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Three-dimensional cell culture technique and pathophysiology ☆

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ABSTRACT

Three-dimensional (3D) tissue constructs consisting of human cells have opened a new avenue for tissue engineering, pharmaceutical and pathophysiological applications, and have great potential to estimate the dynamic pharmacological effects of drug candidates, metastasis processes of cancer cells, and toxicity expression of nano-materials, as a 3D-human tissue model instead of *in vivo* animal experiments. However, most 3D-cellular constructs are a cell spheroid, which is a heterogeneous aggregation, and thus the reconstruction of the delicate and precise 3D-location of multiple types of cells is almost impossible.
In recent years, various novel technologies to develop complex 3D-human tissues including blood and lymph capillary networks have demonstrated that physiological human tissue responses can be replicated in the nano/micro-meter ranges. Here, we provide a brief overview on current 3D-tissue fabrication technologies and their biomedical applications. 3D-human tissue models will be a powerful technique for pathophysiological applications.

Contents

1. Introduction

Quantitative and time-lapse analyses in microenvironments for drug diffusion, drug toxicity, and the release of molecules from drug delivery carriers are useful to explore the physiological processes, mechanisms, and treatments of disease or injury through pathophysiology. Various spatial analytical instruments such as computed tomography, magnetic resonance imaging, and positron emission tomography have been employed for general *in vivo* animal experiments in pathophysiology.

Abbreviations: 3D, three-dimension; LbL, layer-by-layer; ECM, extracellular matrix; FN, fibronectin; G, gelatin; NHDF, normal human dermal fibroblasts; HUVECs, human umbilical vein endothelial cells; iPS, induced-pluripotent stem.

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However, even though their detection limits have been improved day by day, the detection of events in the micron-meter scale is still difficult. Moreover, species differences between animals and humans can cause fundamental confounders such as metabolic processes, enzymes, and membrane proteins. Accordingly, *in vitro* human cell-based drug evaluations, including drug efficacy testing, toxicology, and basic cell biology [1,2], are of great importance as an alternative to animal experiments to solve the significant issue of species differences. In particular, animal testing for cosmetics and chemicals has been prohibited under the 7th Amendment to the Cosmetics Directive (Council Directive 76/768/EEC) and REACH (registration, evaluation, authorization and restriction of chemicals) in the European Union (EU) from March 2013 [3].

Cell-based drug evaluations have generally been performed in a plastic dish under monolayer (two-dimensional; 2D) cell conditions. Since nearly all tissues are integrated three-dimensional (3D) structures of multiple types of cells and extracellular matrices (ECMs), and since intercellular signaling is important for biological functions, it is generally difficult to estimate the actual drug effects on physiologic functions by 2D-culture methods. The development of 3D human tissue constructs consisting of simplified tissue structures with multiple types of cells and ECMs is a key challenge for pharmaceutical and pathophysiological evaluations and tissue engineering.

In general, a cell spheroid has been employed as the 3D-culture model, especially 3D-cancer spheroids for metastasis or pharmacological assays of cancer cells [4–6] and 3D-hepatocyte spheroids for inducing higher activity of hepatocytes [7–9]. Although, interesting 3D-co-culture spheroids have been reported to investigate tumor invasion [10,11], to enhance hepatocyte functions [12] and to mimic *in vivo* conditions [13], the reconstruction of the delicate and precise 3D-location of multiple types of cells has not been achieved yet due to their heterogeneity, the lack of control of the cell number and location, and necrosis inside the cells because of insufficient nutrients.

Here we provide a brief overview of the technologies for the construction of 3D-tissue constructs, mainly our hierarchical cell manipulation technologies, and then discuss how these 3D-tissues could contribute to understanding nano-pathophysiology.

2. Top-down approach for development of 3D-tissue constructs

There are basically two kinds of approaches, top-down and bottom-up approaches, for the construction of 3D-tissue constructs (Fig. 1). A top-down approach has been reported historically, especially the biodegradable scaffold method. Biodegradable scaffolds and hydrogels consisting of biodegradable polymers, such as poly(lactic acid), poly(glycolic acid), alginate and collagens have been used for the construction of 3D-constructs containing living cells [14,15]. The topological control of biodegradable porous scaffolds [16], especially nanofiber scaffolds by electrospinning [17] or self-assembling amphiphilic peptides [18], has attracted much attention due to their high porosity and the controlled alignment of the fibers to control cellular function. The cells encapsulated in the scaffolds can grow actively, and finally formed random cell aggregations inside it. Although their growth rate can be controlled using growth factors in the culture medium, 3D-engineered tissues possessing precisely-controlled cell types, cell alignment, and cell–cell interaction have not been developed yet. These nanofiber scaffolds can contribute to the tentative cell alignment or adhesion due to morphology, but it is difficult to maintain these effects, because the nanofibers are covered completely with cultured cells and expressed ECMs from the cells. Accordingly, a conventional approach using biodegradable matrices such as hydrogels or fiber scaffolds seems to have several limitations in developing 3D-tissue constructs which satisfy the above requirements. A bottom-up approach using multiple cell types as pieces of tissue has recently attracted much attention to solve these problems.

3. Bottom-up approach for the development of 3D-tissues

Various bottom-up approaches such as cell sheets [19], magnetic liposomes [20], hierarchical cell manipulation [21], polymeric aqueous two-phase systems [22] and printing cells and polymers [23] have been reported in constructing a complex tissue structure. These bottom-up approaches are generally categorized into two groups: cell-based methods, and cell and polymer-based methods. Here, the characteristics, advantages, and issues with both approaches are briefly summarized.

3.1. Cellular layer-by-layer approaches

One of the most general methods of the bottom-up approaches is cellular layer-by-layer (LbL). Okano and co-workers have reported the fabrication of monolayers of cell sheets and their stacking to create multilayered structures using temperature-responsive polymer grafted culture dishes [19]. Using temperature-responsive dishes, cultured cells can be harvested as fragile sheets by temperature changes, thereby avoiding the use of proteolytic enzymes. They prepared multiple sheet structures of cardiomyocytes for the reconstruction of 3D-myocardical tissues by stacking the cell sheets [24]. Because the cell sheets have ECM at the bottom underneath the sheet, they can stack easily due to

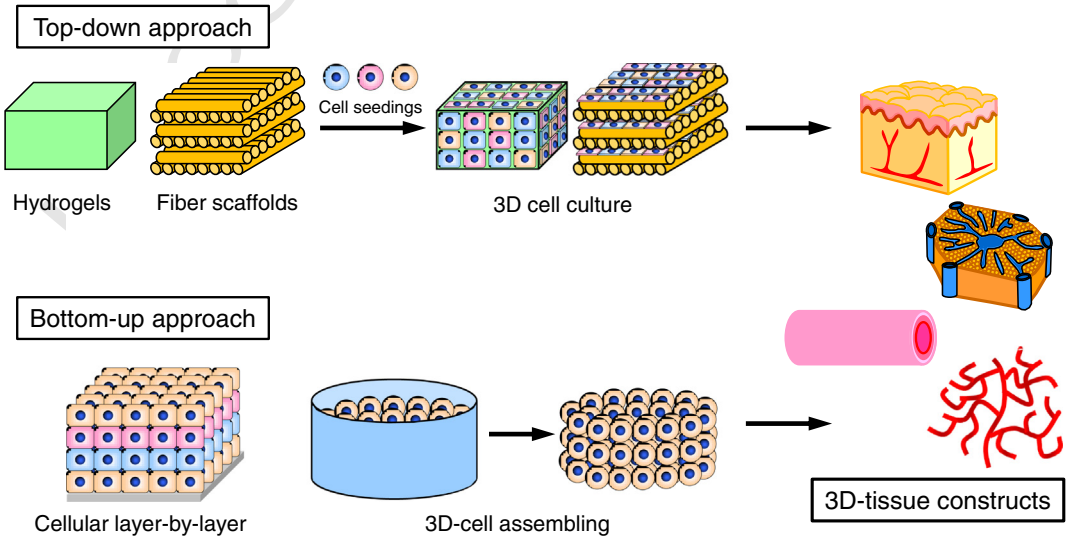

Fig. 1. Schematic illustration of the top-down and bottom-up approaches to fabricate 3D-tissue constructs.

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