A micron scale three dimensional scaffold for colon epithelial intestinal tissue engineering

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Statement of Purpose: Planar porous membranes are commonly used to mimic the intestinal basement membrane and study electrophysiology of the epithelium[1]. Although useful for *in-vitro* testing, the membranes are often non-biodegradable and therefore not suitable for tissue engineering. They also do not accurately model the three dimensional architecture of colonic epithelium. To address this, a new scaffold was designed to mimic intestinal colonic crypts. The scaffold was manufactured from biodegradable poly (D,L-lactic-*co*-glycolic acid) **(**PLGA) based on submicron spheres making it possible to incorporate signalling proteins and test ideal conditions for the maintenance of the intestinal epithelium *in vitro*.

Methods: To create a three dimensional scaffold, a template was manufactured through electron beam lithography and used as a mould for the scaffold. Submicron spheres were prepared using an emulsion method. The spheres were then cast onto the template and sintered to allow fusion. The fused scaffold was then lifted off the template.
Cell sheets were used to culture intestinal cells on the scaffold. The sheets were obtained using a novel technique employing plasma polymer coated surfaces.

Results: The dimensions of the template were based on calculations from various histological sections of mouse colon. These took into account the crypt depth, width and spacing (table 1).

Table 1: Statistical analysis of crypt dimensions based on mouse colon. Error shows mean ± SD for n =20.

Section of Colon	Dimension / μm
Crypt depth	179.44 ± 12.17
Crypt width	61.82 ± 3.06
Spacing between crypts	12.55 ± 1.37

A prototype template was manufactured with an array of ordered pillars with trial dimensions of 50 μm in height and 62 μm in diameter. A distance of 13 μm was set between the pillars (figure 1).

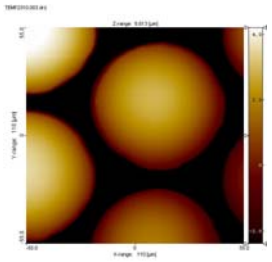

Figure 1: Representative AFM image showing the top view of template (110 x 110 μm).

Spheres cast onto the template and sintered resulted in a scaffold (figure 2) with an accurate reproduction of the patterned surface.

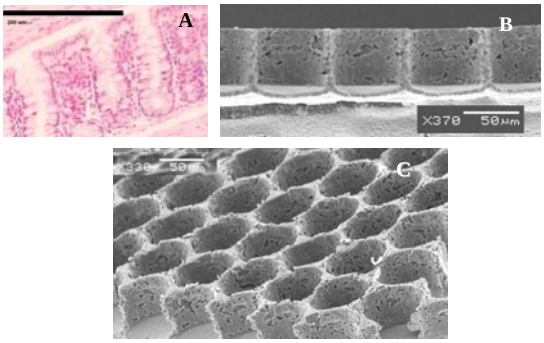

Figure 2: Histological staining of mouse colon (a). Representative scanning electron microscope images showing cross section through the particle scaffold (b) and the top view (c).

Caco-2 cells were cultured on plasma polymer coated glass and at confluence lifted off as cell sheets (figure 3a). The viability of the cell sheet was confirmed using a LIVE/DEAD stain (figure 3b). A single cell sheet was transferred to a scaffold and cell attachment was confirmed after 3 days in culture (figure 3c).

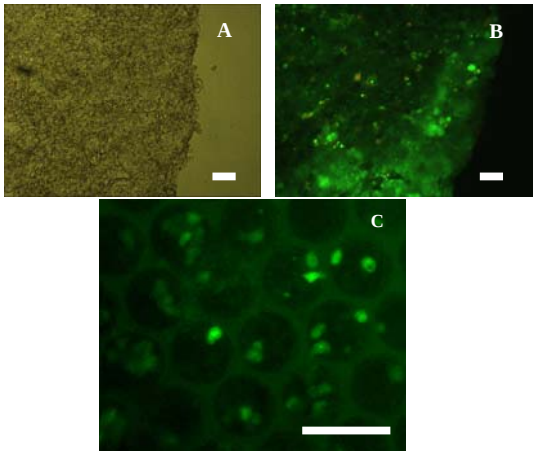

Figure 3: Microscopy images show a cell sheet (a) and a LIVE/DEAD stained sheet (b). Live cells on the scaffold were detected using CellTracker Green (c). The scale bars denote a distance of 100 μm.

Conclusions: We have produced a prototype particle-based scaffold with a three dimensional architectural pattern similar to the mouse colon. We also used a novel plasma polymer method to create cell sheets to transfer cells onto this scaffold. Current studies are focused on optimising the incorporation of proteins into the particles with the aim of creating an *in vitro* crypt signalling gradient similar to that found *in vivo* in order to control the migration of cells.

References: [1]Beltinger J. Am J Physiol Cell Physio. 1999; 277:271-279.