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Review: bioreactor design towards generation of relevant engineered tissues: focus on clinical translation

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Abstract

In tissue engineering and regenerative medicine, studies that utilize 3D scaffolds for generating voluminous tissues are mostly confined in the realm of *in vitro* research and preclinical animal model testing. Bioreactors offer an excellent platform to grow and develop 3D tissues by providing conditions that mimic their native microenvironment. Aligning the bioreactor development process with a focus on patient care will aid in the faster translation of the bioreactor technology to clinics. In this review, we discuss the various factors involved in the design of clinically relevant bioreactors in relation to their respective applications. We explore the functional relevance of tissue grafts generated by bioreactors that have been designed to provide physiologically relevant mechanical cues on the growing tissue. The review discusses the recent trends in non-invasive sensing of the bioreactor culture conditions. It provides an insight to the current technological advancements that enable *in situ*, non-invasive, qualitative and quantitative evaluation of the tissue grafts grown in a bioreactor system. We summarize the emerging trends in commercial bioreactor design followed by a short discussion on the aspects that hamper the 'push' of bioreactor systems into the commercial market as well as 'pull' factors for stakeholders to embrace and adopt widespread utility of bioreactors in the clinical setting. Copyright © 2017 John Wiley & Sons, Ltd.

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1. Introduction

In the past two decades, researchers have been able to generate a wide range of 3D tissues using tissue-engineering strategies including biomaterials, cells and appropriate environmental cues. Some of these tissue-engineered medical products (TEMPs) have also been translated to clinics for *in vivo* tissue repair and regeneration. Despite the emergence of tissue-engineering methods as a promising strategy for overcoming the lack of organs for transplantation, its introduction into the clinical setting is limited to mostly thin tissues such as skin. One main clinical challenge that is hampering the widespread adoption of tissue-engineering strategies is the poor cell survival particularly at the core of thick voluminous grafts (Liu *et al.*, 2015). Growth of thick cellular-grafts in a static culture environment is challenged by mass transport (of oxygen, nutrients, waste products and metabolites) that solely depends on passive diffusion within a cellular-graft, which is often limited to short distances (100–200 μm) and could result in inadequate nutrient supply that is required for maintaining cell viability (Rouwkema *et al.*, 2008). Furthermore, static cultures do not represent the native microenvironment of most tissues. One of the key motivations behind the introduction of bioreactor systems for engineering thick tissues was to mimic the physiological scenario and recapitulate

nutrient transport through blood flow in the human body. Some of the important applications of bioreactors in the field of biomedical sciences and engineering are listed in Figure 1.

1.1. Bioreactors in tissue engineering

The introduction of dynamic culture conditions using bioreactor systems ~~as compared with conventional methods of cell culture in static dishes~~ has thus far shown significant improvement in the quality of the tissue-engineered graft in terms of better cell growth (Nam *et al.*, 2013; Wu *et al.*, 2015; Zhang *et al.*, 2009a,b). In static cultures, cells are often non-uniformly loaded onto and within the scaffold, and the cellular viability and growth is not even throughout the graft. The flow regime generated within bioreactors helps to overcome the limitations of oxygen diffusion within tissue-engineered grafts, which is critical in maintaining cellular survival within the graft and uniform cellular distribution. For instance, Zhang *et al.* (2009b) showed high levels of cell viability within the core of human fetal mesenchymal stem cell (MSC) grafts using a biaxial rotating system ~~as~~ compared with a necrotic centre that was observed in static grafts. The use of a fluidized bed bioreactor to scale up the culture of liver cell biomass for a bio-artificial liver machine achieved a highly dense and viable population of cellular spheroids encapsulated in alginate beads (> 45 million cells mL^{-1} beads) within 11–13 days (Erro *et al.*, 2013). In a recent study, Nguyen *et al.* (2016) had

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Figure 1. Different roles of bioreactor in biomedical applications [Colour figure can be viewed at wileyonlinelibrary.com]

demonstrated that dynamic bioreactor culture supported stem cell viability and functionality throughout a human femoral sized (200 cm³), engineered bone graft.

Other groups have also used bioreactor systems as dynamic cell loading systems for improving seeding efficiency (Ding *et al.*, 2008; Du *et al.*, 2008; Kitagawa *et al.*, 2006; Koch *et al.*, 2010; Zhao and Ma, 2005). Koch *et al.* (2010) had analysed the cell-seeding efficiency and cell distribution after varying fluid flow velocity and perfusion cycle number in a perfusion bioreactor for bone tissue engineering. They had reported cell-seeding efficiency of about 50% with the perfusion bioreactor. The studies so far have demonstrated that dynamic bioreactor culture with improved fluid dynamics can aid in better cell seeding as well as promote tissue maturation.

In addition to providing dynamic fluid flow conditions, bioreactors have now evolved to provide a physiological biomimicking environment for the generation of artificial tissues for human transplantation. There has been increased focus on the mimicry of native microenvironment of the tissue, whether it is biological, physical, electrical or mechanical. Studies are showing that bioreactors offer excellent controlled platforms for providing such biomimicking cues resulting in functionally relevant tissue grafts. Mechanostimulation has been shown to support cellular activity and differentiation for a variety of tissue types (Ishikawa *et al.*, 2011; Liu *et al.*, 2013; Sakai *et al.*, 2009; Valmikinathan *et al.*, 2011), hence maturing the graft to the desired extent before implantation. Bioreactors are also being used for recellularization of decellularized organs/tissues. Decellularized organs are acellular substrates of tissues and have shown promising potential in functional organ replacement (Badyalak *et al.*, 2011). Bioreactors are being used as platforms to recellularize these decellularized tissues under appropriate physiological conditions (Ott *et al.*, 2008; Price *et al.*, 2010; Raredon *et al.*, 2016).

1.2. Bioreactors in drug discovery and implant testing

Recently, bioreactors have become more than just a platform to engineer functionally relevant, three-dimensional tissues. Beyond controlling the fate of tissue growth and development, studies have started exploring bioreactors

as effective *in vitro* platforms for drug/implant testing with a hope to replace or reduce the use of animal models in the future. Pre-clinical animal models are required for pharmaceutical testing, implant testing, and to understand human physiology and disease biology. However, animal maintenance is time consuming, labour intensive and expensive. In consideration of the three Rs (replacement, reduction and refinement; de Vries *et al.*, 2015), there is a need to adopt alternative approaches to animal models and thereby pave a path for humane animal research. This has led to a growing interest towards *in vitro* cell- and tissue-based models that can replace or reduce the usage of animal models.

Many groups have developed 3D *in vitro* models with controlled cellularity, micro-architecture and environmental conditions as platforms for pharmacological testing (Kang *et al.*, 2009; Loessner *et al.*, 2010; Pusch *et al.*, 2011; Subramanian *et al.*, 2010; Zeilinger *et al.*, 2011). With growing interest in these 3D models, it has become essential to import the tools used in tissue engineering like dynamic bioreactor systems for developing these models, particularly 3D disease and cancer models (Hutmacher *et al.*, 2010). The most important advantage of bioreactors would be their ability to maintain tissues under controlled environments and mimic the native tissue's microenvironment, which allows us to possibly create *in vitro* animal models. Some studies have demonstrated the use of bioreactors for growing 3D tissues to test pharmacological agents in substitute of animal models. A study on the biology of multiple myeloma and its response to drug therapy by Ferrarini *et al.* (2013) utilized the commercially available RCCS™ bioreactor to culture myeloma tissues with controlled maintenance of cells, architecture and microenvironment. The ability to sustain cellular viability and functionality for longer durations is an important factor to be considered for drug metabolism and toxicity studies. Leite *et al.* (2011) showed that co-culturing hepatocytes and fibroblasts under dynamic conditions within a bioreactor enabled long-term maintenance of functional cells as compared with conventional 2D monocultures and co-cultures. Ishikawa *et al.* (2011) used the rotating wall vessel bioreactor for cultivating 3D hepatic-like tissues for the purpose of drug testing, and showed the formation of bile duct-like structures and blood vessels from fetal liver cells. More recently, Hirt *et al.* (2015) had shown that

perfused 3D cancer tissues growth within bioreactors shared functional similarities and drug response with *in vivo* cancers.

Further, there is also a strong need for developing better biomimetic tools to study disease models. In addition to drug testing, bioreactor platforms can be used for development of *ex vivo* disease models using human/animal tissues to understand biological mechanisms behind physiological conditions and disease progression. Perfusion bioreactors have been used to emulate the fluid dynamics that represent the arterial/venous parameters in order to study the arterIALIZATION of veins during artery bypass grafting (Longchamp *et al.*, 2014; Model *et al.*, 2014; Piola *et al.*, 2016). Similarly, mechanical loading bioreactors have been used to assess the biological changes upon application of complex mechanical loads on spinal tissues isolated from animals that could lead to the understanding of loading-induced progression of spinal disorders (Chan *et al.*, 2013; Hartman *et al.*, 2015). In another study, a shear stress bioreactor was used to provide controlled haemodynamics to porcine aortic valves and study its role in the development of calcific valve disease (Ling *et al.*, 2012). In addition to *ex vivo* disease models, engineered tissues have also been used for understanding physiological mechanisms using bioreactor systems. An *in vitro* human physiological loading model of cartilage callus was developed by Hoffmann *et al.* (2015) to study fracture healing using a novel perfusion compression bioreactor system. The above-mentioned studies showcase the potential of bioreactors to act as effective bridges between monolayer cell cultures and animal models.

1.3. Bioreactors in clinics

With years of research successes in bioreactor cultivation of engineered tissues, commercial and clinical usage of these systems are evidently not far behind. Bioreactor has been used to apply cyclic radial strain to cells seeded on biodegradable scaffolds to produce functional vascular grafts with well-formed collagenous extracellular matrix (ECM). This was followed by detergent-based decellularization for the generation of clinically relevant decellularized, tissue-engineered vascular grafts that can be eventually stored for future use or seeded with autologous cells and implanted (Dahl *et al.*, 2011; Niklason *et al.*, 1999). The studies have reached clinical trials (NCT01744418, 2012; NCT01840956, 2013; NCT01872208, 2013), and the group's interim press release claims 100% effectiveness (Humacyte, 2013). NeoCart is a commercial product that is currently undergoing clinical trials (Crawford *et al.*, 2012; NCT00548119, 2007) that utilizes a bioreactor to provide conditions that mimic the low oxygen tension and pressure conditions of the knee. Cytograft Tissue Engineering is another company that has a patented bioreactor technology to enable cell sheet tissue engineering to produce vascular grafts (McAllister and

L'Heureux, 2006). The bioreactor provides a cell sheet growth module and a mandrel to roll the sheet to fabricate the engineered blood vessel. The LifeLine graft generated by the company has also been demonstrated to be clinically successful (McAllister *et al.*, 2009; NCT00850252, 2013; Wystrychowski *et al.*, 2011). These studies serve as a promising start for clinical utilization of bioreactor systems for regenerative medicine applications.

Despite the promising start, we need to identify the challenges that are preventing the widespread adoption of these systems in clinics. From a clinician's perspective, certain requirements have to be met for clinical utilization of bioreactor-generated engineered tissue. We need easy-to-use, safe, reproducible, automated, scalable, versatile and regulatory-compliant tissue culture systems with an ability to control and evaluate the growing tissue and microenvironment with a rapid turn-around time. Generation of 3D tissues starts with selection or fabrication of geometrically defined scaffolds, isolation of specific cell types, followed by defining the optimal culture conditions for tissue growth. Firstly, there is lack of an optimized protocol for specific type of tissue in question. Secondly, very few culture systems allow real-time sensing and control of culture conditions. Also, most tissue culture systems do not have the facility to evaluate the end product prior to tissue implantation. Further, most of the systems require manual handling of tissues, which in turn could result in variable quality of the tissues. Hence, the question we are trying to answer is what can be done in terms of bioreactor design to achieve bioreactor utility in clinics. In this review, we aim to provide a summary of key factors in the design of bioreactor systems for tissue engineering eventually enabling clinical translation of bioreactor matured grafts.

2. Design considerations

Three major design factors are going to be discussed in this review, starting with the latest developments in bioreactor design that allow control of physiologically relevant cues on the growing tissue. This would be followed by a brief discussion on the current trends in non-contact sensing of the bioreactor culture conditions and the associated automation features. And thirdly, the current technological advancements that enable non-invasive, qualitative and quantitative evaluation of the growing constructs would be surveyed.

In designing a bioreactor, one of the primary focuses is to evaluate the type of culture and intended application of the system. Upon conceptualizing the bioreactor design, the effectiveness of the bioreactor in achieving the specific roles can be verified using prediction methods to better understand the implications of various parameters, such as fluid flow regime, oxygen tensions, mechanical force stimulation and their influence on cellular behaviour. Bioreactor systems that enable tissue culture on cellular

3D scaffolds under a particular flow condition and/or mechanical condition need to be reproducibly quantified and modelled (Hutmacher and Singh, 2008). Using prediction methods, we can seek to understand, optimize or design bioreactor conditions to verify the intended outcome of the application of interest. These methods could predict media-related flow conditions and chemical gradients for mass transports (Devarapalli *et al.*, 2009), scaffold-related mechanical forces and their responses (Milan *et al.*, 2009; Song *et al.*, 2013), as well as cellular adhesion, morphology, population dynamics, tissue growth, forces and other behavioural patterns (Cheng *et al.*, 2009; Galbusera *et al.*, 2007; Sengers *et al.*, 2007). For instance, Cantini *et al.* (2009) used computational fluid dynamic techniques to design the optimal microenvironment for co-culture of adherent bone marrow stem cells and non-adherent haematopoietic stem cells inside porous scaffolds in perfusion bioreactor, and the results predicted the effects of microchannel-provided scaffolds with different geometries on fluid-dynamics and oxygen transport. This early-staged validation can provide cost savings in development and design of novel bioreactor and scaffold configurations, as well as help to understand scalability-related issues. For instance, Singh *et al.* (2005) had utilized computational methods to predict and understand the fluid flow within scaffolds inside a dynamic bioreactor system under uniaxial and biaxial rotation conditions. Their modelling showed an increase in fluid velocity within the scaffolds under biaxial mode of the dynamic bioreactor system. Subsequently, the experimental validation also showed that biaxial rotation led to a more homogeneous cellular and ECM distribution due to improved fluidics within the system (Zhang *et al.*, 2009b, 2010a).

Another interesting idea would be the utilization of multi-chambered vessels for parametric optimization studies. In attempting to mimic the physiological niche of the tissue-of-interest, several factors and conditions such as varying concentrations and types of soluble factors, cellular density and combination of cell types, different modes and extent of mechanical stimulation and flow rate need to be optimized. Particularly for larger vessels, these studies are expensive, resource- and time-consuming, and often hinder the progress of research. To minimize experimental variations upon optimization of multiple parameters and operate in a more time-efficient and cost-effective manner, it would be helpful to develop an array of scaled-down bioreactors within one system. Essentially, the presence of multiple chambers with separate media conditions will allow the study of effects of different intrinsic parameters (different cell sources, scaffold materials or configurations and media components) and also external factors (mechanical, fluid and electrical stimuli) concurrently in one bioreactor run. For instance, Pavesi *et al.* (2014) developed a multi-chambered device with adaptable, independent culture chambers to test various electrical stimulation protocols simultaneously for stem cell differentiation towards myocardial phenotype. Similarly, Brady *et al.* (2014) designed and validated a high-throughput bioreactor

platform for the application of multiple magnetic fields and mechanical loading regimens simultaneously in multiple chambers with independent control for engineering functional cartilage tissues. In addition to enabling parametric optimization studies, the multi-array configuration could also be potentially used for drug and toxicology studies with disease models (Domansky *et al.*, 2010). This would provide time and cost savings in terms of consumables, manpower and resources, and yield faster results, hence help expedite research and development. In addition to the research studies that have incorporated bioreactors with multiple growth chambers (Cimetta *et al.*, 2009; Domansky *et al.*, 2010; Engelmayr *et al.*, 2003; Juncosa-Melvin *et al.*, 2006; Pavesi *et al.*, 2014; Piola *et al.*, 2013), bioreactor systems with multiple chambers are also gaining commercial importance (BISS TGT, 2016; BISS TGT, 2016; EBERS Medical Technology, 2015a; Flexcell International Corporation, 2016; Kirkstall, 2016; Synthecon, 2016; TA Instruments, 2015).

Once the structural design is ready, aspects such as chamber construction, biocompatibility of its material, size factors and sterility maintenance have to be decided. Concurrent with this design phase, it would be ideal to consider regulatory requirements and industry standards to save time and avoid significant re-designing. This is followed by provision of control and sensing systems for detection and maintenance of the optimal biochemical and biomechanical environment to maximize efficacy of tissue formation. A facility to image the grafts in the bioreactor environment would add value to the system in terms of evaluating real-time tissue growth and dynamics. Particularly for clinical use, the ability to generate grafts with a certain extent of reproducibility is essential for predicting a certain outcome for widespread adoption. This can be achieved by having an automated system that works on a feedback mechanism and enables reduction of manual handling and associated contamination risks. The disposability of the bioreactor chambers has also become an increasingly important consideration. It eliminates labour-intensive tasks like cleaning and storage of bioreactor parts, saves a lot of time taken for starting a bioreactor run and also in between two batches of cultures (Eibl and Eibl, 2009). Thus, their utility in a clinical setting will be high as they have an important role in maintaining high sterility and flexibility of usage.

In weighing the importance of each criterion, some consideration factors might add more value to a clinical set-up, while others might be of higher value in a research laboratory. For instance, introduction of multi-chambered vessels will enable concurrent optimization of parameters to be used in bioreactor. This could be a cost-effective and time-saving design idea in research labs. On the other hand, it might not add significant value in clinics where the primary aim is not concerning judicious use of resources or optimization of bioreactor parameters. And hence for clinical application, designing bioreactors with multi-chambered vessels will be less impactful. Likewise, disposability of bioreactor components will play an important role in ensuring sterility and easier starting up of

bioreactor run at a clinical level, whereas it might not add tremendous value to an existing reusable system in a research lab. Thus, by comparing research use and clinical use (Table 1), it allows us to identify gaps during the phase of research as we keep in mind the overall purpose for clinical utility.

3. Physiological biomimicry

For making tissues of a specific organ, we need to first identify the most optimal combination of cell/scaffold/bioreactor along with the appropriate environmental cues that include biological, chemical, physical, mechanical and/or electrical cues based on the native physiology of the specific tissue in question. At every step in this entire process, physiological biomimicry of the native tissues and the environment could result in tissues with enhanced functional properties (Abbott and Kaplan, 2015). While biological and chemical cues for the regeneration of a specific tissue have been extensively studied and optimized over the past few decades, other types of physiologically relevant cues such as electrical and mechanical cues have also gained a lot of attention in the past few years. Stimulatory effect of electrical signals on *in vivo* tissue growth and regeneration has been well established in the past. Balint *et al.* (2013) have done an excellent review of the physiological relevance of electrical stimuli, the methods of stimulation and resulting cellular effects of electrical stimulation. Non-invasive, ultrasound stimulation has also been widely studied as a tool for tissue regeneration, especially the bones, cartilage and soft tissues (Tanaka *et al.*, 2015). Static cultures for

tissue engineering without appropriate mechanical stimulation may result in poorly cellularized grafts, and require longer culture durations or larger initial cell numbers. By physiological mimicry of mechanical forces experienced by native tissues within the body, accelerated tissue formation has been demonstrated by an increase in proliferation and differentiation of cells. In this review, amongst the various environmental parameters to be optimized, we would like to highlight the role played by mechanical stimulation in tissue development and discuss the current trends in mechanical bioreactors for engineering tissues.

3.1. Understanding mechanical forces on organs

Mechanical forces in the human body play a role in regulating tissue growth and remodelling processes. Cells respond to mechano-stimulation by changes in cellular alignment, rate of proliferation, differentiation, migration, matrix deposition or by apoptosis. The study of mechanical induction of 2D cell cultures showed structural and functional changes like morphology, alignment, polarity, gene and protein expression (Matsugaki *et al.*, 2013; Park *et al.*, 2004; Xie *et al.*, 2012). Literature on single cell studies have aided understanding in downstream signalling events in a mechanically stimulated cell that culminates in differential gene expression, protein synthesis or release of factors, which in turn will indirectly control the aforementioned cell-fate processes (Leipzig and Athanasiou, 2008; Ofek *et al.*, 2010; Rath *et al.*, 2010; Wang *et al.*, 2010).

However, these models do not represent the native 3D environment. Tissues exhibit a hierarchical cellular arrangement based on their physiological functional role,

Table 1. Authors' perspectives of various design factors, and its research and clinical level relevance

Criteria	Key elements	Purpose	Research	Clinical
Modelling	Modelling and prediction of key parameters	Characterize microenvironment	+++	+++
System set-up	Selection of chamber material	Bio-inert, non-corrosive	+++	+++
	Tight sealing mechanism	Prevent leakage and contamination	+++	+++
	Optically transparent windows	Real-time imaging of cellular-grafts	+	+++
	Versatile design	Accommodate various scaffold configurations and cell types	+++	+++
	Autoclavable/single-use tubing, valves, luer fittings, accessories	Prevent contamination	+	+++
System size	Robust system	No fatigue, wear and tear over long term	+	+++
	Smaller system	Save resources, i.e. media	+++	+
	Multiple independent-operating parallel systems	Study of multiple parameters or samples	+++	++
	Bench top	Easier to handle	+++	+++
	Portability	Logistically easier, in case of transport	+++	+++
Flow regime	Appropriate levels of laminar shear stresses	Increase mass transport, stimulate cells without wash off or damage	+++	+++
Environmental control	Control of oxygen levels, pH, temperature, foam levels, gas, flow rate, lighted chamber	Maintain optimal cell growth conditions	+++	+++
Mechanical stimulation	Specific mechanical stimulation of scaffold/ <i>in vivo</i> mimicry	Enhance mass transport and control cell fate processes, mimic physiological loading	+++	+++
Sensors	Gas levels, pH, dissolved oxygen, temperature	Maintain optimal cell growth conditions	+++	+++
	Media sampling for metabolites	Understand metabolic requirements	+	+++
	Media sampling for contamination	Sterility check of media	++	+++
Imaging	Imaging facility integrated with bioreactor chamber	Evaluation of scaffolds, cells, cell viability, tissue growth and dynamics	+	+++
Feedback	Sensor/imaging-based control mechanism	Maintain optimal conditions	+	+++
	Sensor/imaging-based alerts through alarm, messages, e-mails	Alert user during system errors	+	+++
	Video imaging of system	Constant supervision of the system	+	+++

'+' indicates the level of importance in research or clinical set-up; '+++' reflects very high importance.

which could be closely related to the mechanical forces that act on them. To engineer artificial tissues that resemble the native tissues both structurally and functionally, it would be important to provide appropriate *in vitro* conditions that mimic the native microenvironment of the tissue. This has clearly been neglected in our long-standing conventional methods of culturing cells in a static environment. The native biomechanical environment of different tissues in the body has been represented in Figure 2. Engineering functional tissues in conjunction with the use of bioreactor systems focuses on a biomimetic approach where the mimicry of the physiological forces comes into play within the controlled environment in the bioreactor (Butler *et al.*, 2009; Grayson *et al.*, 2009). In the human body, the tissues are exposed to forces like fluid flow, compression, tension, torsion and hydrostatic pressure

(Figure 2). Based on the location and functionality of a specific tissue, it experiences one or a combination of these forces. For example, blood vessels experience both fluid shear stresses and hydrostatic pressure, whereas cartilage tissues are mainly under compression. Various studies have shown that cells respond differently to variations in type of forces, magnitude, culture times and duration of rest periods (Robling *et al.*, 2002; Sen *et al.*, 2011). Clearly, an optimal range of the variables is required for appropriate physiological tissue formation and response. While the different mechanical stimuli experienced by tissues has been summarized before (Polacheck *et al.*, 2013), this could be difficult to benchmark due to variations between anatomical differences physiologically and also different experimental set-ups between research studies. Thus, choosing a 'gold standard' will remain a challenge.

Figure 2. Mechanical forces acting on various organs associated with their inherent functionality. (A) Blood vessel; (B) bone; (C) cardiac tissue; (D) cartilage; (E) heart valve; (F) ligament and tendon; (G) liver and kidney; (H) lungs; (I) muscle; (J) skin; (K) teeth; (L) urinary bladder. Shear forces due to blood flow are a common feature in the majority of the organs. Other key forces include compression, tension, torsion and hydrostatic pressure [Colour figure can be viewed at wileyonlinelibrary.com]

3.2. Mechanical stimulation bioreactor

Bioreactors present an interesting platform to study mechanical forces and their concomitant effect on tissue growth and development for regenerative medicine. To mimic the physiological loading regimes, some bioreactors have incorporated mechanical loading systems to provide compression, tension, torsion forces onto the grafts. Some of the commonly applied mechanical stimuli like compression, torsion, tension and shear stresses on cell-seeded 3D scaffolds within bioreactor systems are T2 represented in Table 2. Research studies that involved

compressive forces have mainly focused on engineering bone and cartilage tissues. Zong Ming *et al.* (2013) demonstrated that the application of cyclic compression on bone explants induced an increase in the expression of osteogenesis-related genes and proteins, resulting in enhanced mechanical properties, mineralization as compared with the unloaded controls. Liu *et al.* (2012) studied the effect of cyclic compression combined with perfusion on mesenchymal stromal cells-seeded polyurethane scaffolds for fibrocartilagenous differentiation. It was found that inclusion of rest periods in between bouts of cyclic compression led to

Table 2. Research studies that applied various mechanical forces on 3D cellular-grafts: (a) compression; (b) tension; (c) torsion; and (d) shear stress within the bioreactor system

Scaffolds	Cells/tissue	Mode	Time	Observed results (in comparison to static conditions)	Reference
(a) Compression					
Collagen/NiTi	MSC/callus model	100 μ m, 1 Hz	14 days	↑ ECM mineralization, tissue maturation	Hoffmann <i>et al.</i> (2015)
PLLA-PCL	NP, AF + MSC/IVD	10%, 1 Hz	21 days	↑ ECM deposition, homogeneous cell distribution	Tsai <i>et al.</i> (2014)
Bone explant	Autologous bone	1000–4000 μ e, 1 Hz	21 days	↑ Osteogenic gene expression, ALP, Ca, mechanical properties	Zong ming <i>et al.</i> (2013)
Polyurethane	BMSC/fibrocartilage	10%, 0.5 Hz	14 days	Synthesis of procollagen, ↑ mechanical properties	Liu <i>et al.</i> (2012)
DBM	EPC/bone	5%, 1 Hz	7 days	↑ Proliferation and formation of microvascular structures	Kong <i>et al.</i> (2012)
Collagen-alginate	MSC/bone/cartilage	10%, 15%, 1 Hz	21 days	↑ Osteogenic (10%) and osteo-chondrogenic gene expression (15%)	Michalopoulos <i>et al.</i> (2012)
Bone-fibrin	BMSCs/bone	4 kPa, 0.05 Hz	1 day	↑ Expression of OPN, TGF- β -R1, ITGB1, SMAD5, PDGF- β	Matziolis <i>et al.</i> (2011)
HA composite	MG-63 cells/bone	3 Hz ($\pm$)	4 days	↑ Collagen, fibronectin, VEGF gene expression	Dumas <i>et al.</i> (2009)
Polyurethane	MLO-A5 cells/bone	5%, 1 Hz	20 days	↑ Collagen, osteopontin, osteocalcin gene expression, stiffness, mineralization	Sittichokechaiwut <i>et al.</i> (2009)
PCL	Osteoblasts/bone	10%/20%, 0.5 Hz	4 h	↑ Osteogenic gene expression, ALP and matrix proteins	Rath <i>et al.</i> (2008)
(b) Tension					
Tissue matrix	Heart cells/cardiac	5%, 1 Hz	2 days	↑ Cardiomyocyte and endothelial gene expression	Lux <i>et al.</i> (2016)
Collagen I	Dermal fibroblasts/skin	10%, 0.1 Hz	1 day	↑ Active TGF- β 1 gene expression	Peters <i>et al.</i> (2015)
Decell. tendon	BMSC/tendon	3%, 0.2 Hz	7 days	↑ Collagen I, decorin and tenomodulin gene expression	Qin <i>et al.</i> (2015)
Collagen I	ESC,iPSC/cardiac	5%, 1 Hz	14 days	Differential contractile gene expression, calcium dynamics	Ruan <i>et al.</i> (2015)
PCL nanofibres	MSC/fibrocartilage	6%, 1 Hz	14 days	↑ Fibrous gene expression, collagen deposition, mechanical properties	Baker <i>et al.</i> (2011)
Polyurethane	SMC/vascular tissue	10%, 1 Hz	28 days	↑ Proliferation, cell area, cell distribution, tensile properties	Sharifpoor <i>et al.</i> (2011)
PCL	BMSC/ligament	6%, 0.125 Hz	21 days	↑ Collagen I, III, tenascin C gene expression	Petrigliano <i>et al.</i> (2007)
PET	Synovial cells/ligament	0.6–4.5% 1 Hz	35 days	↑ Cell proliferation	Raif and Seedhom (2005)
(c) Torsion					
Bovine IVD	NA/IVD	Torsion: 0° $\pm$ 2°/5°/10°, 0.1 Hz	4 days	Differential cell viability and metabolic activity	Chan <i>et al.</i> (2011)
PCL	Fibroblasts/ligament	Torsion: 100°, 600°/min	3 days	↑ Collagen type I, tenascin C, collagen type III gene expression	Scaglione <i>et al.</i> (2010)
Chitosan-hyaluronan fibres	Fibroblasts/ligament and tendon	Rotational (90°), 0.5 Hz	21 days	↑ Proliferation, ECM synthesis	Sawaguchi <i>et al.</i> (2010)
Silk matrix	BMSC/ligament	Rotational (90°), 0.0167 Hz	21 days	↑ Cell spreading, growth and differentiation into ligament-like cells	Altman <i>et al.</i> (2002)
(d) Shear stress					
Silk fibroin	hASC	Pulsatile flow: 400, 1200 μ m/s, 0.5 Hz	35 days	↑ Expression of osteogenic genes, matrix protein components	Correia <i>et al.</i> (2013)
Polyurethane	Gingival fibroblasts	Flow: 500 μ l/min, Shear: 10 dyne/cm ²	28 days	↑ Proliferation, expression of collagen I	Cheung <i>et al.</i> (2013)
Polyglycerol sebacate	Cardiac cells	18 μ l/min	8 days	↑ Cell proliferation, distribution, cardiac protein expression, tissue organization	Maidhof <i>et al.</i> (2012)
Alginate	HUVEC	1–13 dyne/cm ²	17 days	Differential expression of ICAM-1 and p-eNOS, cell sprout lengths	Rotenberg <i>et al.</i> (2012)

NiTi, nickel-titanium-based constructs; MSC, mesenchymal stem cell; ECM, extracellular matrix; PLLA, poly-L-lactic acid; PCL, polycaprolactone; NP/AF, nucleus pulposus/annulus fibrosus; IVD, intervertebral disc; ALP, alkaline phosphatase; BMSC, bone marrow stromal cell; DBM, demineralized bone matrix; EPC, endothelial progenitor cell; OPN, osteopontin; TGF- β -R1, transforming growth factor- β -receptor 1; ITGB1, integrin- β 1; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; HA, hydroxyapatite; MG-63, human osteosarcoma cell line; mRNA, messenger ribonucleic acid; VEGF, vascular endothelial growth factor; MLO-A5, osteoblast cell line; SMC, smooth muscle cell; PET, polyethylene terephthalate; hASC, human adipose tissue-derived stem cells; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule 1; p-eNOS, phosphorylated endothelial nitric oxide synthetase.

higher expression of ECM component Procollagen Type I N-terminal Propeptide compared with both free-swelling static conditions and rest-free loading, possibly due to increased subcellular communication as a result of decreased intracellular spaces. Kong *et al.* (2012) had studied dynamic compression of endothelial progenitor cells (EPC)-loaded scaffolds for engineering vascularized bone tissue. They showed an increase in proliferation of EPCs and formation of tube-like microvascular structures for BTE. *In vivo* rat studies showed that scaffolds under compression had increased numbers of EPCs and more evenly distributed vessel-like structures compared with the unloaded control scaffolds. Matziolis *et al.* (2011) demonstrated an increase in gene expression that contributed to osteogenic predifferentiation of bone marrow stromal cells seeded on fibrin matrix on application of pressure of 4 kPa at 0.05 Hz for a day relative to controls that were not mechanically stimulated (Table 2a). In another study, MSCs were mechanically preconditioned by syringe-based pressure loading towards nucleus pulposus-like cells for intervertebral disc regeneration and loading led to upregulated gene expression of nucleus pulposus-related marker genes SOX9, COL II and ACAN (Zeng *et al.*, 2015).

Tensile forces are more commonly experienced in muscles, tendons, ligaments, blood vessels and cardiac tissues. Nagai *et al.* (2012) had analysed molecular level changes like increase in ERK phosphorylation levels on application of uniaxial static strain of skeletal muscle cell-seeded scaffold. Baker *et al.* (2011) demonstrated an increased collagen production and fibrous gene expression on dynamic tensile loading of MSCs seeded on polycaprolactone (PCL) nanofibres. Sharifpoor *et al.* (2011) demonstrated higher DNA mass, better cell area coverage, better cell distribution and improved tensile mechanical properties on application of cyclic uniaxial tensile strain on smooth muscle cell (SMC)-seeded polyurethane scaffolds (Table 2b). Torsional forces have been used in engineering ligament tissues and intervertebral discs. Scaglione *et al.* (2010) showed that application of torsional forces to fibroblast seeded PCL-based scaffolds that resulted in increased expression levels of collagen type I, collagen type III and tenascin C. Sawaguchi *et al.* (2010) demonstrated that application of torsional strains enhanced ECM synthesis and proliferation. Chan *et al.* (2011) showed an improved cell viability in the inner region of the disc on application of torsional forces of low magnitude that lies within the physiological range (Table 2c). Shear is one of the most commonly studied forces in the bioreactor likely due to the mimicry of blood flow in the human body. Different groups have thoroughly reviewed the role of shear stresses in bioreactor systems and concentrating on different aspects like the current state for organ engineering (Bijonowski *et al.*, 2013), a specific interest on the impact of shear stresses on osteogenesis and angiogenesis (McCoy and O'Brien, 2010) and influence of dynamic shear stress in bone tissue engineering (Yeatts and Fisher, 2011). In general, application of shear stresses on cell-seeded 3D

scaffolds shows increased proliferation and ECM synthesis (Bhumiratana *et al.*, 2011; Cheung *et al.*, 2013; Liu *et al.*, 2012) or differentiation (Bhumiratana *et al.*, 2011; Maidhof *et al.*, 2012; Table 2d).

In sum, the application of functionally relevant mechanical signals inside the chamber of a bioreactor has clearly demonstrated improved tissue functionality owing to the recapitulation of the native biophysical microenvironment. Although it will be a technical feat to exactly mimic the *in vivo* conditions per se in a bioreactor, our progress in the understanding of mechanical effects on tissue engineering is leading us closer to the development of clinically relevant engineered tissues/organs.

4. Sensing and automation

4.1. Sensing culture conditions

In cell and tissue culture, growth media provides the necessary nutrients for cell proliferation and/or differentiation, while metabolites and by-products such as lactate are being released into the media. The ability to systematically ascertain specific culture conditions within the bioreactor vessel and subsequently regulate a feedback response is essential for achieving effective tissue growth. Slight changes in vital parameters like dissolved O₂, CO₂, pH and glucose in the culture media could adversely affect the cellular functions like proliferation and differentiation. With respect to tissue culture in bioreactors, on-line monitoring of culture conditions is important to ensure accuracy and reproducibility of the bioreactor runs. Commercially available bioreactor systems possess integrated sensor systems that can perform on-line measurements of gas and metabolite levels, etc.

There are three ways of sensing bioreactor culture conditions, namely, invasive, shunt and non-invasive sensing (Wendt *et al.*, 2009). Shunt sensing is a simple technique where culture media would be sampled at regular intervals and measured off-line using benchtop sensors. This has obvious disadvantages of not being real-time. Direct invasive sensors that can perform on-line sensing in bioreactors are generally sterilisable/single-use, electrochemical sensors that are in contact with the culture media, mostly restricted to the media reservoir, culture media loop or inlet/outlet of bioreactor chambers (Janssen *et al.*, 2006; Li *et al.*, 2014; Mundargi *et al.*, 2015). This method is widely popular owing to the abundance in commercially available pH and DO (dissolved O₂) electrochemical probes. In fact, microelectrodes are not only used to measure the surrounding milieu but also inside the growing grafts to assess the conditions of cellular microenvironment (Malda *et al.*, 2004). The major limitation with these invasive sensors is the possible risk of contamination in addition to high costs, fouling and consumption of analytes in the culture media (Starly and Choubey, 2008).

Utility of bioreactors in clinics necessitates contamination-free processes with real-time sensing facilities that allow automated feedback to maintain optimal

conditions. Over the past few years, there has been an increasing interest in the non-invasive imaging modalities, especially luminescence-based sensors for sensing environmental conditions within the bioreactor. While direct contact is required for electrochemical sensing, it is possible to non-invasively detect fluorescence-based signals through the walls of a transparent vessel. Fluorescence-based sensing for detection of media conditions in the bioreactor started almost two decades ago (Vunjak-Novakovic *et al.*, 1999). A number of fluorescent dye-based adhesive patches have been developed and are available commercially that can be adhered on to transparent bioreactor vessel surfaces. Changes in fluorescence signal are detected using optical probes that are not in direct contact with the sterile culture media (Leah *et al.*, 2005). Papkovsky and Dmitriev (2013) have explained the working principle of these sensors in depth and reviewed the different types of these luminescence-based sensors. CellPhase®, HP5 and PSt3/PSt6 (PreSens®), RedEye® are some of the commercial fluorescent sensor patches that are suited for sensing DO/pH/metabolites inside bioreactor vessels. Some of these sensors come in single-use packages making it safe for clinical usage. Santoro *et al.* (2012) had used PSt3® disposable, micro-oxygen sensors to measure the oxygen levels at the inlet and outlet of a perfusion bioreactor used for engineering cartilage grafts. The consumption of oxygen in the bioreactor chamber gave an indication of the number of chondrocytes on the graft, which in turn enabled non-invasive evaluation of quantitative quality of engineered cartilage graft. Recently, Raimondi *et al.* (2015) demonstrated the use of SF-RPSu4® sensor foils within a miniature perfusion bioreactor system to quantify the oxygen concentrations within the tissue-engineered construct in a non-destructive way. In addition to sensor foils, microbeads and nanoparticles are also being used as oxygen-sensing systems within the tissues. In a recent study, oxygen-sensitive, phosphorescent microbeads were placed inside the scaffolds to non-destructively determine the oxygen levels in the centrum of the scaffold under static and dynamic culture conditions (Weyand *et al.*, 2015). Although these bead/nanoparticle-based sensing of metabolites within scaffolds provide valuable information, they are invasive and redundant for clinical application.

As close as we are in incorporating these useful sensing systems for clinical use, we need to address some of its limitations, including complexity of optical set-ups required for detection and lesser flexibility in aligning the set-up with dynamic, moving bioreactor parts. Nevertheless, these luminescence-based non-invasive sensors exhibit

great potential for rapid sensing of environmental parameters in the bioreactor culture for clinical applications.

4.2. Automation in bioreactor systems

Sensing of the bioreactor culture conditions must be accompanied with a feedback mechanism to maintain the relevant conditions at an optimal level. And, if this feedback mechanism is automated, it would add more value for clinical usage. For that matter, right from the initial setting up of the bioreactor system to its operation, manual handling can be labour intensive, time-consuming, prone to contamination and could result in the reduction of graft reproducibility. Automation of bioreactor systems will allow better control of the bioreactor processes, provide higher efficiency and reproducibility of graft outcomes. This is an extremely important criteria to fulfil for clinical relevance of bioreactor-cultured engineered grafts. Automation not only improves productivity but also maintains consistency of the artificial tissue to be formed as well as the environment it is in. Professor Teruo Okano, one of the pioneers in the area of automation for cell culture technologies, has demonstrated the importance of an automated process control in clinical acceptance and utility of the technology. Briefly, his technology generates cell sheets that are detached from temperature-responsive cell culture-ware based on temperature changes. Unlike conventional methods that require an enzymatic reaction (Kushida *et al.*, 2001, 2005; Yamato and Okano, 2004), this method of automation has significantly increased productivity, and minimized the number of procedural steps and risk of contamination during the handling of cell sheets. This technology has since progressed into the pre-clinical models for use in the repair of various tissues, such as cornea, skin, oesophagus, heart and periodontal tissue (Iwata *et al.*, 2009; Kobayashi *et al.*, 2013; Nishida *et al.*, 2004; Sawa *et al.*, 2012; Takagi *et al.*, 2010).

Media exchange, harvest of cellular-grafts during imaging and even transfer of the bioreactor from the incubator into the biosafety hood are potential routes for contamination. Automation of the bioreactor systems would help minimize the risk of tissue graft contamination. In addition, software and controllers that are designed for use in conjunction with these automated systems require a user-friendly interface for user's preference and for capturing real-time data with automated data processing into graphical or numerical formats. Table 3 shows some examples of automated,

Table 3. Automated bioreactor cultivation of cells and tissues with minimal manual handling

Type of bioreactor/company	Features of automation*	Cell/tissue used	Reference
Fraunhofer TEB1000 3D bioreactor (EBERS Medical)	Cell extraction, expansion and tissue cultivation Cell seeding on 3D grafts, exchange of media	Skin MSC	Fraunhofer (2010) EBERS Medical Technology (2015b)
Parallel Bioreactor Systems(DASGIP)	Temperature, pH, pO ₂ control based on feedback	Stem cells	Olmer <i>et al.</i> (2012)

*Automation here refers to the non-requirement of manual handling for the processes described.
MSC, mesenchymal stem cell.

commercial bioreactor systems used with scaffolds for different applications. Automation in bioreactors has been introduced at different steps of cell culture, such as medium change, expansion, dynamic seeding of scaffolds and automated sequencing of user-defined protocols (Ding *et al.*, 2016). Some bioreactors have sensors to provide feedback on the culture conditions (Volkmer *et al.*, 2012), while others also have the ability to record, analyse and transfer data to local network.

5. Evaluation of engineered tissue

During the generation of artificial tissues *in vitro*, it would be ideal to monitor and study the extent of tissue growth, particularly in determining whether the graft is substantially mature and ready for patient implantation. Currently, most bioreactors require frequent halting of the entire dynamic process and dismantling parts of the bioreactor to harvest the cellular-grafts, which could result in contamination. The most commonly utilized techniques are scanning electron microscopy (SEM) and confocal systems for imaging 3D tissue grafts, and this is most often limited to an end-point harvest due to the post-processing and dye-staining involved. The field and depth of view of the tissue of investigation is also difficult to track due to either the limited capabilities of the imaging system or because the images have been sectioned. Having compatible imaging systems incorporated with bioreactor systems would allow real-time and non-destructive monitoring of the quality of the cellular-graft, its tissue characteristics, properties and growth dynamics. Real-time, non-invasive imaging methods reduce the source of errors and noise within the experiments in longitudinal studies, and increase the ease of handling and user convenience. They also aid in saving time and prevention of contamination of the system, which is essential especially for clinical analysis prior to implantation.

In our classification of the different imaging modalities, the cultured tissue of interest may be subjected to destructive or non-destructive testing upon imaging (Table 4). Various parameters that can be obtained using these imaging modalities include cellular morphology, viability and distribution, scaffold architecture, mechanical properties of the tissue graft, collagen organization, mineralization and metabolite distribution. In recent years, there has been significant advancement in the various sophisticated technologies for imaging on both cellular and tissue level. Near infra-red imaging, ultrasound, magnetic resonance imaging (MRI), computed tomography (CT), single positron emission computed tomography (SPECT), positron emission tomography (PET) and confocal systems have been used for imaging different cellular processes like proliferation, angiogenesis perfusion, and apoptosis with varying levels of detection capabilities due to the differences in their depth of sample penetration. Table 4 also lists the various research studies that utilized imaging systems for analysis of tissue

development in bioreactors. The use of the individual imaging modalities such as PET, CT and MRI have their own advantages and disadvantages. Hence, by combining the desired features of individual imaging technologies, multimodality imaging technologies could improve the quality of information obtained.

In spite of the advantages associated with real-time imaging within a bioreactor, like achievement of images with greater details, high resolution, opaque structures of scaffold architecture and cell profile in 3D under dynamic culture conditions, *in situ* dynamic bioreactor imaging has not gained much popularity due to the high costs involved in incorporating sophisticated imaging technologies complemented with the bioreactor and the need to maintain and miniaturize these imaging systems. The future lies in the development of a miniaturized imaging unit that could be incorporated into the bioreactor system for real-time imaging without compromising on the ease of use and bioreactor operations.

6. Emerging trends in clinically relevant bioreactor design and future directions

In recent years, there has been a rising trend in the number of bioreactor-related studies with the introduction of a variety of new bioreactor designs for different applications. Currently available commercial systems for generation of artificial 3D tissues provide dynamic flow environment in addition to application of specific mechanical forces depending on the tissue type. Table 5 summarizes a selected list of commercially available bioreactors for generating 3D tissue-engineered grafts. The table showcases the current trends in commercial bioreactor design, including provision for application of mechanical forces and availability of sensing modalities to sense culture conditions inside the bioreactor system.

In the past decade, there have been several studies that showcased the possibility of tweaking bioreactor designs to make them relevant in clinics. The three major bioreactor design factors discussed in this review will be important requirements for their future utility in hospitals. Biomimicry is a well-known strategy in tissue engineering (Grayson *et al.*, 2009). Several groups have reviewed the importance of studying developmental biology of tissues to aid functional tissue engineering (Liu *et al.*, 2011; Stock and Vacanti, 2001). Almost a decade ago, Martin and Vermette (2005) had suggested modelling bioreactors based on the natural reactor 'uterus'. Our group had developed the biaxial bioreactor system that has the ability to spin (rotate about the z-axis) and tumble (rotate about the x-axis) simultaneously, which mimics the 'fetal gyroscopic motion' inside the womb. This biaxial rotation has been reported to aid in increased mass transport through thick scaffolds leading to efficient nutrient and waste exchange (Zhang *et al.*, 2009b), and showed increased bone formation and greater neo-vasculogenesis *in vivo* (Zhang *et al.*, 2010b). Recently, Morgan and Black

Table 4. Destructive and non-destructive imaging modalities to evaluate tissue-engineered grafts cultured in bioreactor systems (Appel *et al.* 2015)

Imaging modality	Functionality	Usage in tissue engineering	Disadvantages	Type of bioreactor	Reference
Destructive techniques					
Electron MS • SEM • TEM	SEM: 1–5 nm TEM: 0.2 nm	Cell-material interactions, scaffold architecture, material surface topography	• Not real-time imaging, limited time points • Long processing steps, no 3D information	Biaxial rotation bioreactor, Rotating chamber bioreactor	Zhang <i>et al.</i> (2009b) Gonfiotti <i>et al.</i> (2014)
Histology via phase contrast MS	Sub-micron resolution	Cellular alignment, morphology, deposition of ECM contents	• Difficult to interpret structure–function correlation		
AFM	10–20 nm	Scaffold architecture, surface topography	• Destruction of samples due to sample preparation • EM and AFM: expensive and high maintenance		
Non-destructive techniques					
Ultrasound imaging and photoacoustic MS	1–50 MHz acoustic waves, 250–500 µm	• Assessment of vascularization, ECM and mineral deposition • Longitudinal assessment of regeneration • Changes in compressive moduli • Oxygen saturation	• Speckle noise • Limited tissue contrast • Trade-off between imaging depth and spatial resolution • Limited penetration depth (in mm)	Mechanical (Ultrasound and video microscope)	Popp <i>et al.</i> (2012)
Optical imaging: Fluorescence MS, confocal MS, raman confocal MS (RCM), multiphoton fluorescence MS, SHG MS, OCT, bioluminescence imaging	Visible, IR and ultraviolet	Cellular viability, proliferation, differentiation, scaffold architecture RCM: monitor dynamic <i>in vitro</i> and <i>in vivo</i> scaffold degradation kinetics	• Thickness restriction due to out of focus light • Cannot study cell–cell interaction, cell–scaffold interaction	Static (OCT), parallel plate flow chamber (fluorescence), hollow fibre membrane bioreactor (bioluminescence), mechanical (confocal and MPM + SHG)	Hofmann <i>et al.</i> (2012) Khan <i>et al.</i> (2015) Gurjarpadhye <i>et al.</i> (2015) Kluge <i>et al.</i> (2011)
MRI Diffusion tensor imaging	1.5–11.7 T, 60–125 µm	ECM deposition, mineralization, scaffold architecture	• Need to assess safety of contrast agents	Perfusion (MR spectroscopy), hollow fibre bioreactor perfusion (MRM)	Crowe <i>et al.</i> (2011) Chesnick <i>et al.</i> (2007) Mertens <i>et al.</i> (2015)
X-ray • Conventional X-ray • Micro-CT • X-ray + phase contrast	CT: 12–50 µm	ECM deposition and mineralization, hydrogel (scaffold) architecture, vascularization	• Excluding mineralization-related studies, drying of sample or exogenous contrast agents required • Long data-acquisition times (X-ray + phase contrast)	Axial perfusion (micro-CT imaging), mechanical (micro-CT imaging)	Porter <i>et al.</i> (2007) Hagenmüller <i>et al.</i> (2010) Appel <i>et al.</i> (2015)
Nuclear imaging • PET • SPECT	1–3 mm	Cell viability, metabolic activity, tracking	• Structural information not provided • Poor spatial resolution	Perfusion bioreactor (PET)	Whitehead <i>et al.</i> (2013)

MS, microscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy; AFM, atomic force microscopy; ECM, extracellular matrix; CT, computed tomography; PET, positron emission tomography; SPECT, single positron emission computed tomography; OCT, optical coherence tomography; MPM, multiphoton fluorescence microscopy; SHG, second harmonic generation; MR, magnetic resonance; MRI, magnetic resonance imaging; IR, infrared.

(2014) performed bioreactor studies for cardiac tissue engineering by mimicking the native biophysical environment during ventricular contraction of the heart. They demonstrated that the delayed electrical stimulation after the start of mechanical stimulation mimicking the *in vivo* scenario promoted generation of functional cardiac tissues in comparison to other modes of stimulation. In another study by Heher *et al.* (2015), bioreactor was used to apply mechanical stimulation protocols that mimic *in vivo* muscle development during embryogenesis for engineering functionally relevant skeletal muscle tissues. The results demonstrated that strain led to aligned myotube formation and also temporal expression of functional genes similar to patterns observed *in vivo*.

While it is a commendable scientific feat to achieve a functional, *in vitro* tissue through bioreactor conditioning, a strong need for bioreactor-matured tissues in place of premature grafts in a clinical scenario has not been established. Firstly, there is no defined *in vitro* end-point for tissue maturation. There are varying degrees of maturation for each type of tissue. If we consider the bone tissue, there are four progressive stages of bone regeneration (Liu *et al.*, 2015), starting from precipitation of mineralized nodules in the first stage, and the final stage is defined by formation of dense and compact lamellar bone. Secondly, even if we do specify an end-point for the tissue in question, we need to establish the superiority of highly matured tissue grafts for clinical implantation

Table 5. Selected commercially available bioreactors and associated features used for the production of 3D tissue-engineered grafts

Company	Product cells/tissue*	Mechanical forces				Sensing and/or control	Reference
		Fluid flow/perfusion	Compression	Tension	Chamber		
Biometric ADMET TA Instruments	IAXSYS bioreactor	✓	✓	✓	SU	NA	Biometric (2016)
	BioTense Bioreactor	✓		✓	RU	T, strain, force	ADMET (2015)
	BioDynamic 5500 test instrument		✓		RU	Pressure, DO, T, pH, CO ₂ , force, displacement, metabolites, etc.	TA Instruments (2015)
	Multi-specimen BioDynamic test instruments	✓	✓	✓			TA Instruments (2015)
	BioDynamic 5900 Test Instrument	✓	✓				TA Instruments (2015) Q7
	BioDynamic 3DCulturePro	✓					TA Instruments (2016)
	Bioreactor tendon, ligament, bone, cardiac muscle, stem cells, vascular tissue, dental						
Ebers Medical	TEB 1000 flow bioreactor stem cells, other cells	✓			SU, RU	DO, T, pH, CO ₂ , metabolites	EBERS Medical Technology (2015a)
	TC-3 mechanical stimulator bioreactor stem cells, vascular tissue	✓	✓	✓		Not specified	EBERS Medical Technology (2013)
Flexcell International Corporation	Flexcell Tissue Train Culture System and FX-5000 compression system tendon, bone, cardiovascular, muscle, dental, ligament, cartilage, stem cells		✓	✓	SU	Not specified	Flexcell International Corporation (2016)
Glenmills/Zellwerk	Z@RP Bioreactor 500 cartilage, bone, stem cells	✓			SU, RU	pH, T, pO ₂ , perfusion rate	ZellWerk (2016)
Harvard Apparatus	InBreath bioreactor, Hollow organ bioreactor trachea	✓			RU	Pressure, DO, T, pH, CO ₂	Harvard Apparatus (2016)
	LB-2 bioreactor stem cells, lung	✓				Perfusion and airway pressure, tidal volume	Harvard Apparatus (2016)
	KLB-2 Kidney and Liver bioreactor kidney and liver	✓				Pressure, DO, pH, CO ₂ , flow rate	Harvard Apparatus (2016)
	Accelerator 1000	✓				Flow	Harvard Apparatus (2016)
	Multi-well Imaging Bioreactor	✓					Harvard Apparatus (2016)
Kirkstall	ORCA Bioreactor	✓				Pressure, DO, T, pH, CO ₂	Harvard Apparatus (2016) Q8
	Quasi-vivo system	✓			RU	pH, T, pO ₂ , airflow, flow	Kirkstall (2016)
Quinxell Technologies	TisXell Regeneration Systems stem cells, bone	✓			RU	pH, T, pO ₂ , rotation speed, flow rate	Quinxell Technologies (2016)
Synthecon	Rotatory, perfusion, stem cell culture systems skin, skeletal muscle, neural tissue, liver, pancreas, cartilage, cardiovascular, bone, stem cells	✓			SU, RU	Speed of rotation	Synthecon (2016) Synthecon (2016) Synthecon (2016) Q9
Tissue Growth Technologies	Osteogen, bone stem cell LumeGen, vascular progenitor cells	✓	✓	✓	RU	Flow, pressure, real-time stiffness	BISS TGT (2016) BISS TGT (2016) BISS TGT (2016) BISS TGT (2016) Q10
	LigaGen, ligaments/tendons						
	CartiGen, stem cell, chondrocyte						
	DermiGen, skin						

All information was obtained from the respective company website.

*Literature available for the culturing of these cells/tissue with the particular bioreactor.
DO, dissolved oxygen; T, temperature; SU, single use (disposable); RU, reusable.

over premature grafts that depend on *in vivo* remodelling for tissue maturation. Especially, the efforts taken to construct a system complex enough to provide conditions

that mimic the biochemical, physical and mechanical microenvironment of the native tissue need to be justified. Also, it has been suggested that biophysical stimulation

Figure 3. Schematic showing the ideal workflow of the procedure from bench to transplantation of the bioreactor-cultured tissue-engineered graft. The laboratory phase comprises of bioreactor design, computational modelling, and is followed by *in vitro* and *in vivo* validation experiments. *In situ* imaging systems that allow real-time monitoring of grafts are necessary prior to human implantation [Colour figure can be viewed at wileyonlinelibrary.com]

probably results in effects that lead to tissue fibrosis when implanted (Carver and Goldsmith, 2013; Parvin Nejad *et al.*, 2016). Continued studies in this aspect can only further our understanding on the practical relevance of physiological biomimicry in bioreactors for clinical implantation. With regards to monitoring culture conditions, we are already close to adapting the currently available commercial modalities in clinical settings. Sensing provides us ways to ensure accuracy of the microenvironmental conditions for tissue growth, which is very important for subsequent clinical implantation. On the other hand, bioreactor-integrated imaging systems for real-time evaluation of tissue growth and development have not shown as much translation even to the commercial arena owing to the complexity and the costs involved.

Despite being able to predict successful utilization of bioreactor in clinics by making suitable design modifications, there are limitations associated with the translation of laboratory-designed bioreactors into industrial-grade units for utility in the clinical setting. High financial costs incurred from research studies and the development of bioreactor systems, with increased complexity (i.e. automation, process control and sterilization systems), as well as the long wait-time for compliance with the regulations are the primary hurdles that hinder fast introduction of new bioreactor systems into the commercial and clinical scene. Good manufacturing practice (GMP) ensures quality assurance and control in accordance with the stringent guidelines throughout the development and production phases. The International Organization for Standardization (ISO) standards have also been introduced for companies to comply in design, manufacturing followed by subsequent product installation and validation. Over the years, the push factor for more stringent regulation has indirectly led to higher levels of technological advances such as automation, which helps in facilitating

validation tests. Ultimately, the intent of having these tightened regulations is to achieve quality and safety in the final cell-cultured product. However, the long processing times may have resulted in the exit of non-validated bioreactor systems and is likely to be a long journey before the bioreactor will be adopted widespread in the clinical setting. The clinical implementation of bioreactors is expected to be faced with challenges to a certain extent, also due to the logistical set-up and the need for a strong team of well-trained and dedicated cGMP-certified personnel. In different geographical jurisdictions, the governmental requirements for cGMP, hospital layouts and flow processes differ. The need for sterility and long preparation time of bioreactor requires qualified and well-trained technical personnel. Issues like safety, scalability and ease of handling also need to be considered while designing bioreactor systems. Ultimately, the successful clinical utilization of the design strategies will depend on constant developments in our understanding of tissue-engineering approaches, identifying the relevant clinical needs and solving the logistical challenges in bringing bioreactors to clinics.

7. Conclusion

For effective utilization of laboratory research, scientists need to constantly work towards implementing the research successes in a hospital (Chen *et al.*, 2012). With respect to bioreactor studies, we are still far from the era of widespread utility of bioreactors in the clinical setting. The key design factors highlighted in this article are worthy of consideration for future utility of these tissue culture systems in medicine. From an engineering point of view, the bioreactor technology has shown significant development over the years, with precise control of the various parameters of the microenvironment and real-

time sensing and imaging facilities, which allow greater control and reproducibility of the tissue graft generation. In addition, closer interactions among clinicians, scientists, engineers and other stakeholders such as the regulatory bodies and industrial partners are required to integrate the various requirements needed for a clinically

relevant bioreactor (Martin *et al.*, 2009). The future trend of bioreactors lies in an approach directed towards the mimicry of physiological environment, which incorporates the key design considerations discussed in this review and is envisaged to be of effective clinical utility (Figure 3).

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