



A novel glass-based 3D cell matrix for the culture of hepatocytes.

The pharmaceutical and biotechnology industries are increasingly seeking methods that replace the use of animals in drug testing. Many such methods involve the use of laboratory-grown cells to mimic the behaviour of organs, providing alternatives to animal experimentation in research and toxicological testing.

Hepatocytes are one of the most commonly used cells in these experiments. In the liver, hepatocytes exist in a complex 3D environment, but when they are cultured by traditional 2D methods the cells lose much of their characteristic behaviour, thus creating a less effective model system.

This problem has led to the development of 3D cell culture systems to allow cells to grow under conditions more closely resembling those encountered *in vivo*. One such system is supplied by Orla Protein Technologies and is based on a matrix from Nippon Sheet Glass (NSG) made from the same high optical grade borosilicate glass used for the production of 2D microscope slides and coverslips.

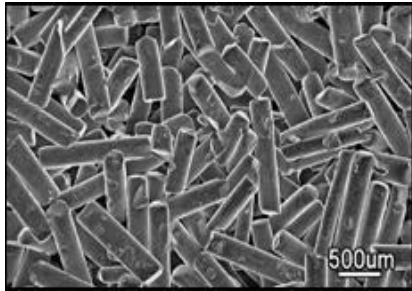
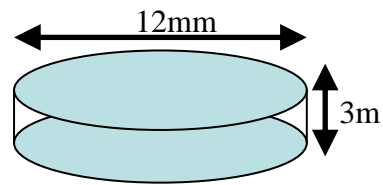


Fig 1 - Image of cylindrical, randomly-oriented glass particles.
The result is a highly porous material, with pores of variable size making up approximately 60% of the total volume.

The resulting disk-shaped products of Orla CellCarrier™ (see Fig 2a) fit into standard 12 or 24 well plates to allow the easy culture of cells using standard culture techniques.



Figs 2a and 2b - Image of a 12 x 3mm disk for use in a 24 well plate.
Analogous 18 x 3mm disks are used in 12 well plates

The increased amount of surface area available for cell adhesion and growth allows the culture of a significantly greater number of cells in the same sized well compared to a standard 2D surface (see Fig 3 below).

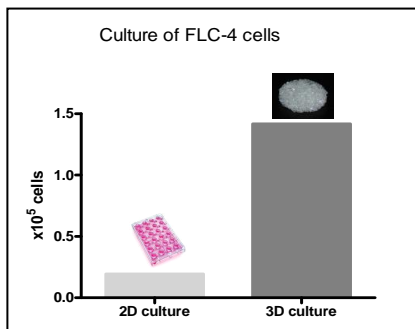


Fig 3 – Number of cells per well in cultured FLC-4 cells grown on 2D and 3D borosilicate glass substrates.



In addition, cells grown on 3D CellCarrier showed more hepatocyte-like characteristics compared to those grown on 2D surfaces (see Figs 4 and 5). For example, one *in vivo* hepatocyte function is the production of serum albumin; so this is one measure that can be used to estimate how successful *in vitro* culture conditions are at inducing *in vivo*-like behaviour. When a range of hepatocyte cell lines were tested, there was a clear increase in albumin production in 3D-grown cells compared to 2D.

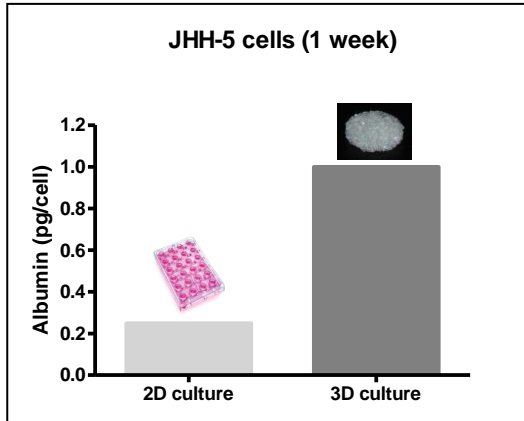


Fig 4: Albumin production in 2D versus 3d grown JHH-5 cells

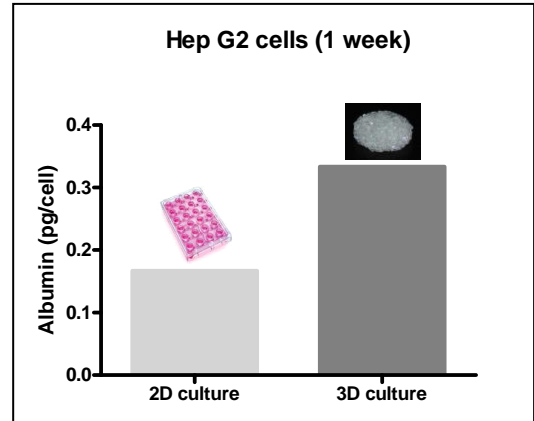


Fig 5: Albumin production in 2D versus 3d grown Hep G2 cells

A vital function of the liver is the removal and processing of toxins. One of the most important enzymes in this process is cytochrome P450-3A4 (CYP-3A4). Because CYP-3A4 is heavily involved in the processing and removal of many pharmaceutical agents, it is important that any hepatocyte model systems used in pharmaceutical research or toxicological testing express appropriate levels of CYP-3A4. When the mRNA levels of CYP-3A4 were assessed there was a large increase in CYP-3A4 mRNA when cells were grown on 3D Cell Carrier compared with cells grown on a 2D surface of the same material.

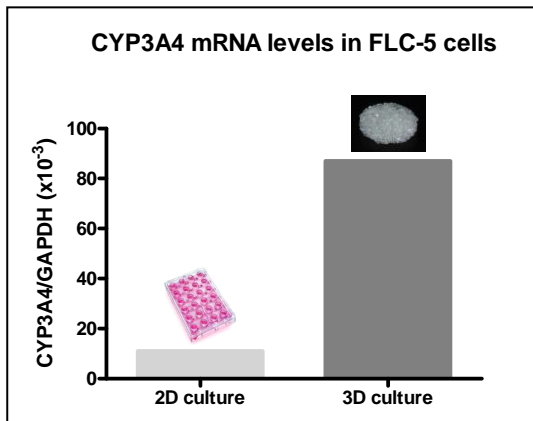


Fig 6 - CYP-3A4 mRNA levels in 2D versus 3D grown FLC-5 cells.

These results show (i) that more cells could be cultured per well in 3D growth and (ii) that the resulting cells exhibited enhanced hepatocyte *in vivo* behaviour, including higher levels of albumin production and CYP-3A4 mRNA expression. Products like 3D CellCarrier therefore offer an environment that better replicates *in vivo* conditions for those carrying out activities such as research and toxicological testing.

3D Cell Carrier is available together with other 2D and 3D cell culture systems and animal-free cell culture proteins from Orla Protein Technologies Ltd, Bioscience Centre, International Centre for Life, Times Square, Newcastle Upon Tyne, NE1 7EP, UK

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