The culture of hESCs is further complicated by the gold standard method that produces heterogeneous colonies in undefined culture media using animal components. This state of affairs is not tenable as a platform for regulated therapeutic products where cells of measurable and reproducible purity and potency from a GMP compatible production system are regulatory necessities. If this is ever to be realised, the standardisation of large-scale culture systems capable of achieving consistent cell populations will need to be developed.

This project is driven by the requirements of the project partner, the UK Stem Cell Bank (UKSCB), to achieve reproducible and scalable culture methods for the distribution of stem cells and, builds on the recently published success from Thomas et al( 2009, Biotechnology and Bioengineering)demonstrating the capability of a large scale robotic system (CompacT Select) at maintaining both pluripotency and a consistent proliferation rate of hESC lines Hues-7 and Nott-1. The project aims to further characterize the processing of hESCs under different culture conditions by systematically investigating the responses (hESC critical to quality marker profiles) and interactions between several key processing parameters, identified through the creation and analysis of high-detail process maps in an attempt to determine optimal windows of operation for the consistent large scale production of high quality hESCs.

**Keywords.** Human embryonic stem cells, pluripotentcy, large scale, automation, optimisation, quality, process control

**(2.P7) POROUS GELATINE-HYDROXYAPATITE COMPOSITE SCAFFOLDS VIA GAS-IN-LIQUID FOAM TEMPLATING**

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**Introduction.** Gelatin and hydroxyapatite (HAp) sponges because of their biocompatibility and biodegradability have the potential to be used as scaffolds to support osteoblasts and to promote bone regeneration in defective areas. In this work gelatine and HAp composites were fabricated in a foam type via a novel foam templating technique.

**Materials and Methods.** A dispersion of nano HAp particles in a concentrated solution of gelatine and an appropriate surfactant was foamed using hexafluoroethane as the blowing agent. The foam, once formed, was frozen in liquid nitrogen and then freeze-dried. Subsequently it was cross-linked with a carbodiimide derivative to retain its chemical and thermal integrity. X-ray computed microtomography was used to nondestructively and quantitatively measure the three-dimensional porosity and the morphometric parameters. The samples were scanned with a Skyscan 1072 μ-CT imaging system (Belgium) at 7,32 μm resolution and with following settings: 40 kV and 250 μA. Image reconstruction and analysis were conducted using the software package provided by Skyscan.

**Results.** All the scaffolds synthesised exhibited an excellent, totally interconnected trabecular morphology. A content of HAp up to 40 % w/w was achieved. Through μ-CT it was shown that HAp particles are distributed homogeneously within the gelatine framework (fig.1). In order to achieve a higher level of HAp content, similar to that of natural bone (~ 70% w/w), the composite scaffold characterised by a HAp content of 40 % w/w was subjected to four cycles of deposition of HAp on the scaffold walls. The final content of HAp as determined by thermogravimetry was very close to 70 % w/w.

**Conclusion.** The foaming technique described, associated with the deposition procedure permits the preparation of scaffold that fulfil both from a morphological and compositional point of view the main characteristic of trabecular bone and as a consequence are promising as constructs for bone tissue engineering.

**Keywords.** Micro-computed tomography, biomaterials, bone substitutes, scaffold

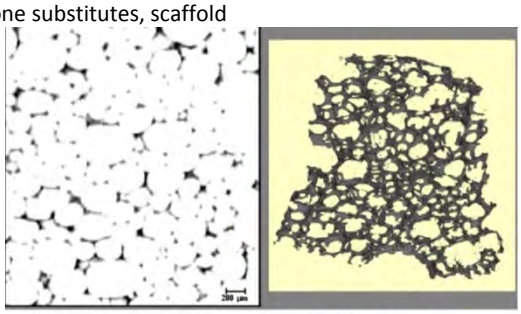

Fig. 1: 2D microtomography slice (left) and 3D reconstruction (right) of a series of 15 slices of a sample.

**(2.P8) LAYER-BY-LAYER BIOFABRICATION USING LASER-ASSISTED-BIOPRINTING AND ELECTROSPINNING ENHANCES CELL PROLIFERATION IN VITRO AND IN VIVO**

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**Introduction.** Laser-Assisted-Bioprinting (LAB) is an effective printing technology for patterning cells, biomolecules and biomaterials, and electrospinning may be used to build thin membranes of polymers. The aim of this work was to associate LAB and electrospinning to achieve three-dimensional cellularized materials and to evaluate the influence of layer-by-layer bio-fabrication on MG63 cell proliferation in vitro and in vivo.

**Materials and Methods.** The LAB setup comprised an infra red laser (Nd:YAG 1064 nm, 30 ns) controlled by scanners, and focused onto glass ribbons coated with a gold absorbing layer (30 nm). Space between ribbon and quartz substrate was 400μm. The Polycaprolacton (PCL) scaffolds (100μm thick) were prepared using a PCL solution (20% w/v in CHCl3) loaded into a syringe and electrospun using a pump and a high voltage generator. MG63 osteoblastic cells transfected with luciferase were cultured in complete medium (IMDM supplemented with 10% FBS). The concentration of cell bio-ink was 50.106 cells/ml, suspended in 1% alginate solution (w/v) and culture medium. The building sequence of the test group comprised three sequential layers of cells and PCL scaffolds stacked. In the control group, a similar amount of cells was printed over three PCL membranes stacked. Then, the materials were cultured in vitro during 3 weeks or implanted 2 months in bone calvarial defects of 20

NOG mice. Follow-up was done using photon imager quantification in vitro and in vivo and histological analyses.

**Results.** In vitro and in vivo results have shown that layer-by-layer bio-fabrication significantly enhanced cell proliferation. Histological analyses confirmed that the tissues retrieved after sacrifices were thicker in the layer-by-layer group.

**Conclusions.** We have demonstrated in this model that a layer-by-layer bio-fabrication using LAB and PCL scaffold is an efficient combination to improve cell proliferation in vitro and in vivo.

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**Keywords.** Layer-by-Layer; Electrospinning; Laser Assisted Bioprinting

**(2.P9) IN VITRO ENGINEERING OF A TRACHEAL EPITHELIUM: CO-CULTIVATION OF TRACHEAL EPITHELIAL CELLS AND FIBROBLASTS ON SMALL INTESTINAL SUBMUCOSA SEGMENTS**

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**Introduction.** Surgical correction of large tracheal defects remains to be a tackling problem. Lesions that cannot be treated by an end-to-end anastomosis and need an interponate which often fail to regenerate a functional tracheal epithelium. Here we investigated whether suturable decellularized small intestine submucosa (SIS) may serve as matrix for the in vitro generation of tracheal epithelium.

**Materials and Methods.** Primary tracheal epithelial cells and fibroblast were harvested from porcine trachea by Protease XIV and Collagenase A digestion, respectively, and cultured in their appropriate culture media. For seeding purposes decellularized SIS, generated from porcine small intestine by decellularization, was clamped in stainless steel frames. Primary isolates of epithelial cells were seeded onto the sub-mucosa side of the SIS after reaching 80% of confluency in culture flasks. Stimulatory effects of tracheal fibroblasts were tested by seeding cells onto the sub-serosa side of the SIS (constructs without fibroblast served as controls). SEM and Histology analysis of constructs were conducted after five days of culture of which three were spent as air liquid interface culture.

**Results.** SEM examination and Phalloidin stains show a completely covered SIS with orientated respiratory epithelium. Immunohistochemistry against Cytokeratin 14 (basal cell marker), Mucin 5AC (goblet cell marker) and β-Tubulin IV (ciliate cell marker) demonstrated a pseudostratified-like epithelium. The production of glycosaminoglycan and Mucin 5AC was more pronounced after fibroblast co-culture.

**Conclusion.** Decellularized SIS is suited for culturing tracheal epithelium and may serve as useful matrix for tracheal tissue engineering purposes for the generation of surgical implants.

**Keywords.** tracheal epithelium, tissue engineering, SIS, air liquid interface culture

**(2.P10) BIOFABRICATION OF TISSUE ENGINEERED VASCULAR GRAFTS**

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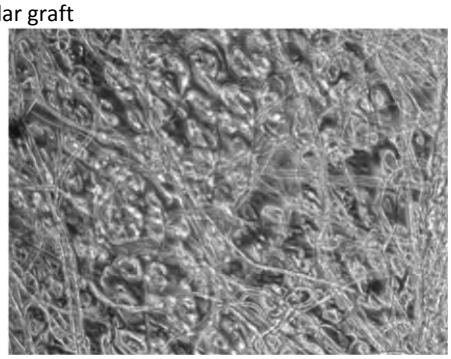
Cardiovascular disease is the largest contributor to mortality in the world claiming nearly 30 percent of all deaths. Tissue engineered scaffolds are essential for small diameter vascular grafts to avoid the fatal risk of thrombosis of the synthetic vascular grafts. In this work, biomimetic gelatine/elastin fibrous scaffolds are proposed, fabricated by electrospinning as tubular constructs. Tissue engineering then takes place in vitro in a bioreactor, in which the tubular scaffold is rotated in a bioreactor surrounded by a smooth muscle cell (SMC)-culture medium suspension, while a suspension of endothelial cells (ECs) flows axially inside the tubular scaffold in a recirculating flow. A novel fluorescence quenching type of sensor has been developed to be embedded at different positions in the scaffold for continuously monitoring the oxygen concentration in the growing tissue. Adherence, growth and proliferation of both types of cells is examined for different scaffold structures and different processing conditions, such as cell concentration, flow rate of the cell-culture medium suspension and rotation speed of the scaffold. The fibrous scaffolds have been crosslinked using glutaraldehyde as a crosslinking agent. Cytotoxicity studies are also carried out to investigate the effect of glutaraldehyde on the cell growth and proliferation.

**Keywords.** Scaffold, Fluorescent quenching, bioreactor, vascular graft



**(2.P11) FABRICATION OF THREE-DIMENSIONAL CELL-LADEN HYDROGEL FOR SOFT TISSUE ENGINEERING**

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Three dimensional (3D) scaffolds should be porous to transfer oxygen and nutrient for cell proliferation and differentiation in tissue engineering. Scaffolds have been fabricated using various conventional techniques of salt leaching, freeze drying, fiber bonding, phase separation, and gas expansion. However, they have a limitation of homogeneous cell distribution on the scaffold. Scaffold fabrication techniques need to control 3D pores inside scaffold. In these methods, solid freeform fabrication (SFF) of rapid prototyping (RP) technology has been adopted to 3D scaffold design with controllable and reproducible porosity and well-defined 3D structures for tissue engineering. Especially, soft tissue has a very high