

determines whether this cross-linking density can be achieved. The density of extruded liquid was found higher than theoretical density of the buffer solution by our calculation, which suggested a physicochemical change inside the cross-linked matrix as well as possible leaking of uncross-linked polymer, which supported our hypothesis regarding HRP and cross-linking density. Additionally, the ultrastructure of Gtn-HPA with goat marrow stem cells embedded was directly observed, for the first time, by high resolution electron microscopy without coatings or environmental controls. Extracellular matrix surrounding seeded cells was found remodeled and densified on Day 7.

### Developing Three-dimensional Human Brain Tumor Models for Personalized Drug Screening

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Pediatric cancers remain the leading cause of non-traumatic deaths among children. In an effort to increase survival and decrease the toxicity of current treatment regimens, current research focuses on the aggressive and treatment-resistant cell populations residing within pediatric tumors. Recent evidence indicates that a clonal population of neoplastic cells in the tumors exhibits marked heterogeneity in proliferation and differentiation. In order to target the heterogeneous drug-resistant sub-populations of tumor cells, development of a personalized medicine approach aimed at patient-specific drug screening would be timely. We hypothesize that a three-dimensional (3D) brain tumor model containing patient-specific tumor cells could utilize the self-renewal property of the stem-like cells to generate clonal copies of tumor cells that could be used for personalized drug screening. An *in vitro* 3D human brain tumor model was generated using silk protein-based scaffolds that were seeded with cells isolated directly from a surgically resected pediatric brain tumor. Initial results indicate key differences between cells in 3D versus 2D cultures. Expression of self-renewal markers was higher in tumor cells cultured in the 3D brain tumor model when compared with conventional 2D culture. Inversely, expression of neural differentiation markers was decreased in 3D systems compared to 2D cultures. The 3D tumor models were maintained for at least one month in culture which would enable development of a platform for high throughput drug screening. The 3D human brain tumor model could serve a useful tool for personalized drug development for pediatric brain tumors.

### The Role of Autophagy on the Differentiation Potential of Adipose Stromal Cell Sheets under Normoxia vs Hypoxia

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It has been demonstrated that the effects of low oxygen tensions in cultures of human adipose stromal cells (hASC) range from increased cell death to changes in proliferation and differentiation potential. Specifically, hypoxia-induced autophagy promotes cell survival as result of Hypoxia Inducible Factor-1 $\alpha$  activation and downstream signalling. In the present project, we intended to verify the existence of a correlation between the levels of hypoxia-induced autophagy and changes in the differentiation potential of hASC sheets. Cell sheets (CS) of hASC were produced and cultured in normoxic and hypoxic conditions (5% O<sub>2</sub>) for 1 to 4 days. At

each time point, samples were collected for western blot and qPCR analysis or further cultured in osteogenic or adipogenic medium. Gene expression analysis of LC3, ULK1, BECLIN1 and ATG5 revealed overexpression of these autophagy players at day 4, independently of the culture oxygen tension. LC3II protein quantification revealed an increase in the autophagic flux at day 4, regardless of the oxygen tension, confirming the qPCR data. This suggests that the state of hyperconfluency of CS, increasing from day 1 to 4, may modulate the autophagic state of cells, masking the effects of low-oxygen tensions. Importantly, CS cultured in hypoxia for 4 days had increased osteogenic differentiation in comparison with day 1 hypoxic samples but decreased adipogenic differentiation, which hints at a possible correlation between higher levels of autophagy and a tendency towards the osteogenic phenotype. This hypothesis is being confirmed using autophagy modulators.

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### Nerve Tissue Engineering using Blends of Polyhydroxyalkanoates for Peripheral Nerve Regeneration

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Polyhydroxyalkanoates blend films with varying ratios of poly(3-hydroxyoctanoate)/poly(3-hydroxybutyrate) (P3HO)/P(3HB) were produced and evaluated to investigate their use as base materials for the manufacture of nerve guidance conduits to assist peripheral nerve repair. Neat films of P(3HO), P(3HB) along with 75:75, 50:50 and 25:75 P(3HO)/P(3HB) blend films were chemically, physically, and biologically characterized. In the surface analysis, the blends exhibited the highest values of roughness compared with the neat films. XRD analysis of the blends showed an increase in the crystallinity with the increase of P(3HB) content. Mechanical analysis showed that 75:25 P(3HO)/P(3HB) and 25:75 P(3HO)/P(3HB) blends presented suitable tensile strength and percentage of strain for their application in peripheral nerve repair.

Live/dead measurement and immunolabelling of NG-108-15 neuronal cells were performed to study cell attachment and differentiation on the evaluated flat substrates using confocal microscopy. Although all of the blends were biocompatible with cultured neuronal cells, the 25:75 P(3HO)/P(3HB) blend showed significantly better support for their growth and differentiation. Aligned electrospun fibres of 25:75 P(3HO)/P(3HB) blend were produced using varying polymer concentrations (5, 10, 15, 20% w/v) under different conditions of voltage (12 kV, 18 kV) and collector speed (1000, 1500 and 2000 rpm). The thickest diameter of electrospun fibres of 25:75 P(3HO)/P(3HB) blend was then chosen as a substrate for cell culture studies using neuronal cells. Live/dead cell tests showed that the growth of cells on the 25:75 electrospun scaffolds was significantly better and cells were more uniformly distributed when compared with 25:75 P(3HO)/P(3HB) blend flat substrate.

### Development of a Three-Dimensional *In Vitro* Model to Study Neovascularization

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One of the greatest challenges currently faced in tissue engineering is the incorporation of vascular networks within tissue-engineered constructs. Knowledge of the major factors that drive vascular ingrowth is key to overcoming this hurdle. The aim of this study was to develop a technique for producing a perfusable, three-dimensional