Immobilisation of Heparan Sulphate on Electrospun Meshes for Optimal Embryonic Stem Cell Culture and Directed Differentiation

Kate Meade¹, Dr Claire Johnson, Dr Rebecca Holley, Professor Sandra Downes¹, Dr Jason Whittle², Professor Anthony Day³ and Dr Catherine Merry¹

¹Stem Cell Glycobiology, School of Materials, University of Manchester, UK. ²BD Biosciences, Sheffield, UK. ³Faculty of Life Sciences, University of Manchester

Kate.a.meade@postgrad.manchester.ac.uk

Introduction

Embryonic stem (ES) cells, derived from the inner cell mass (ICM) of the pre-implantation embryo, have the capacity to form any adult cell type. Both self renewal of pluripotent cells and differentiation to specific cell lineages are governed by intimate interplay of growth factor signalling, cell:extracellular matrix (ECM) interactions and cell:cell contacts in which heparan sulphate (HS) plays a pivotal role. ES cells express an unusually low sulphated form of HS whilst differentiation of ES cells to both neural and mesodermal lineages is accompanied by, and dependent on, production of specific patterns within HS chains. HS deficient ES cells remain pluripotent in standard ES culture conditions but cannot differentiate to a neural lineage unless media is supplemented with exogenous HS. We aim to enhance the rate and efficiency of ES cell differentiation by combining specific HS species with three dimensional scaffolds, effectively manipulating the ES cell microenvironment.

Materials and Methods

20% (w/v) PLGA (85:15, Sigma) dissolved in Hexafluoroisopropanol (HFP) was electrospun onto a rotating mandrel (100 rpm) at 25 kV, with a 0.5ml/hr flow rate and a 20 cm working distance. Electrospun scaffolds (average fibre diameter 580nm) were treated with cold plasma polymerization enabling immobilization of heparin and HS. Functionality of the bound sugars were detected using a binding assay with biotinylated Link_TSG6, the heparin-binding module of tumour necrosis alpha stimulated gene-6 (TSG-6)¹. For neural differentiation, EXT1⁺/-ES cells were seeded at 1x10⁴ cells/cm² onto scaffolds with and without immobilised HS and cultured in neural induction media for 8 days².

Results

Fig. 1. An increase in absorbance, and therefore TSG-6 binding, is observed with increasing heparin concentrations immobilised on treated PLGA scaffolds. No binding is observed on untreated PLGA scaffolds.

Fig. 2. EXT1⁺ ES cells lacking endogenous HS could not commit to a neural lineage on scaffolds without immobilised HS (A). However, treated PLGA scaffolds coated with immobilised HS were able to compensate for the lack of endogenous HS allowing neural differentiation (Green: BIII Tubulin, Blue: Nucleus).

Discussion and Conclusions

Our PLGA scaffolds enable binding of HS in a biologically active form that is competent to direct ES cell fate. By pairing HS with electrospun meshes, the biological and architectural cues of the ES microenvironment can be manipulated and controlled and used to enhance both ES cell culture and differentiation.

References