] J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, S. N. Bhatia, C. S. Chen, *Nat. Mater.* **2012**, *11*, 768 – 774.
[134] X. Cui, T. Boland, *Biomaterials* **2009**, *30*, 6221 – 6227.
[135] C. Khatiwala, R. Law, B. Shepherd, S. Dorfman, M. Csete, *Gene Ther. Regul.* **2012**, *07*, 1230004.
[136] J. B. Robbins, V. Gorgen, P. Min, B. R. Shepherd, S. C. Presnell, *FASEB J.* **2013**, *27*, 872.12 .
[137] R. Chang, K. Emami, H. Wu, W. Sun, *Biofabrication* **2010**, *2*, 045004.
[138] G. Gao, T. Yonezawa, K. Hubbell, G. Dai, X. Cui, *Biotechnol. J.* **2015**, *10*, 1568 – 1577.
[139] D. F. Duarte Campos, A. Blaeser, A. Korsten, S. Neuss, J. Jäkel, M. Vogt, H. Fischer, *Tissue Eng. Part A* **2015**, *21*, 740 – 756.
[140] L. A. Hockaday, K. H. Kang, N. W. Colangelo, P. Y. C. Cheung, B. Duan, E. Malone, J. Wu, L. N. Girardi, L. J. Bonassar, H. Lipson, C. C. Chu, J. T. Butcher, *Biofabrication* **2012**, *4*, 035005.
[141] R. Gaetani, P. A. Doevendans, C. H. G. Metz, J. Alblas, E. Messina, A. Giacomello, J. P. G. Sluijter, *Biomaterials* **2012**, *33*, 1782 – 1790.
[142] P. Xie, Z. Hu, X. Zhang, X. Li, Z. Gao, D. Yuan, Q. Liu, *PLoS ONE* **2014**, *9*, e109373.
[143] M. Mirazul Islam, V. Cėpla, C. He, J. Edin, T. Rakickas, K. Kobuch, Ž. Ruželė, W. Bruce Jackson, M. Rafat, C. P. Lohmann, R. Valiokas, M. Griffith, *Acta Biomater.* **2015**, *12*, 70 – 80.
[144] M. S. Mannoor, Z. Jiang, T. James, Y. L. Kong, K. A. Malatesta, W. O. Soboyejo, N. Verma, D. H. Gracias, M. C. McAlpine, *Nano Lett.* **2013**, *13*, 2634 – 2639.
[145] J.-S. Lee, J. M. Hong, J. W. Jung, J.-H. Shim, J.-H. Oh, D.-W. Cho, *Biofabrication* **2014**, *6*, 024103.
[146] F. Pati, J.-H. Shim, J.-S. Lee, D.-W. Cho, *Manuf. Lett.* **2013**, *1*, 49 – 53.
[147] Y. Rui, Z. Renji, Y. Yongnian, W. Xiaohong, *J. Bioact. Compat. Polym.* **2009**, *24*, 5 – 24.
[148] M. V. Berridge, P. M. Herst, A. S. Tan, in *Biotechnology Annual Review, Vol. 11* (Ed.: M. R. El-Gewely), Elsevier, Amsterdam, **2005**, pp. 127 – 152.
[149] L. Crigler, A. Kazhanie, T.-J. Yoon, J. Zakhari, J. Anders, B. Taylor, V. M. Virador, *FASEB J.* **2007**, *21*, 2050 – 2063.
[150] A. Astashkina, B. Mann, D. W. Grainger, *Pharmacol. Ther.* **2012**, *134*, 82 – 106.
[151] N. Arya, V. Sardana, M. Saxena, A. Rangarajan, D. S. Katti, *J. R. Soc. Interface* **2012**, *9*, 3288 – 3302.
[152] L. Huyck, C. Ampe, M. Van Troys, *Assay Drug Dev. Technol.* **2012**, *10*, 382 – 392.
[153] H. Fatakdawala, L. G. Griffiths, S. Humphrey, L. Marcu, *J. Biomed. Opt.* **2014**, *19*, 080503.
[154] C. Idleburg, E. DeLassus, D. Novack, in *Osteoporosis and Osteoarthritis, Vol. 1226* (Eds.: J. J. Westendorf, A. J. van Wijnen), Springer, New York, **2015**, pp. 87 – 95.
[155] M. Uhlén, L. Fagerberg, B. M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, Å. Sivertsson, C. Kampf, E. Sjöstedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C. A.-K. Szigyarto, J. Odeberg, D. Djureinovic, J. O. Takanen, S. Hober, T. Alm, P.-H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J. M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Pontén, *Science* **2015**, *347*, 394.
[156] V. Gautam, A. Sarkar, *Mol. Biotechnol.* **2015**, *57*, 299 – 308.
[157] P. Pinzani, C. Orlando, M. Pazzagli, *Mol. Aspects Med.* **2006**, *27*, 140 – 159.
[158] A. Kriete, K. Boyce, *Methods Inf. Med.* **2005**, *44*, 32 – 37.

[159] Z. Ting, Y. Karen Chang, O. Liliang, S. Wei, *Biofabrication* **2013**, *5*, 045010.
[160] R. Hannivoort, V. Hernandez-Gea, S. Friedman, *Fibrog. Tissue Repair* **2012**, *5*, 1.
[161] G. Chen, T. G. Gharib, C.-C. Huang, J. M. G. Taylor, D. E. Misek, S. L. R. Kardia, T. J. Giordano, M. D. Iannettoni, M. B. Orringer, S. M. Hanash, D. G. Beer, *Mol. Cell. Proteomics* **2002**, *1*, 304 – 313.
[162] Q. Tian, S. B. Stepaniants, M. Mao, L. Weng, M. C. Feetham, M. J. Doyle, E. C. Yi, H. Dai, V. Thorsson, J. Eng, D. Goodlett, J. P. Berger, B. Gunter, P. S. Linseley, R. B. Stoughton, R. Aebersold, S. J. Collins, W. A. Hanlon, L. E. Hood, *Mol. Cell. Proteomics* **2004**, *3*, 960 – 969.
[163] J. B. Kim, *Semin. Cancer Biol.* **2005**, *15*, 365 – 377.
[164] K. Kim, K. Ohashi, R. Utoh, K. Kano, T. Okano, *Biomaterials* **2012**, *33*, 1406 – 1413.
[165] A. I. Astashkina, B. K. Mann, G. D. Prestwich, D. W. Grainger, *Biomaterials* **2012**, *33*, 4700 – 4711.
[166] V. Y. Soldatow, E. L. LeCluyse, L. G. Griffith, I. Rusyn, *Toxicol. Res.* **2013**, *2*, 23 – 39.
[167] L. E. O'Brien, W. Yu, K. Tang, T. S. Jou, M. M. P. Zegers, K. E. Mostov, in *Methods in Enzymology*, *Vol. 406* (Eds.: W. Balch, C. Der, A. Hall), Academic Press, San Diego, **2006**, pp. 676 – 691.
[168] E. Hadjipanayi, V. Mudera, R. A. Brown, *J. Tissue Eng. Regener. Med.* **2009**, *3*, 77 – 84.
[169] A. J. Engler, H. L. Sweeney, D. E. Discher, J. E. Schwarzbauer, *J. Musculoskeletal Neuronal Interact.* **2007**, *7*, 335.
[170] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677 – 689.
[171] D. L. Butler, S. A. Goldstein, F. Guilak, *J. Biomech. Eng.* **2000**, *122*, 570 – 575.
[172] B. L. Seal, T. C. Otero, A. Panitch, *Mater. Sci. Eng. R* **2001**, *34*, 147 – 230.
[173] W. Xu, R. Mezencev, B. Kim, L. Wang, J. McDonald, T. Sulchek, *PLoS ONE* **2012**, *7*, e46609.
[174] K. M. Yamada, E. Cukierman, *Cell* **2007**, *130*, 601 – 610.
[175] R. S. Kane, S. Takayama, E. Ostuni, D. E. Ingber, G. M. Whitesides, *Biomaterials* **1999**, *20*, 2363 – 2376.
[176] R. Chang, J. Nam, W. Sun, *Tissue Eng. Part C* **2008**, *14*, 157 – 166.
[177] Organovo, **2014**.
[178] J. A. Kim, H. N. Kim, S.-K. Im, S. Chung, J. Y. Kang, N. Choi, *Biomicrofluidics* **2015**, *9*, 024115.
[179] J. A. Magida, L. A. Leinwand, *EMBO Mol. Med.* **2014**, *6*, 482 – 495.
[180] J. Thundyil, D. Pavlovski, C. G. Sobey, T. V. Arumugam, *Br. J. Pharmacol.* **2012**, *165*, 313 – 327.
[181] M. A. Jordan, R. J. Toso, D. Thrower, L. Wilson, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 9552 – 9556.
[182] C. Ellingsen, I. Natvig, J.-V. Gaustad, K. Gulliksrud, T. M. Egeland, E. Rofstad, *J. Cancer Res. Clin. Oncol.* **2009**, *135*, 1177 – 1184.
[183] M. J. Bissell, D. Radisky, *Nat. Rev. Cancer* **2001**, *1*, 46 – 54.
[184] V. Mironov, T. Trusk, V. Kasyanov, S. Little, R. Swaja, R. Markwald, *Biofabrication* **2009**, *1*, 022001.
[185] Y. Zhao, R. Yao, L. Ouyang, H. Ding, T. Zhang, K. Zhang, S. Cheng, W. Sun, *Biofabrication* **2014**, *6*, 035001.
[186] T. Xu, J. Rohozinski, W. Zhao, E. C. Moorefield, A. Atala, J. J. Yoo, *Tissue Eng. Part A* **2009**, *15*, 95 – 101.
[187] F. Guillemot, V. Mironov, M. Nakamura, *Biofabrication* **2010**, *2*, 010201.
[188] F. Marga, K. Jakab, C. Khatiwala, B. Shepherd, S. Dorfman, B. Hubbard, S. Colbert, G. Forgacs, *Biofabrication* **2012**, *4*, 022001.
[189] S. Wüst, R. Müller, S. Hofmann, *J. Funct. Biomater.* **2011**, *2*, 119 – 154.

Received: June 3, 2015
Published online: February 19, 2016

