Porous Polystyrene Scaffolds Enabling Routine Three-dimensional Cell Culture

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Statement of Purpose: Altering the environment in which cells can grow can radically affect their function and capacity for growth and differentiation. In various cell culture applications, it has been shown that the growth and function of cells as multi-cellular three dimensional (3D) structures, is superior to their growth as two dimensional (2D) monolayer cultures as in standard plasticware[1,2]. Here, we present a novel technology that provides an environment for routine 3D cell growth *in vitro*. We have developed a thin membrane of polystyrene scaffold with a well defined and uniform porous architecture[3]. We have adapted this material specifically for cell culture applications for use in existing culture plates. We have exemplified the application of this technology by growing numerous cell types in 3D including liver tissues and the development of an artificial skin construct composed of differentiating keratinocytes that mimic the barrier function of the epidermis.

Methods: *Preparation of scaffold materials:* This method has been described previously and involves emulsion templating technology[3]. *Cell Culture:* Human HepG2 carcinoma cells were cultured as described previously[4]. Populations of human keratinocyte cell line HaCaT cells were initially expanded in 2D plates in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100µg/ml streptomycin, 100µg/ml penicillin until they reached 80-100 % confluence. The cells were then detached and seeded on to the 3D substrate at a seeding density of $1x10^6$ cells/cm^2, and cultured in keratinocyte specific medium Quantum 153 for 2 days under submerged conditions and then raised to the air/liquid interface and cultured for a further 7-35 days. *Metabolic Activity of HepG2 Cells:* This was performed as described previously[4]. *Electron microscopy:* Analysis for transmission electron microscopy (TEM) was performed as described previously[4].

Results: TEM was used to study the ultra-structural features of HepG2 liver cells grown on 2D and 3D polystyrene substrates.

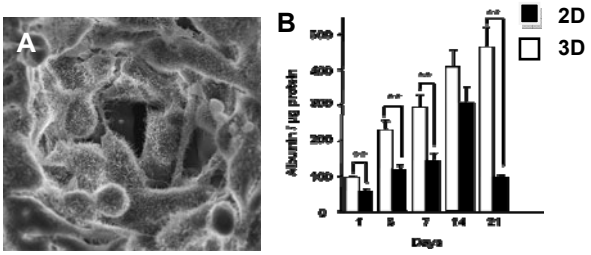

Fig (1):
Liver cells cultured on 3D substrates grew homogeneously (Fig 1A). They showed numerous morphological features that are found in native liver tissue. The presence of such features corresponded to the enhanced metabolic activity of HepG2 cells grown in 3D compared to 2D supports. The detection of peroxisomal clusters which are unique to mammalian liver was highly encouraging. Micrographs of cells grown in 3D also revealed adjacent hepatocytes sharing microvilli-lined channels with tight junctions indicating cell polarization and the formation of channels resembling bile canaliculi. Such features were not observed in 2D cultures.

The liver is a major source of for the production of albumin and as such is often used as a measure of hepatocyte metabolic activity *in vitro*. It can be seen clearly that there is a significantly higher albumin concentration in the 3D culture at all time points measured, compared to the 2D culture (Fig 1B, data normalized taking into account cell number).

We have also investigated the formation of the epidermal barrier using our 3D cell culture technology. There is demand for robust and representative *in vitro* skin constructs that possess barrier function, especially as the use of intact human and animal skin from biopsies or autopsies is limited by availability and regulatory constraints.

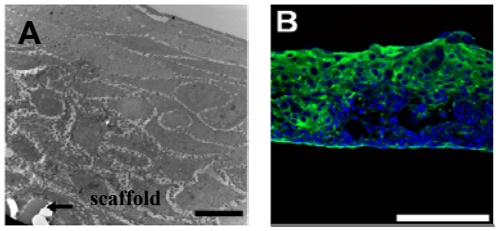

Fig (2):
Keratinocytes cultured on 3D polystyrene scaffolds organized themselves and formed structures resembling that of real skin. TEM analysis shows skin cells migrating and differentiating toward the surface of the culture, acquiring keratin, loosing their nucleus and undergoing stratification (Fig 2A). This transition from the spinous layer to the larger flattening cells occurs in real skin and ultimately results in the formation of the stratum corneum. Tight junction complexes are differentially regulated during this migration, leading to cells being lost on the surface. Markers of mature keratinocytes such as involucrin are therefore detected in the higher levels of the culture (Fig 2B). These data demonstrate the formation of a robust *in vitro* skin construct.

Conclusions: Porous polystyrene scaffolds have been shown to support routine 3D cell culture. Evidence demonstrates enhanced performance of cells grown in 3D compared to standard 2D cultures. This technology represents an important step to improve in vitro assays, and the accuracy and relevance of the data they produce.

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