Biomaterials 33 (2012) 1414–1427

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

The differentiation and isolation of mouse embryonic stem cells toward hepatocytes using galactose-carrying substrata

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ARTICLE INFO

Article history:
Received 15 October 2011
Accepted 7 November 2011
Available online 26 November 2011

Keywords:
Embryonic stem cells
Hepatocyte differentiation
Galactose-carrying polymer
E-cadherin
Extracellular matrix

ABSTRACT

A simple culture system to achieve the differentiation of embryonic stem (ES) cells toward hepatocytes with high efficiency is crucial in providing a cell source for the medical application. In this study, we report the effect of a matrix-dependent enrichment of ES cell-derived hepatocytes using immobilized poly(*N*-*p*-vinylbenzyl-4-*O*-β-D-galactopyranosyl-D-gluconamide) (PVLA) with E-cadherin-IgG Fc (E-cad-Fc) as a galactose-carrying substratum. PVLA and E-cad-Fc were confirmed to be stably co-adsorbed onto polystyrene surface by quartz crystal microbalance (QCM). We showed that the E-cad-Fc/PVLA hybrid substratum was efficient in culturing primary hepatocytes and maintaining liver functions, on which the undifferentiated ES cells also maintained high proliferative capability. Furthermore, ES cell-derived hepatocytes on this hybrid matrix expressed elevated level of liver specific genes and functions together with early expression of definitive hepatocyte marker, asialoglycoprotein receptor (ASGPR). Finally, we isolated a high percentage of cells (about 60%) with ASGPR expression after re-seeding onto PVLA-coated surface, and observed the elimination of the poorly differentiated cells (Gata6$^+$ and Sox17$^+$) and the ones toward another cell lineage (brachyury$^+$ and Pdx1$^+$). The system uses a glycopolymer as an extracellular substratum for isolation and enrichment of ES cell-derived hepatocytes with adequate homogeneity and functionality.

1. Introduction

The methods of liver cell implantation or artificial liver were developed in order to assist the recovery in liver damage [1,2], the current technology of which, though does not permit long-term expansion and highly functional expression in hepatocytes [3]. High-quality cell sources remain in an urgent need, among which the embryonic stem (ES) cell-derived hepatocyte provides a potential candidate in prolonging cellular life time with a large population [4]. However, the conventional processes, including the embryoid body (EB) formation, the culture on natural substrates or feeder layers, result in forming cell colonies, in which the chaotic differentiation into all three germ lineages was stimulated with a low efficiency [5,6]. Therefore, the recovery of hepatocyte population with adequate purity and functions is now being brought to the forefront for clinical applications.

Currently most of the research is focused on the effect of cytokine-cocktails and targeted gene delivery together with flow cell sorting to achieve homogeneous population of differentiated cells [7,8]. However, there has not been a successful strategy reported to produce a relatively homogeneous population of cells with less damage at the time of enrichment. Moreover, the cell sorting approach depended on fluorescence activated cell sorting (FACS), seriously affects the yield and viability of differentiated ES cells and allows the recovery of unwanted cells [9]. So far, little progress has been made toward defining the cell surface markers for guiding the differentiation of ES cells despite recent findings of the importance in cell adhesion molecules for maintenance of pluripotency, self-renewal, and differentiation [10,11]. For example, E-cadherin has been implicated in not only to maintain homogeneous population of ES cells, but also directing stem cells to a hepatic cell fate [12–15]. On the other hand, the asialoglycoprotein receptor (ASGPR) on the hepatocyte membrane plays a critical role in functionality and maturation of hepatocytes. The hepatocytes bind specifically to β-galactose residues via ASGPR [16], and form multilayered spheroids on galactose-carrying substratum [17,18]. For instance, primary hepatocytes on a glycopolymer, namely poly(N-p-vinylbenzyl-4-O-β-D-galactopyranosyl-(1→4)-D-gluconamide) (PVLA), maintain a reinforced liver functionality

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doi:10.1016/j.biomaterials.2011.11.007

[19,20]. Moreover, the expression of ASGPR is highly restricted in hepatic populations [21], indicating that enrichment based on ASGPR expression may be more selective than sorting based on previously-described approaches [22].

In this study, we established a monolayer culture system in inducing mouse ES (mES) cells into hepatocytes in vitro, using PVLA together with E-cadherin in the purpose to unite the advantages of both materials. Furthermore, we applied the PVLA matrix for the isolation of differentiated hepatocytes with preserved functions. We focused on the efficiency of the matrix dependency of galactose-carrying matrices in the induction and enrichment of endoderm-derived population.

2. Materials and methods

2.1. Preparation of PVLA

PVLA (Fig. 1A) was synthesized by the method reported previously [23]. Briefly, the p-vinylbenzylamine was achieved by Gabriel synthesis and the oligosaccharide was oxidized to obtain a gluconamide. The N-p-vinylbenzyl-O-β-D-galactopyranosyl-(1→4)-D-gluconamide (VLA) was later dissolved in distilled water and

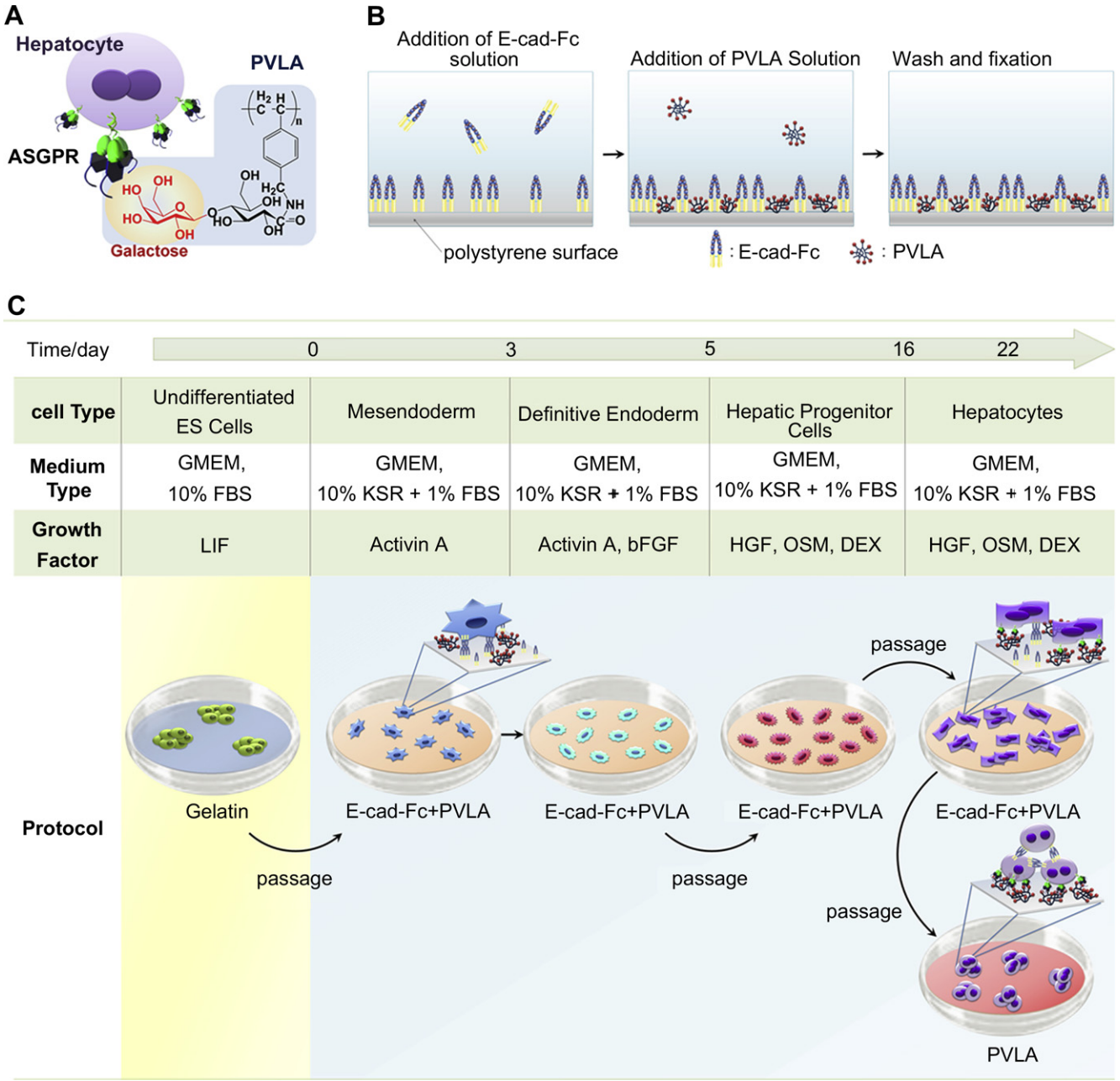

Fig. 1. Schematic representation of the strategy to prepare the hybrid substratum of PVLA and E-cad-Fc and induce the ES cells differentiation to hepatocytes on the co-immobilized matrix. Fig. A shows the molecular formula of the PVLA polymer, on which the galactose residues bind to the ASGPRs on the hepatocyte membranes. Fig. B demonstrates the method to prepare the co-coating layer of E-cad-Fc and PVLA on PS surface. The illustration of the protocol for the differentiation of mouse ES cells to hepatocytes includes the cellular states of undifferentiated (yellow area) and differentiated ones (blue area) (C). The state of differentiation is divided into four different stages: mesendoderm, definitive endoderm, hepatic progenitor cell and finally hepatocyte. Separated by the days of differentiation, the conditions, including the medium and growth factors in various stages, are listed in the figure. The amplified figures demonstrate the types of cell adhesion on the matrices of E-cad-Fc/PVLA hybrid matrix (orange surface) and PVLA matrix (red surface). Abbreviation: LIF: leukemia inhibitory factor; bFGF: basic fibroblast growth factor; HGF: hepatocyte growth factor; OSM: oncostatin M; DEX: dexamethasone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)