Acta Biomaterialia 54 (2017) 1–20

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Review article

Scaffolds for 3D *in vitro* culture of neural lineage cells

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ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 28 February 2017
Accepted 28 February 2017
Available online 1 March 2017

Keywords:
Neural stem cells
Scaffolds
3D cell culture
Polymers
Hydrogels

ABSTRACT

Understanding how neurodegenerative disorders develop is not only a key challenge for researchers but also for the wider society, given the rapidly aging populations in developed countries. Advances in this field require new tools with which to recreate neural tissue *in vitro* and produce realistic disease models. This in turn requires robust and reliable systems for performing 3D *in vitro* culture of neural lineage cells. This review provides a state of the art update on three-dimensional culture systems for *in vitro* development of neural tissue, employing a wide range of scaffold types including hydrogels, solid porous polymers, fibrous materials and decellularised tissues as well as microfluidic devices and lab-on-a-chip systems. To provide some context with *in vivo* development of the central nervous system (CNS), we also provide a brief overview of the neural stem cell niche, neural development and neural differentiation *in vitro*. We conclude with a discussion of future directions for this exciting and important field of biomaterials research.

Statement of Significance

Neurodegenerative diseases, including dementia, Parkinson's and Alzheimer's diseases and motor neuron diseases, are a major societal challenge for aging populations. Understanding these conditions and developing therapies against them will require the development of new physical models of healthy and diseased neural tissue. Cellular models resembling neural tissue can be cultured in the laboratory with the help of 3D scaffolds – materials that allow the organization of neural cells into tissue-like structures. This review presents recent work on the development of different types of scaffolds for the 3D culture of neural lineage cells and the generation of functioning neural-like tissue. These *in vitro* culture systems are enabling the development of new approaches for modelling and tackling diseases of the brain and CNS.

Contents

Abbreviations: 2D, two dimensional; 3D, three dimensional; BDNF, brain derived neurotrophic factor; bFGF, basic fibroblast growth factor; BMP, bone morphogenic protein; CNS, central nervous system; CNTF, ciliary neurotrophic factor; d, days; ECM, extra cellular matrix; ESC, embryonic stem cell; EGF, epidermal growth factor; FDA, Food and Drug Administration; FGF, fibroblast growth factor; FGF8, fibroblast growth factor 8; GSK3βi, glycogen synthase kinase-3β inhibitor; hiPSC, human induced pluripotent stem cell; hPSC, human pluripotent stem cell; iPSC, induced pluripotent stem cell; NGF, nerve growth factor; NPC, neural progenitor cell; NS/PC, neural stem/progenitor cell; NSC, neural stem cell; PEG, poly(ethylene glycol); PHEMA, poly(hydroxyethyl methacrylate); PLA, poly(lactic acid); PSC, pluripotent stem cell; RA, retinoic acid; RG, radial glial stem cell; SGZ, subgranular zone; SHH, sonic hedgehog; SVZ, subventricular zone; VEGF, vascular endothelial growth factor; vmIPN, variable moduli interpenetrating polymer network.

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http://dx.doi.org/10.1016/j.actbio.2017.02.046
1742-7061/

1. Introduction

The brain is the least understood organ in the human body. It is difficult to access, highly susceptible to damage and complex in structure and function. The poor understanding of the human brain is reflected in the lack of effective treatments for various neurological disorders such as Parkinson's and Alzheimer's disease and motor neuron disorders. To address this research gap, new methods for the culture of human neural (neuronal and glial) lineage cells, particularly *in vitro* 3D culture, are being developed to more accurately reconstruct the complex *in vivo* structure and function of the human brain.

Human somatic cells cultured in flat, stiff, 2D environments typically display an irregular morphology and form unnatural cell-cell interactions [1]. Traditional monolayer cell cultures are simple and convenient to analyse, however tissue specific architecture, mechanical and biochemical cues and cell-cell communication are lost to various degrees. This can lead to physiological inaccuracies that can be extremely problematic for disease modelling and pre-clinical drug screening. In cancer research, it has been found that studies with animal models often do not result in successful translation to human trials because of the limited similarity to human physiology [2]. Both these scenarios can have considerable detrimental impacts on the progression of new drug candidates from pre-clinical trials to clinical trials, and can be particularly evident when modelling complex disease states such as those found in the central nervous system (CNS) [3].

Three dimensional (3D) cell culture systems aim to replicate the *in situ* functions of living tissue, by providing a more physiologically relevant environment for cell growth and function. Engineering neural tissue that is truly representative of that found in the human brain and central nervous system requires a scaffold to recreate the 3D *in vivo* microenvironment. Various materials (natural and synthetic) in different formats (gels, porous solids and fibres) can be used as scaffolds to aid the 3D culture of replicating and terminally differentiated cells. To aid tissue growth and increase physiological relevance, scaffolds can be surface modified, mechanically tuned or chemically/biologically functionalised all of which have been shown to aid cell attachment, proliferation and differentiation. This review describes recent progress on developing scaffolds for the *in vitro* 3D culture of neural stem cells and their derivative neuronal and glial lineage cell types. To give the reader a deeper understanding of the topic, it also provides some background information on neurogenesis and the neural stem cell (NSC) niche in the embryonic and adult human brain.

2. The neural stem cell niche and neural differentiation *in vitro*

Somatic/adult stem cells reside in specialized microenvironments that provide specific extracellular conditions, primarily to maintain quiescence to prevent cell exhaustion, but also to induce differentiation and cell specialisation when required. Various stem cell niches exist within the adult human body including the bone marrow, the bulge of the hair follicle, the apex of the testis and the subventricular zone (SVZ) of the brain [4]. Much like any extracellular environment, the stem cell niche influences cell behaviour by a combination of signals from the extracellular matrix (ECM), various factors, nutrient and waste gradients, oxygen concentration, shear stress and temperature. The stem cell niche provides the ideal set of conditions for the maintenance and differentiation of stem cells. Understanding the makeup and function of the niche is critical in the construction of environments that mimic it, particularly in the field of tissue engineering. [5].

2.1. The embryonic neural stem cell niche and human brain development

In the developing embryo, four weeks post-conception, invaginating epithelial neural plate cells form what is known as the neural tube, a single layer of proliferating columnar neuroepithelial cells that eventually give rise to the CNS (brain and spinal cord). Through careful spatial and temporal environmental control, these primary neural stem cells can form neurons and glial cells, and organize themselves into the CNS. Neuroepithelial cells extend from ventricular (apical) to pial (basal) surfaces in an orientation known as apical-basal polarity. Neuroepithelial cells initially divide symmetrically producing two identical multipotent daughter cells [6]. Later in development they divide asymmetrically generating a self-renewing radial glial (progenitor) cell and a differentiating neuroblast [7] at the apical surface, and a basal progenitor and differentiating neuroblast at the basal surface [8,9]. Outer radial glial cells, of the SVZ and beyond, act as a guide for the migration of newly formed neurons, which then is critical for the formation of the cortex layer (Fig. 1) [10].