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# *In vitro* platforms for tissue engineering: implications to basic research and clinical translation

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## Abstract

Due to the current clinical translation of the field of tissue engineering into regenerative medicine and the diminishing use of animals for the screening of drugs and other compounds, there is an increasing demand for *in vitro* engineered tissues which can be used for implantation, as well as for *in vitro* screening systems and basic research. However, current *in vitro* models are generally not capable of delivering the necessary output and further optimization of the models is a necessity. To facilitate this, bioreactor platforms and microscale technology will play an important role. Here we present a short overview regarding the current status and future development of *in vitro* tissue models, based on the findings presented at a recent international symposium.

## Introduction

*Biophysical stimuli and mass transport for tissue development* was the focus of a recent symposium in the Netherlands organised by Jos Malda (University Medical Center Utrecht), Jeroen Rouwkema (University of Twente) and Frank Baaijens (Technical University of Eindhoven). The meeting was held to discuss the use of bioreactor platforms in studies of biophysical signalling and mass transport in engineered tissues. The meeting consisted of 5 sessions: (1.) Utility of *in vitro* models, (2.) Mass transfer in developing tissue, (3.) Biophysical stimuli, (4.) Microscale technologies, and (5.) a discussion session moderated by Prof. Clemens van Blitterswijk (Twente University). The meeting was sponsored by the Translational excellence in Regenerative Medicine (TeRM) consortium, Dutch Platform for Tissue Engineering (DPTE) and Biomedical Materials (BMM) program. This opinion paper summarizes the most interesting conclusions of this meeting, and some of the current research needs related to the effective use of advanced bioreactor platforms in basic research and clinical translation. A more comprehensive review of the state of the art of the field can be found in several recent reviews (Martin et al. 2004; Martin and Vermette 2005; Freed et al. 2006; Khetani and Bhatia 2006; Wendt et al. 2008; Burdick and Vunjak-Novakovic 2009; Martin et al. 2010; Sung and Shuler 2010; Tandon et al. 2010).

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## Current status

### Utility of *in vitro* models

Tissue equivalents can be engineered for both clinical applications and *in vitro* testing. The development of *in vitro* screening systems based on human cells and tissues, has recently taken off due to a number of fundamental and practical reasons. First, there are ethical reasons for limiting the number of test animals to a necessary minimum. The 3Rs initiative (Reduce, Refine and Replace animal experiments) instituted by the European Union resulted in a total ban on animal use for screening of cosmetic ingredients since 2009, with an extension granted until 2013 for assessment of sensitizers and repeated dose toxicity. Cosmetics containing ingredients tested on animals can no longer be marketed after 2013 (http://ec.europa.eu/). Animals may still be used for screening of therapeutics, but only with the implementation of stringent reduction and refinement protocols. *In vitro* three-dimensional (3D) models based on engineered human tissues are now emerging as a viable alternative to two-dimensional (2D) cell culture assays (which often give false predictions due to the oversimplified cell environment) and *in vivo* experiments (which do not necessarily capture the important aspects of the human condition).

In addition, human tissue models are now thought to have higher predictive power than *in vivo* animal models, because different species often respond differently to treatments or compounds. In particular, data from rodent models that are most practical and most frequently used in screening studies, cannot be scaled up easily (if at all) to the human system due to major differences in size, time constants for transport and signalling, and the overall physiology (*e.g.,* the heart rate in a mouse is 6 times faster than in human). Finally, *in vivo* experiments are generally more labor-intensive, expensive and time-consuming than *in vitro* studies. Most importantly, about 92 percent of all drugs that enter clinical trials following extensive animal testing fail to achieve FDA approval, either because they are not safe or not effective in humans. In addition, of the 8 percent drugs that are approved, half are withdrawn or relabeled due to adverse effects not detected during animal testing (http://www.opposingviews.com/).

The symposium participants stressed the value of *in vitro* model systems for reducing the costs and time needed to bring a pharmaceutical product to the market, and develop new modalities of personalized medicine tailored to a patient and her/his medical condition. They also emphasized that many of currently used *in vitro* models lack predictive power, in most cases due to the lack of critical molecular and physical cues in the cell/tissue environment. One of the most important suggestions was that the field should focus more on integrating true 3D environments and that advanced bioreactor systems are critically needed to provide physiologically relevant cues to the cells/tissues in the *in vitro* screening platforms.

### Mass transfer in developing tissue

*In vivo,* most cells are in the vicinity of blood vessels providing nutrient supply and waste removal. In contrast, tissues engineered *in vitro* are generally not vascularized and not connected to blood flow. An understanding of the mass transfer principles in bioreactors is necessary to ensure that nutrient limitations and/or build-ups of waste products do not occur. Mass transport between tissue and blood, as well as culture medium and engineered tissue, is governed by two processes: diffusion of molecules over short distances (in most cases <100 μm) and convective flow over larger distances. It was emphasized that the concepts used to increase nutrient supply *in vitro* fall into three categories: increasing the effective diffusion coefficient, decreasing the diffusion distance, or increasing the role of convective transport (Rouwkema et al. 2009). Perfusion bioreactors have been widely employed in the field of tissue engineering (Martin et al. 2004; Radisic et al. 2008; Grayson et al. 2010),

enabling the production of large (millimeter to centimetre size) pieces of viable tissue, an outcome not possible when relying on diffusion alone. However, it should be noted that perfusion bioreactors can have negative side effects that should not be overlooked, such as the induction of shear stresses or the washing out of growth factors and cytokines that are produced by the developing tissue. Other strategies for enhancing mass transport that have been discussed include the optimization of scaffold architecture (increasing the overall diffusion coefficient) (Malda et al. 2004), the use of oxygen carriers (Radisic et al. 2006; Centis and Vermette 2009) and oxygen-generating scaffolds (Oh et al. 2009), and approaches that decrease diffusion distances by growing micro tissues that are later combined into larger units (McGuigan and Sefton 2006; Du et al. 2008; Rivron et al. 2009).

## Biophysical stimuli

Besides improving the supply of biochemical stimuli such as nutrients and growth factors in the perfused medium to the cells, bioreactors were shown to allow for the modulation of tissue development by controlled application of physical forces. Clearly, physical stimulation can influence cell fate, altering for example the secretion of extracellular matrix by the cells and the organization and alignment of the matrix and cells within the developing tissue (Freed et al. 2006). While historically physical stimulation has been employed at the macro-scale as a method to control cell activity, for example by subjecting the tissue to mechanical compression, recent studies suggest that the effects of physical signals at the micro-scale may be even stronger (Rehfeldt et al. 2007; Discher et al. 2009). Moreover, the biophysical stimuli do not necessarily have to be administered from the exterior as is the case with compression or extension, but can also be included in the matrix of the tissue construct itself. Stem cells, for instance, can sense small environmental changes such as stiffness and will differentiate upon these biophysical stimuli (Reilly and Engler 2010).

## Microscale Technologies

Clearly, proper tissue development requires tight control of the cellular environment, both on micro and macro scales. Several presentations at the symposium discussed emerging technologies designed to control and monitor cellular environments in order to control cell fate and, ultimately, tissue development. With regards to the latter, multi-and pluripotent (human) stem cells were considered as key cellular sources for these endeavors. Their unique ability to renew themselves (self-renewal) and to give rise to specialized progeny (differentiation), as well as new methodologies for their generation (i.e. induced pluripotent stem cells (Yamanaka and Blau 2010)), opens up truly exciting avenues for bioreactor-based tissue engineering. However, one hurdle to overcome is the difficulty to control the behavior of (stem) cells in culture. Conventional *in vitro* culture platforms only poorly mimic the physiological context, and often suffer from imprecise spatial and temporal control of extracellular cues, low throughput, lack of scalability and reproducibility, and limited inclusion of biophysical regulatory factors. Novel platforms based on microfabrication and microfluidics were discussed as means to overcome these issues and, in particular, offering a better representation of the complexity of the *in vivo* situation, as they could provide a greater control of environmental parameters (Gupta et al. 2010). Moreover, apart from the advantage of very low reagent consumption, the scale and transparency of lab-on-a-chip systems allows for on-line imaging of cellular processes, which makes these systems particularly suitable for high-throughput screening. This is especially important when studying complex phenomena as for instance the regulation of stem cells by their niches (Lutolf and Blau 2009).

## Future directions

One of the conclusions from this meeting is that tissue engineering for implantation and for *in vitro* research and screening applications can pose distinctly different requirements to the design of bioreactor systems. Another conclusion was that *in vitro* model systems need to be more sophisticated – to expose cells to a physiological 3D environment and include well-controlled stimulation of signalling factors - in order to replicate the *in vivo* environments to the extent necessary for biological relevance. One notable example are skin model systems that tend to become more complex: from dermal and epidermal skin equivalents to full skin equivalents and currently still not existing skin models containing melanocytes, capillary networks, and Langerhans cells (Gibbs 2009). The common notion is that an increased complexity in most cases brings the *in vitro* models closer to the actual *in vivo* system. However, the question is how much complexity one needs for a specific application. An assay to test for the cytotoxicity of a compound will most likely not require a capillary network or melanocytes, whereas an assay to test for the immunological response to a compound is useless if the model system is not immune-competent.

For engineered tissues used in regenerative medicine applications, the goal is not necessarily to mimic a natural tissue, but rather to generate a graft with the ability to replace or regenerate damaged tissue. In this case, the 'maturation' of the tissue can occur entirely *in vivo*. Obviously, this will have an effect on the requirements of the in vitro system (Martin et al. 2009). The key recommendation in this sense was: "If you engineer a tissue for clinical application, keep it as simple as possible". The meeting participants agreed that simple tissues will facilitate translation by decreasing the time and investment into regulatory approval, and by improving reproducibility. Apart from that, the tissue construct should be designed by bioengineers working hand-in-hand with clinicians, to properly address all fundamental and practical requirements.

Another major recommendation at the meeting was that the design criteria of bioreactors should depend upon the niche characteristics of the cells. To fully employ this concept, it was suggested to continue efforts to understand the transport and signaling at all scales: from the macro-scale of the actual device right to the micro-scale of the cellular environment. Efforts in this field should thus be directed towards "opening the black box" of *in vitro* model systems. To this end, new developments in sensor technology and mathematical modeling are becoming necessary. Microscale technologies are also proving to be an important tool, as they allow tight control of cellular environment, and precise monitoring of processes within the system. Moreover, microtechnologies enable the creation of an array of cellular environments for high-throughput screening. However, as is the case with model systems at the macro-level, microscale models currently often offer a 2D environment to the cells. Since the natural environment of a cell is three-dimensional in nature, *in vitro* model systems that offer a 3D environment are more suitable to replicate the *in vivo* situations.

One of the most important developments in the field of *in vitro* model systems may thus be enhanced replication of the *in vivo* environment. It is clear that technological developments and bioreactors will play an important role, by allowing the culture of large and viable 3D tissue equivalents due to medium perfusion through the tissue, or by precisely controlling and perturbing the cellular environment using microscale technologies. This new generation of *in vitro* model systems will provide us with the information needed to better understand regenerative processes *in vivo*, identify and overcome the factors that limit the natural healing or regeneration of tissues. Clearly, this information is critical for the currently ongoing translation from tissue engineering into regenerative medicine (Leeuwenburgh et al. 2008). Through this transition, *in vitro* model systems can play their role in clinical

applications. Not only as implantable tissues, but also as platforms to identify the critical factors for tissue regeneration.

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