Review

CellPress

Strategies for improving the physiological relevance of human engineered tissues

Rosalyn D. Abbott and David L. Kaplan

Department of Biomedical Engineering, Science and Technology Center, Tufts University, 4 Colby Street, Medford, MA 02155, USA

This review examines important robust methods for sustained, steady-state, *in vitro* culture. To achieve 'physiologically relevant' tissues *in vitro* additional complexity must be introduced to provide suitable transport, cell signaling, and matrix support for cells in 3D environments to achieve stable readouts of tissue function. Most tissue engineering systems draw conclusions on tissue functions such as responses to toxins, nutrition, or drugs based on short-term outcomes with *in vitro* cultures (2–14 days). However, short-term cultures limit insight with physiological relevance because the cells and tissues have not reached a steady-state.

Defining 'physiological relevance' in tissue engineering approaches

The goal of tissue engineering is to generate living tissue constructs *in vitro* that are morphologically and functionally similar to native tissue. Growing physiologically relevant tissues requires multidisciplinary research where the resulting tissues can be used for the study of human development and disease, to test the efficacy and toxicity of compounds and treatments, and for regenerative medicine applications. In tissue engineering reports, many terms are commonly used to describe outcome measurements of these tissues, including 'physiological relevance', 'mature', and 'stable.' While all of these terms imply that the *in vitro* tissues behave in a similar manner to *in vivo* tissues, they may not describe the essential details accurately unless the terms are properly defined for each case.

To make a general, broad definition, 'physiological relevance' is the characteristic of (or corresponding to) healthy or normal biological functioning. However, in different situations this will mean different things in relation to tissue engineering. For instance, if the goal of the study is to screen drug candidates during preclinical drug development for liver treatment, recreating general cellular functions (oxygen uptake, amino acid metabolism, and substrate consumption) and liver-specific functions (drug-metabolizing capacities and the production of liver-specific metabolites) can qualify as physiologically relevant [1]. However, for implantation in a patient suffering from liver failure, the liver will have to additionally contain bile ducts, a functional vascular network, and a hepatic microarchitecture, as well as have a substantial regenerative capacity, to be considered physiologically relevant [2].

In the same context, physiologically relevant tissues should contain 'mature' cells specific to the tissue and goal of the study. However, this brings up the question – what is a mature cell? Each tissue contains different cell types that vary depending on the tissue and the state of maturation of that tissue. Therefore, a 'mature cell' can be defined as a cell that exhibits normal biological functions in the 'developed' form of the tissue. 'Developed' in this case refers to the stage of the desired tissue, which can be embryonic, young, aged, diseased, etc., depending on the goals of the study.

After establishing the targeted or required 'mature' status of cells within the tissue, it is important to establish when the tissue has become 'stable'. Importantly, having mature cells does not mean the tissue is stable because the tissue could still be adjusting, expanding, and forming. Therefore, stability can be defined as a tissue that is not changing with time. This can be determined by tracking material properties [3], matrix content [4], or by other markers of function such as secreted proteins [5–8] or endogenous signals [9,10]. A homeostatic, 'stable' tissue is essential for tissue engineering as a baseline for *in vitro* studies of the efficacy and toxicity of compounds, or to maintain phenotype upon implantation for regenerative applications. It is important to note the goals of the study, however, in some disease states such as tumors, 'stable' tissues would not be the goal.

In this review we describe strategies for improving the physiological relevance of tissue engineered constructs, acknowledging that 'physiological relevance' will vary in definition in different contexts. We will touch upon some of the more common strategies for forming 'stable' biological functions with 'mature' cells that are more in line with *in vivo* function, with a specific focus on the temporal component of culturing engineered tissues *in vitro*.

Strategies for improving the physiological relevance of tissue engineered constructs

While the endpoint criteria are specific to the tissue of interest and the desired application common strategies to improve the physiological relevance of tissues (Figure 1)

Corresponding author: Kaplan, D.L. (david.kaplan@tufts.edu).
Keywords: long-term culture; tissue engineering; microfluidics; 2D culture; 3D culture; bioreactors.

0167-7799/
© 2015 Elsevier Ltd. All rights reserved. http://dx.doi.org/10.1016/j.tibtech.2015.04.003

CrossMark

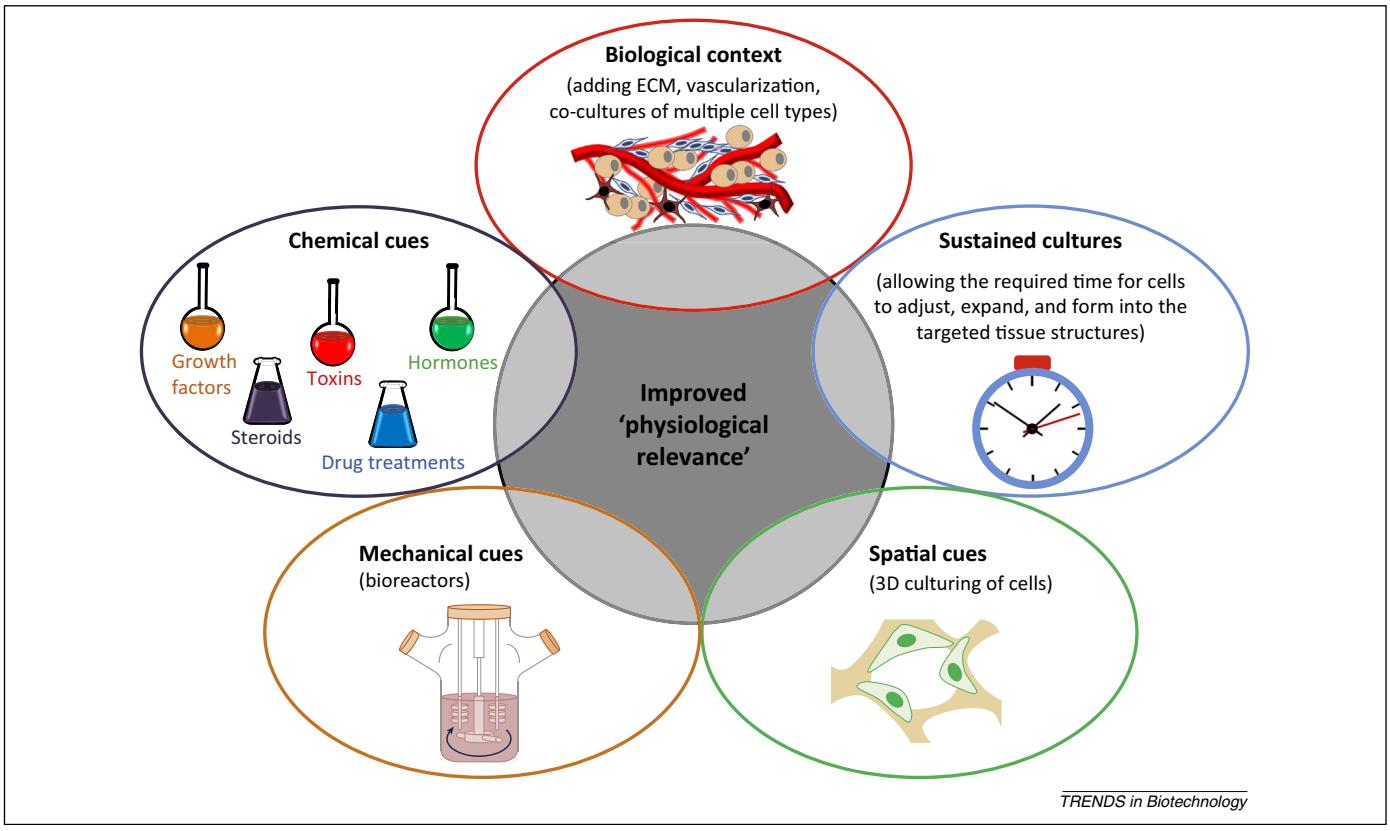

Figure 1. To improve the 'physiological relevance' of engineered tissues biological context (extracellular matrix, vascularization, and cell types), chemical cues, mechanical cues (bioreactors), spatial cues (culturing cells in 3D), and the temporal timing of cultures should be considered.

include recapitulating the biological context [such as the extracellular matrix (ECM), vascularization and cell types] and chemical and mechanical cues (through the use of reagents and bioreactors), as well as incorporating spatial cues (by culturing cells in 3D). All of these strategies require the optimization of culture conditions in an attempt to form mature, stable tissues.

Recapitulating biological context

Each tissue exhibits variability in the amount and type of ECM components [11,12]. Therefore, for the *in vitro* environment the matrix should be carefully considered for each tissue to mimic the tissue content and properties. Biomaterial scaffolds predominately consist of ceramics (examples: hydroxyapatite or tri-calcium phosphate), synthetic polymers (examples: polystyrene, poly-L-lactic acid, polyglycolic acid, poly-D,L-lactic-co-glycolic acid), or natural polymers (examples: collagen, alginate, silk) with varying physicochemical properties, architecture, and degradability [13]. In particular, the porosity, pore dispersal, surface area, mechanical properties, and surface chemistry influence the attachment, migration, proliferation, and production of ECM by the seeded cells within the scaffold. In addition, to mimic other aspects of the ECM the process can be aided with a hydrogel (examples: Matrigel, collagen), or the hydrogel can be used as a standalone 3D matrix lacking the structural integrity of a more robust, rigid porous scaffold.

One of the major challenges of generating matrix-rich, dense tissues, however, is the limited mass-transfer distances for nutritional supply and waste removal. To address this issue, tissue vasculature (which provides and removes nutrients *in situ*) can be recreated (Box 1). Moreover, to recapitulate the biological context, cellular interactions within a tissue must be considered to help maintain tissue specificity and homeostasis which is fostered through cell–cell signaling. Enhanced differentiation and survival has been achieved in many organ systems by co-culturing relevant cell types, for example: skin [14], neural tissue [15,16], bone [17], and liver [18]. Co-cultures lead to increased ECM deposition over mono-cultures, including fibronectin deposits in glomerular tissue [19], and collagen deposition and mineralization in bone tissue constructs [20]. Improved function has been demonstrated by co-cultures including beating cardiomyocytes, which increased fluctuations in intracellular calcium ion concentrations not achieved in mono-cultures [21]. In addition, proper morphology has been observed in co-cultures for cardiomyocytes [21], endothelial cells [18], and epithelial cells [19] that was not observed when the cells were cultured individually. Improvements in vascular structures can also be achieved with co-cultures over mono-cultures [17,18,22]. While co-culture systems enhance physiological relevance, they increase the complexity of the culture system and require special design considerations. For instance, the media components, ratios of cell types, and timing of differentiation need to be optimized to obtain proper tissue formation. In addition, differential labeling of each cell type is helpful to evaluate cellular interactions and contributions [23].

Download English Version:

https://daneshyari.com/en/article/36962

Download Persian Version:

https://daneshyari.com/article/36962

Daneshyari.com