Biomaterials 35 (2014) 1816–1832

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Review

A review of microfabrication and hydrogel engineering for micro-organs on chips

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ARTICLE INFO

Article history:
Received 3 October 2013
Accepted 7 November 2013
Available online 4 December 2013

Keywords:
Artificial extracellular matrix
Hydrogel
Micro-environment
3D scaffold
Physiology
Tissue engineering

ABSTRACT

This review highlights recent trends towards the development of *in vitro* multicellular systems with definite architectures, or "organs on chips". First, the chemical composition and mechanical properties of the scaffold have to be consistent with the anatomical environment *in vivo*. In this perspective, the flourishing interest in hydrogels as cellular substrates has highlighted the main parameters directing cell differentiation that need to be recapitulated in artificial matrix. Another scaffold requirement is to act as a template to guide tissue morphogenesis. Therefore specific microfabrication techniques are required to spatially pattern the environment at microscale. 2D patterning is particularly efficient for organizing planar polarized cell types such as endothelial cells or neurons. However, most organs are characterized by specific sub units organized in three dimensions at the cellular level. The reproduction of such 3D patterns *in vitro* is necessary for cells to fully differentiate, assemble and coordinate to form a coherent micro-tissue. These physiological microstructures are often integrated in microfluidic devices whose controlled environments provide the cell culture with more life-like conditions than traditional cell culture methods. Such systems have a wide range of applications, for fundamental research, as tools to accelerate drug development and testing, and finally, for regenerative medicine.

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1. Introduction

Langer has described tissue engineering as 'an interdisciplinary field that applies the principles of engineering and the life sciences to the development of biological substitutes that restore, maintain, or improve tissue function' [1]. This field is currently advancing rapidly, combining progress in biology and technology, and has raised many hopes in several areas of biology and medicine. First and foremost it has a strong potential as an application in regenerative medicine, for developing life-like replacement tissues and organs. But it also has an important role to play in fundamental biological research: it allows users to reproduce physiological microenvironments more closely in *in vitro* settings than traditional culture methods, offering a way to bridge the gap between *in vivo* experiments and conventional *in vitro* studies. On a more operational side, the development of new *in vitro* models based on human cells has raises the possibility of tackling many of the obstacles that currently hinder pharmaceutical research and drug development. Engineered tissues could reduce the limitations related to the transposition of findings from one organism to another, alleviate ethical problems related to animal testing, increase standardization, and allow more thorough studies of toxicity, metabolism and life cycle of putative drugs before entering clinical testing. This could, in turn, reduce the duration, cost failure rate and risk of clinical trials.

In vivo, the formation of organs and tissues is based on the coordination in time and space of cell differentiation, polarity, shape, division and death. This coordination relies on the cellular integration of signals from the microenvironment, mainly consisting of the extracellular matrix (ECM), and intercellular communication [2]. The transduction of a typical combination of these factors coupled to specific cytoplasmic components can induce the three progressive steps in differentiation. First, stem cells are specified towards a certain fate, then they shift from a specified state to a determined state, in which the cell fate cannot be reversed, and finally reach their differentiated state.

The main challenge of tissue engineering is to reconstitute '*in vitro*' an environment that induces the differentiation of cells and their organization in an ordered functional tissue. The cell substrate is of particular importance, since *in vivo* the extracellular

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http://dx.doi.org/10.1016/j.biomaterials.2013.11.021

space is occupied by the ECM. The chemical composition of the ECM and the resulting mechanical properties are both important aspects, as the transduction of both chemical and physical signals via cellular adhesion molecules affects cell shape, polarization, migration and differentiation [3–5]. Furthermore, the ECM topography orients tissue polarity and the morphogenesis of new organs [5,6].

Crude *in vitro* substrates that lack these features are therefore insufficient for guiding the assembly and coordination of isolated cells to form a coherent tissue with a common global orientation and polarity. New devices that mimic the microscale structures of the ECM are needed and are now being developed thanks to the fast evolving fields of microfabrication and microfluidics technologies that have the ability to structure space on the scale of micrometers to centimeters. Microfabrication is allowing researchers to structure space at the right order of scale for positioning individual cells according to architectures experienced *in vivo*. Microfluidics also provides new tools for controlling the transport and availability of chemical and biochemical signals on such micron scales. The cells can be seeded in an enclosed area with volume restrictions and transport properties favoring autocrine and paracrine communication, allowing users to build more realistic and/or more specific culture conditions than can be achieved in traditional cell culture. The resulting autonomous and functional tissues shaped with a morphology that attempts to recapitulate conditions seen *in vivo* organs has led to the concept of 'organs on chip'.

Many existing methods seed cells in microfluidics systems with purposes as diverse as performing massively parallel single cell analysis [7], studying populations of unicellular organisms [8], or even using cells as "actuators" for purely technical functions, e.g. fluid transport or actuation by myocytes [9]. In this review, however, we focus on techniques that attempt to reproduce physiological environments and therefore induce cells to proliferate, grow and differentiate into specific tissues that acquire the related functionality.

We first describe the main chemical and mechanical features needed to create an artificial extracellular matrix that recapitulates *in vivo* ECM as closely as possible, in order to induce cell differentiation. In general, however, this is not sufficient, since cells grown on a crude, non-structured artificial extracellular matrix substrates rarely organize spontaneously into tissues. We therefore also review how to use two-dimensional systems to position, orient or polarize cells, from single cells to multicellular entities. We subdivide these two-dimensional systems into two subcategories, « purely 2D » ones involving the chemical patterning of a flat surface, and another one we call "2D ½". This concept of "2D ½" describes substrates presenting a discontinuity in the third dimension, but in which cells grow in 2D. Finally, we address the most complex architectures, in which cells are arranged in 3D tissues with dimensions consistent with the *in vivo* multicellular units of organs. These last types of scaffolds attempt to generate substrates that are physiological or "biomimetic" both in topography and biochemical formulation.

2. Artificial extracellular matrices

The *in vivo* ECM consists mainly of collagen fibers, elastin fibers, glycoproteins and polysaccharides. It acts as a mechanical support to the cells it surrounds and plays an important role in cell shape, cell polarity, cell migration, resistance to external forces, and signal transduction. The primary focus of tissue engineering is to achieve an *in vivo*-like cellular environment, by developing *in vitro* artificial ECM. In regenerative medicine applications, such scaffolds could either be directly introduced into an injured organ in order to induce *in vivo* genesis, or first seeded with cells *in vitro* and then transplanted once those cells have differentiated. For *in vitro* research and testing applications, they are generally directly used as a substrate. In all cases, the artificial structures need to recapitulate the *in vivo* environment signals responsible for cell differentiation into the desired tissue. In the following sections, we discuss the different ways of generating and controlling artificial ECM properties. We also underline how the possibility of uncoupling biochemical and physical parameters has opened the route for a deeper understanding of the influence of cell microenvironment on cell fate.

2.1. Chemical composition

Biomaterial scaffolds are essentially made of hydrogels. Based on their ability to retain water by swelling, they mimic the high water content of the extracellular matrix [10]. Hydrogels can be classified into two distinct categories: the natural and the synthetic hydrogels [10–12]. Natural hydrogels include collagen [13–15], fibrin [16], hyaluronic acid [17], Matrigel [18], and derivatives of natural materials such as chitosan [19], alginate [20] and silk fibers [21]. They remain the most physiological hydrogels as they are components of the ECM *in vivo*. Two main drawbacks of natural hydrogels, however, make their final microstructures and properties difficult to control reproducibly between experiments. First, the fine details of their mechanical properties and their dependence on polymerization or gelation conditions are often poorly understood [22]. Second, due to their natural origin (bovine fibrinogen [16], rat tail collagen I [23]…) their composition may vary from one batch to another.

In contrast, synthetic hydrogels such as poly(ethylene glycol) diacrylate [24,25], poly(acryl amide) [26,27], poly(vinyl alcohol) are more reproducible, although their final structure can also depend on polymerization conditions in a subtle way, so that a rigorous control of the preparation protocol, including temperature and environment control, may be necessary. Generally speaking, however, synthetic hydrogels offer more flexibility for tuning chemical composition and mechanical properties; users can, for example vary the concentration or molecular weight of the precursor, or alter the percentage of crosslinkers. They can also be selected or tuned to be hydrolysable or biodegradable over variable periods of time.

Cell adhesion ligands must be present in hydrogels to allow cells to adhere, spread, migrate and proliferate. There is a large variety of adhesion molecules, such as laminin [28] and its derivatives [29], fibronectin [30], RGD peptide sequence carried by fibronectin [11] and collagen [31,32]. It is therefore crucial to select the adhesion molecules for which the seeded cell type has the largest affinity to make adhesion effective. Natural hydrogels are bioactive and usually provide native adhesion sites. Conversely, synthetic hydrogels are inert, since their carbon skeleton presents no adhesion molecules or endogenous factors inducing proliferation and cell differentiation.

To enrich their potential as "bioactive" materials, synthetic hydrogels are generally supplemented with adhesion molecules [33], either by covalent grafting [34], adsorption [35] or electrostatic interaction [36]. Adhesion molecules can be grafted after hydrogel polymerization, or added to the pre-polymerized mixture and either physically trapped or chemically incorporated during polymerization. Finally, in the case of photoactivatable materials such as PEGDA, adhesion molecules can be chemically modified to covalently attach to the hydrogel backbone. The grafting of PEG polymer has been thoroughly described in the review of Zhu et al. [37].

The inertness of synthetic hydrogels may appear to be a disadvantage, since additional manipulation is needed to promote cell adhesion. However it allows a more flexible tuning of the different factors at play in cell fate. It can, for example, allow researchers to decouple mechanical and biochemical factors to assess independently the effect each factor has on cell behavior. This kind of

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