CELL REGENERATION

REVIEW Open Access

Defining synthetic surfaces for human pluripotent stem cell culture

Jack W Lambshead[1,2], Laurence Meagher[1], Carmel O'Brien[1,2] and Andrew L Laslett[1,2,3*]

Abstract

Human pluripotent stem cells (hPSCs) are able to self-renew indefinitely and to differentiate into all adult cell types. hPSCs therefore show potential for application to drug screening, disease modelling and cellular therapies. In order to meet this potential, culture conditions must be developed that are consistent, defined, scalable, free of animal products and that facilitate stable self-renewal of hPSCs. Several culture surfaces have recently been reported to meet many of these criteria although none of them have been widely implemented by the stem cell community due to issues with validation, reliability and expense. Most hPSC culture surfaces have been derived from extracellular matrix proteins (ECMPs) and their cell adhesion molecule (CAM) binding motifs. Elucidating the CAM-mediated cell-surface interactions that are essential for the *in vitro* maintenance of pluripotency will facilitate the optimisation of hPSC culture surfaces. Reports indicate that hPSC cultures can be supported by cell-surface interactions through certain CAM subtypes but not by others. This review summarises the recent reports of defined surfaces for hPSC culture and focuses on the CAMs and ECMPs involved.

Keywords: Human embryonic stem cells, Induced pluripotent stem cells, Cell adhesion molecules, Pluripotency

Introduction

Human pluripotent stem cells (hPSCs) include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) and have enormous potential for applications to drug screening, disease modelling and cellular therapies [1,2]. These applications will necessitate the use of cell culture conditions that are consistent, chemically-defined and/or non-xenogenic for reasons of scale, reproducibility and safety. hPSCs are adherent cells and have long been cultured on poorly-defined, complex surfaces of xenogenic origin. Such surfaces present a wide range of ligands and interact with hPSCs via poorly understood mechanisms through many different cell adhesion molecules (CAMs) on the cell surface. CAM-ligand interactions are restricted by the types of CAMs and ligands available and are governed by the physical properties of the culture surface. Specific CAM-ligand interactions mediate various intracellular signalling pathways thought to be involved in maintaining the homeostasis and self-renewal of hPSCs. The involvement of CAM-mediated intracellular signalling pathways in the maintenance of hPSCs are addressed in the following reviews [3-5]. A detailed understanding of the effects of CAM-surface interactions on hPSC phenotype and behaviour in culture should facilitate the optimisation of defined culture conditions to support both hPSC self-renewal and somatic differentiation pathways. A wide variety of chemically-defined surfaces that engage different CAM subtypes have been reported to support the long-term self renewal of hPSCs [for examples 6-13]. It is challenging to elucidate the roles of CAMs from these reports due to the diverse physicochemical properties of the culture surfaces as well as the inter-laboratory variation in cell culture protocols and in the cell and surface characterisation methods utilised. Non-specific protein adsorption to many "defined" surfaces can also confound results [14]. Direct comparisons between culture surfaces and the hPSCs cultured thereon are limited and have been focussed on identifying systems able to support culture of hPSCs as defined by minimal criteria including gene expression and qualitative differentiation assays [15-17]. Detailed characterisation and direct comparison of hPSCs cultured on defined surfaces that specifically engage different CAMs is required to elucidate the roles of CAMs in maintaining

* Correspondence: andrew.laslett@csiro.au
[1]CSIRO Materials Science and Engineering, Clayton, Victoria 3168, Australia
[2]Australian Regenerative Medicine Institute, Monash University, Victoria 3800, Australia
Full list of author information is available at the end of the article

pluripotency. The following review describes published reports of defined culture surfaces for hPSC self-renewal with a focus on the CAMs and extracellular matrix proteins (ECMPs) thought to be involved in mediating cell-surface interactions and maintaining pluripotency (Figure 1).

Human pluripotent stem cells

Pluripotency describes the ability of single cells to differentiate into every cell type in the developing and adult body [18]. Pluripotent stem cells are also capable of indefinite self-renewal *in vitro* under appropriate conditions. hPSCs are therefore a potential cell source for myriad regenerative medicine approaches and *in vitro* disease models, for example hPSC-derived cardiomyocytes could be used to repair damaged tissue following a myocardial infarction [1,2]. Pluripotency is a complex state that is maintained *in vitro* by large transcriptional networks that are yet to be fully elucidated [reviewed by 19]. Although many genes are involved in the regulation of pluripotency, cell line variation and population heterogeneity have hampered the identification of reliable molecular markers of pluripotency [20,21]. To further complicate matters, murine studies have identified multiple pluripotent states that are maintained by different signalling networks [22]. It has been suggested that many of the differences between murine pluripotent stem cells (mPSCs) and hPSCs could be attributed to mPSC and hPSC cultures representing different states of pluripotency and that hPSCs can move between these states with changes in culture conditions [22,23]. All of these factors make correct identification and characterisation of hPSCs a challenging task. Adequate characterisation of hPSCs is essential for the unambiguous identification of surfaces capable of supporting hPSC expansion.

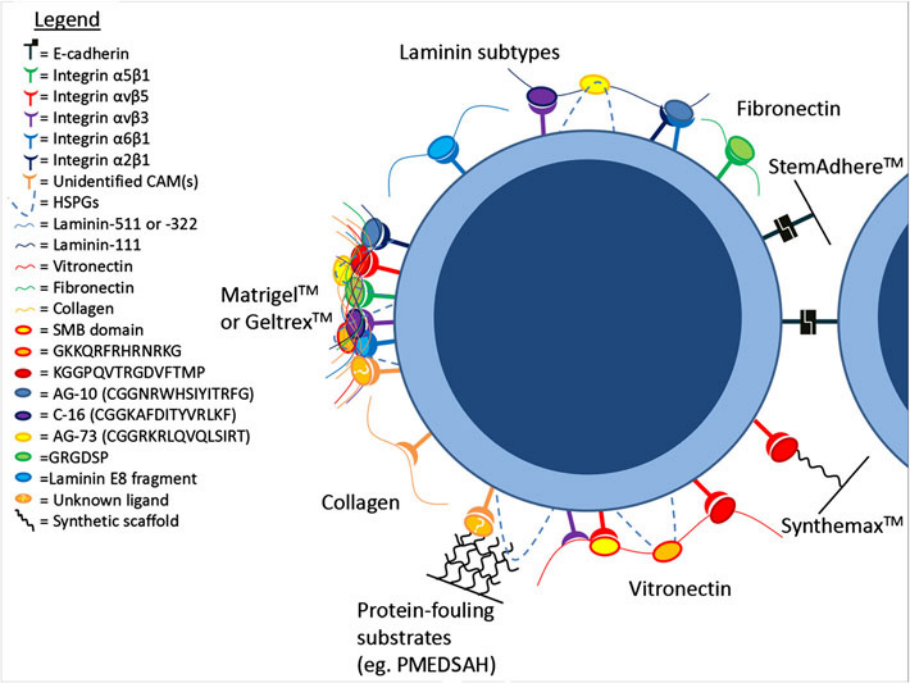

Figure 1 Molecular interactions between human pluripotent stem cells (hPSCs) and culture surfaces. A schematic diagram of a single hPSC illustrates molecular interactions with reported hPSC culture surfaces through different ligands and CAM subtypes. Specific ligands and cell adhesion molecules (CAMs) are included if they have been reported in hPSC attachment and/or culture studies. CAMs involved in hPSC adhesion include integrin subtypes α5β1 (green), αvβ5 (red), αvβ3 (purple), α6β1 (blue) and α2β1 (navy blue), E-cadherin (black blocks), heparan sulphate proteoglycans (HSPGs; dashed blue lines) and unidentified CAMs (orange). Ligands are portrayed as coloured ovals and include the SMB domain of vitronectin (yellow/red), GKKQRFRHRNRKG (orange/red), KGGPQVTRGDVFTMP (red/dark red), AG-10 (CGGNRWHSIYITRFG; blue/dark blue), C-16 (CGGKAFDITYVRLKF; purple/navy blue), AG-73 (CGGRKRLQVQLSIRT; yellow/orange), GRGDSP (green) and laminin E8 fragments (light blue/blue). The ligands are presented by ECMPs [represented by curved coloured lines: laminin-511 or −322 (blue), laminin-111 (navy blue), vitronectin (red), fibronectin (green) collagen (yellow)] or synthetic surfaces (thick black lines) including Synthemax™, StemAdhere™ and PMEDSAH. On the left of the image complex extracellular matrix extracts (eg. Matrigel™ and Geltrex™) are illustrated as combinations of ECMPs, and on the right cell-cell adhesion is simplified in the extreme to illustrate homophilic E-cadherin binding. Where specific ECMP ligands are poorly-defined, CAMs are shown to interact with the ECMP line. Where specific CAMs have not been identified the orange CAM is used, and undefined, adsorbed ligands are represented by orange ovals with a white question mark. This figure is a greatly simplified and stylised representation of the cell-surface and cell-cell adhesion interactions important for hPSCs and discussed in this review.