Supplementary Material (ESI) for Journal of Materials Chemistry

Supplementary Information

Sugar functionalised PEGA surfaces support metabolically active hepatocytes

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S1: Fluorescence Viability Staining

S1.1 Methodology

Unless otherwise stated, reagents, enzymes and solvents were obtained from commercial suppliers. The mouse hepatocyte viability was evaluated by a double staining fluorescence assay at 1, 3 and 7 days. The sample surfaces were seeded with hepatocytes at a density of 6 x 10^4 cells/ml onto either: 1) glass, 2) PEGA 3) PEGA-LA (0.1mmol), or 4) PEGA-GlcA (0.1mmol). At each time point, the media was aspirated and the surfaces were rinsed twice with pre-warmed PBS. A working solution of 2 µl/ml ethidium homodimer-1 (red fluorescence) (Molecular Probes, UK) and 0.5 µl/ml Calcein AM (green fluoresecence) (Molecular Probes, UK) was prepared in PBS.

Each sample was covered with the working solution for 10 minutes in the dark, after which the excess dye was removed. Each of the samples was then visualised by fluorescence microscopy (Nikon Eclipse 50i).

S1.2 Fluorescence Microscopy

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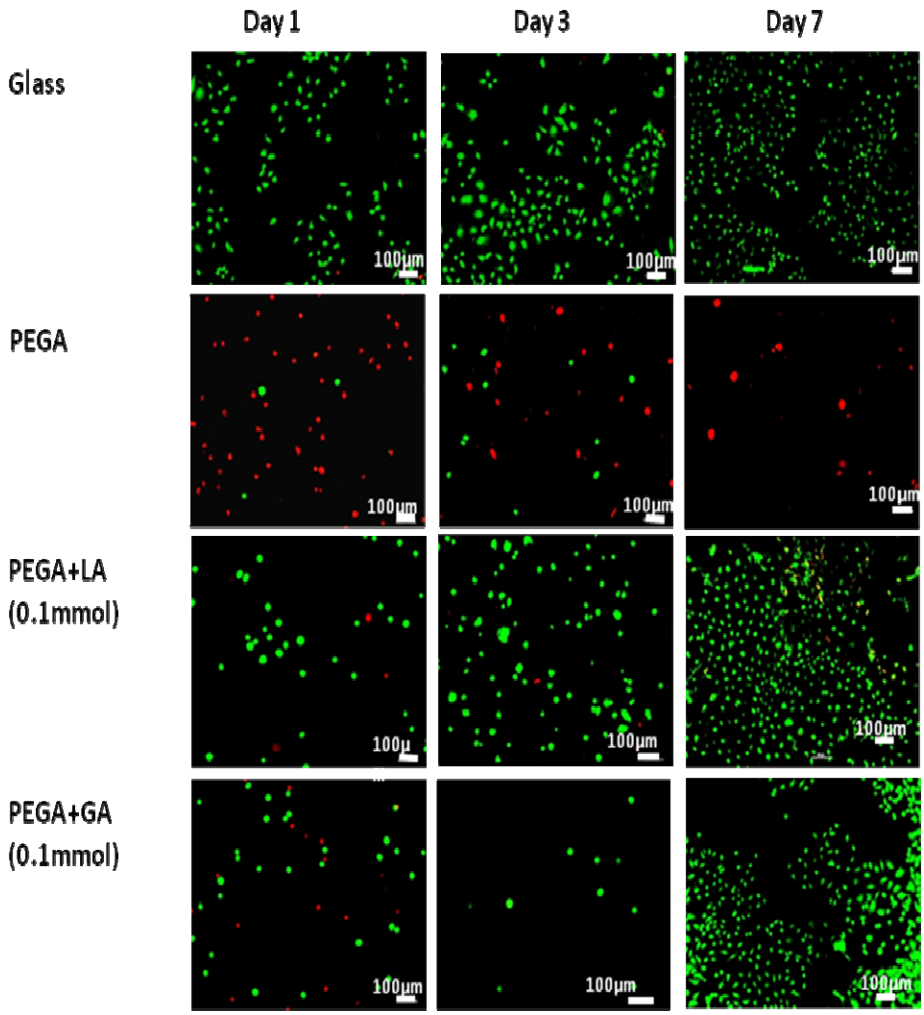

Figure S1: Live /Dead staining evaluating the cellular viability of hepatocyte cell line FL83B grown on selective surfaces (Glass, PEGA, PEGA-LA and PEGA-GlcA) over the course of 7 days. Green fluorescence is an indication that the cells are alive, whilst red fluorescence is a sign that the cells have either died or are not viable.

S2:Flow Cytometry

S2.1 Methodology

A sub-confluent culture of FL83B mouse hepatocytes was used to assess the cell line for the presence of ASGP-R. The media was removed from the flask and the cells were rinsed twice with pre-warmed sterile PBS. 3 ml of dissociation buffer was

added and the flask was returned to the incubator at 37ºC for 10 minutes. Any cells which did not detach from the tissue culture plastic were dislodged gently with a cell scraper. The cells were then transferred into a falcon tube. A sample of the cell suspension was retained for a cell count, and the remainder was then spun for 5 minutes at 2000 RPM to create a cell pellet. The supernatant was then carefully removed and the cell pellet was resuspended in the appropriate volume of PBS to obtain 2 million cells per ml in eppendorf tubes. The sample was divided into two eppendorf tubes; one tube for the negative control and the other to test for the presence of ASGP-R using a fluorescein isothiocyanate (FITC) conjugated monoclonal antibody to rat ASGP-R (Clone 8D7, Hycult Biotechnology, The Netherlands).

Please note that this antibody shows cross reactivity between rat and human ASGP-R and was was used against mouse cells in this study as it has been shown previously by Park *et al* that there is a strong sequence homology between human and mouse ASGP-R[1]. Rat ASGP-R consists of 3 polypeptide subunits, Rat Hepatic Lectin (RHL)-1, RHL-2 and RHL-3. The monoclonal antibody 8D7 recognises a subunit-specific epitope on RHL-1, which is highly homologus between mouse and human proteins[1].

The cell samples were spun down in a bench top centrifuge for one minute at 5000 RPM and the supernatant was then removed. 100 µl of FTIC conjugated monoclonal antibody to rat ASGP-R, diluted 1 in 10 with fluorescent activated cell sorter (FACS) buffer (0.2 % BSA , 0.1 % Sodium Azide in PBS) was added to the eppendorf and the cells were carefully resuspended. Cells being used for the negative control were resuspended in FACS buffer without any antibody present. The cells were incubated on ice for 30 minutes in the dark, after which the cells were spun at 5000 RPM for one minute and the supernatant removed. The cells were then washed with 900 µl of PBS (non-sterile) per eppendorf, and then spun at 5000 RPM for one minute. The supernatant was then carefully removed and the cells resuspended in 250 µl of PBS. The samples were kept at 4ºC until they could be analysed by flow cytometry.

A BD FACSCalibur Flow Cytometer with Cell Quest Pro software was used to evaluate the cell population. Cells were gated using the forward vs sidescatter graph. 10,000 events in the cell gate were counted and the fluorescence in the FL1 or FL2 regions was registered and plotted against the cell count.

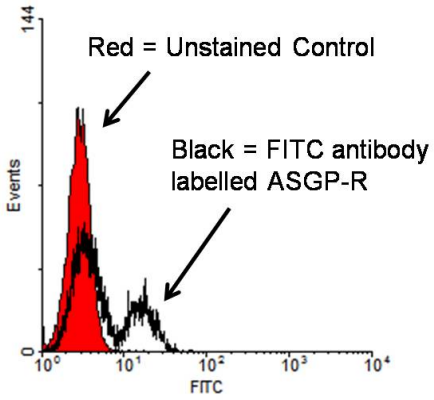

Figure S2: Flow cytometry of ASGP-R of a typical cell population of hepatocytes. Data shows a split population of cells with 29 % positive for ASGP-R.

References:

1. Park, J.H., et al. 2006. Detection of surface asialoglycoprotein receptor expression in hepatic and extra-hepatic cells using a novel monoclonal antibody. Biotechnol. Lett. 28: 1061-1069.