currently being performed to evaluate endothelial cells (ECs) proliferation and confluence on the lumen side and to verify smooth muscle cells (SMCs) adhesion on the outer surface and their migration inwards.

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**Endothelialisation of Bio-functionalised Nanocomposite with Progenitor Stem Cells**

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**Objective/Aims:** Endothelial dysfunction/ the lack of an endothelium associated with cardiovascular grafts is a major cause of graft failure which is linked to thrombosis and related complications. This study aimed to 1) bio-functionalise a nanocomposite biomaterial, Polyhedral Oligomeric silesquioxane (POSS PCU) which has ideal properties for cardiovascular grafts and to 2) induce endothelialisation by capturing progenitor cells from peripheral blood.

**Methods:** 1) Bio-functionalisation of the nanocomposite polymer: bioactive RGD peptide, which is a functional domain of an extracellular matrix component, fibronectin was synthesised using fmoc chemistry. A lauric acid hydrophobic “tail” was also attached to optimise the RGD orientation on the biomaterial. The peptide was covalently attached to POSS PCU. The presence and the biophysical effect of RGD on the nanocomposite was tested. 2) Progenitor cells were extracted from peripheral blood, which was obtained from adult healthy volunteers and cultured on bio-functionalised nanocomposite biomaterial. The degree of cell adhesion, proliferation was tested with Alamar Blue assay. Endothelialisation was confirmed with electron microscopy and SEM. Immunostaining was also performed with endothelial cell markers, CD34, CD31 and eNOS.

**Results:** Water contact angle measurement indicated that bio-functionalisation has increased hydrophilicity of the nanocomposite polymer. EPC cell counting indicated that more cells were adhered to bio functionalised nanocomposite and Alamar blue indicated a greater presence of cells on bio-functionalised nanocomposite. Electron microscopy and SEM provided evidence for endothelial colony formation. Immunostaining confirmed the presence of endothelial cell markers.

**Conclusion:** Bio-functionalised nanocomposite polymer induced endothelialisation by capturing progenitor cells from peripheral blood.
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**Multilayered chitosan scaffold for bile duct reconstruction**

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Bile duct injuries are associated with upper abdominal operations and biliary tract surgical procedures. Their overwhelming majority is caused by laparoscopic cholecystectomy and are considered a true health and financial emergency (1). Their repair carries a significant mortality rate and can run 4.5 to 26.0 times the cost of the uncomplicated procedure. The standard treatment of neoplastic or degenerative/flogistic diseases that cause stenosis of the main biliary duct involves the resection of the extrahepatic biliary duct and the subsequent tension-free anastomosis between the bile duct stump and the intestine (Roux-en-Y hepaticojejunostomy). Often, this treatment is burden with septic complications (cholangites) and/or anastomotic stenosis. Post-operative cholangitis, one of the most common complication in hepatobiliary surgery, accounts for 8-22% of patients following hepaticojejunostomy (2), and affects more than 50% of pediatric patients following Kasai’s operation for biliary atresia. Considering these common post-operative complications (cholangites and stenosis), the present work aimed at developing a novel highly biocompatible chitosan-based polymeric scaffold in form of a tube to be used as a substitute of the human main bile duct. Polymeric tube-shaped scaffolds were manufactured by casting a chitosan solution, prepared, as previously described (3), into cylindrical mold constituted by two coaxial plastic tubes. The solution was, then, frozen and gelified (3). The reproducibility of the method was evaluated by assessing physical parameters of the scaffold such as swelling index, while porosity and microstructural characteristics were assessed by using electron-scanning microscopy. Permeability experiments through the gelled scaffold were carried out in a Resomat II apparatus using a concentrated bovine bile solution in the donor compartment and measuring the concentration of the permeated bile acids in the receptor compartment. Dynamometric measurements were performed to determine the elastic modulus and the elongation to break in axial direction, as well as the resistance to
Preparation and Characterization of Retinoic Acid Loaded Nanoparticles for Cancer Therapy

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Retinoic Acid (RA) is now included in many antitumor therapeutic schemes for the treatment of various diseases such as acute promyelocytic leukaemia, ovarian carcinoma and bladder cancer. However, due to its hydrophobicity, RA parenteral administration is very difficult and to date no commercial parenteral formulation is available. Nanoparticles based on Poly (lactic-co-glycolic acid) (PLGA) and Poly (3-hydroxybutyrate) (PHB) loaded with Retinoic Acid (RA) were prepared by nanoprecipitation method using various solvents and surfactants. The poor solubility of RA in water hampered the evaluation of the drug release kinetics in phosphate buffer solution (PBS), commonly used as releasing medium. Human Serum Albumin (HSA), one of the protein carriers of RA in the aqueous environment of cells and plasma, was added to the suspension of nanoparticles in PBS. This method allowed for the evaluation of RA in PBS by using simple UV spectrophotometry. In order to improve nanoparticles half life, a formulation based on PLGA and HSA for the delivery of RA was developed. A cryoprotective agent was added to the formulation to avoid the agglomeration of the particles during the purification process and liophilization. HSA had a strong effect nanoparticles stability in vitro. A possible correspondence within in vivo condition is assumable. Efforts will be aimed at developing nanoparticles formulations stable in HSA containing medium.

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In-vitro engineering of the liver using multiple axis stimuli

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A great deal of effort is being made to preserve liver specific function in vitro. There are several driving forces for this, perhaps the two most important are drug testing and bioartificial liver devices. For the moment, the prevailing opinion in the literature is that it is impossible to maintain an adequately differentiated hepatic phenotype after isolation from the liver for more than a week. Several parameters are known to play a role in maintaining liver function, among these are the presence of an adequate nutrient supply, an extra cellular environment rich with adhesion ligands, and a spatial architecture resembling that of the native liver, as well as a multicomponent medium. These parameters form what we call here the tripartite of cues: a trio of biochemical, biophysical and biomechanical signalling systems which interact synergically to support both form and function in all living tissues.

We describe here the results of a study in which a step by step approach was used to study the influence of these three factors on hepatocyte function in vitro. HepG2 was used as a model cell line. Firstly the influence of a three dimensional topology was assessed using three-dimensional microfabricated polymer scaffolds realised with the Pressure Activated Microsyringe (PAM). Hep2 cells were seeded and cultured on hexagonal-three-dimensional polymeric poly-lactic-co-glycolic-acid (PLGA) scaffolds, initially sterilized and function-alised with murine collagen type IV. Hepatocyte cell density, glucose consumption, and albumin secretion rate were measured daily over a week. The results of this study confirmed that cell proliferation is enhanced on the scaffolds, while albumin production per cell remains fairly constant. Interestingly cells on the scaffolds consume about half as much glucose as on 2 dimensional films on the first few days. While albumin production per cell remains fairly constant. Having confirmed that scaffolds increase cell density, we added a second stimulus, that of flow, through the use of a dynamic cell culture system, the MCB (MultiCompartment Bioreactor) system. Cells under dynamic conditions and seeded on scaffolds showed an increase in cell density compared with static monolayer controls. Moreover, cell...