CSI-05 – Stem Cell Differentiation

SUN-444
3D collagen scaffolds hosting neural stem cells: developing neuroimplants for spinal cord injury (SCI) repair

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Many studies have investigated the effects of engraftment of *in vitro*-propagated Neural Stem Cells (NSC) or committed neuronal or glial progenitors on the recovery of injured Spinal Cord (SC). Most of the studies performed in rodents demonstrate that grafted cells display a short-term beneficial effect on locomotor function. One of the major setbacks of these efforts is that transplantation of NSC cell suspensions results in cell spreading and dispersal within the SC, limiting their efficacy for successful local integration and functional networking. Transplantation of a solid scaffold containing NSCs may successfully overcome these limitations. We are developing neuroimplants using 3D collagen I scaffolds hosting embryonic neural stem cells, isolated from the knock in *sox2-egfp* mice strain. A sox2-positive population more than 90% pure is isolated, using fluorescence-activated cell sorting (FACS) analysis. Scaffolds were prepared with homogenization of microfibrilar bovine collagen I in acetic acid, then freeze-drying: freeze-dry parameters control pick porosity and cross-link with various additives control stiffness and pick degradation rate. Collagen I scaffolds are much more stiffer and degradation-resistant than gels scaffolds have an open foam structure, 0.5% mass fraction and 30 to 400 μm pores and *in vivo* degradation rate at 90 days half life. Confocal and electron microscopy analysis of NSC have shown that composition and microstructure of 3D scaffolds play a significant functional role: scaffolds with a combined composition (50% collagen-50% gelatine or 92% collagen-8% chondroitin-6-sulphate) support more effectively NSC survival and proliferation as measured by TUNEL and Ki67 staining respectively, maintaining stemness (sustained sox2 expression) and propagating neurosphere formation. On the other hand, primary sensory neurons from mouse embryonic dorsal root ganglia, grew better in pure collagen scaffolds with less rigid structures. We are now testing the 3D collagen I scaffolds populated with mouse neural stem cells in mice with experimental spinal cord injury.

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Keywords: Collagen scaffolds, Neural Stem Cells, Spinal Cord Injury.

SUN-445
3D microcarrier cell culture affects proteomic signature of exosomes derived from human dental pulp stem cells

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Microcarrier technology is an efficient method for scaling-up cell production in small volumes, while promoting improved cellular phenotypes in a three-dimensional environment. The main advantage of this system is the possibility to cultivate up to 100 times more cells in the same amount of medium. In addition, these systems could be potentialy used as a factories for the small scale production of different therapeutic factors. Here we present results on the use of BioLevitator™ (Hamilton), a commercially available three-dimensional culturing platform and alginate microcarrier cell culture system (Global Cell Solutions) for the propagation of stem cells derived from the dental pulp of human exfoliated deciduous teeth (SHEDs). Exosomes were purified by ultracentrifugation from SHEDs cultivated under two conditions: standart two-dimensional culture flasks, or from SHEDs grown on the laminin-coated microcarriers in bioreactor. In both cases cells were grown in serum- and xeno- free medium (MSC Nutri-Stem XF, Biological Industries). For proteomic studies liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) analysis was carried out on an EASY-nLC (Thermo Fisher Scientific) connected to a Velos Pro-Orbitrap Elite hybrid mass spectrometer with nano electrospray ion source (Thermo Fisher Scientific). In total we identified 80 proteins in exosomes from standart SHED cultures and 60 proteins in exosomes from microcarrier cultures. The majority of the identified proteins are included in the vesiclepedia database. Importantly, only 28 proteins were common between exosomes from different preparations. These findings indicate, that 3D microcarrier cell culture have a profound impact on the proteomic composition and possibly physiological properties of exosomes.
Keywords: Bioreactor, exosomes, stem cells.

SUN-446
A multidisciplinary approach to studying crypt-villus homeostasis and regeneration in the intestinal epithelium

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The luminal surface of the gastrointestinal tract is folded to form invaginations or crypts, and evaginations, or villi. The epithelial cell layer lining these structures performs a sophisticated dual role in maximising nutrient exchange while maintaining a barrier to intestinal pathogens. The epithelial layer is continuously regenerated; stem cells located at the base of crypts proliferate and differentiate into a variety of cell types which migrate upwards along crypts and villi before being shed into the gut lumen. Maintenance of the functional integrity of the intestinal barrier requires tight coordination of cell proliferation, migration and shedding along the crypt-villus axis, with dysregulation of these processes leading to tumourigenesis and inflammatory disease. How these processes are regulated in homeostatic, disease and recovery states is incompletely understood.

Using a multidisciplinary approach combining experimental measurement and mathematical models we aim to define the mechanisms involved in homeostasis of the intestinal epithelium and its recovery after perturbation. Specifically, the mechanisms controlling cell migration along the crypt-villus axis, those regulating cell shedding, and those involved in translating events on the villus to responses in the crypt. We have developed individual based models (IBMs) to study the spatio-temporal dynamics of

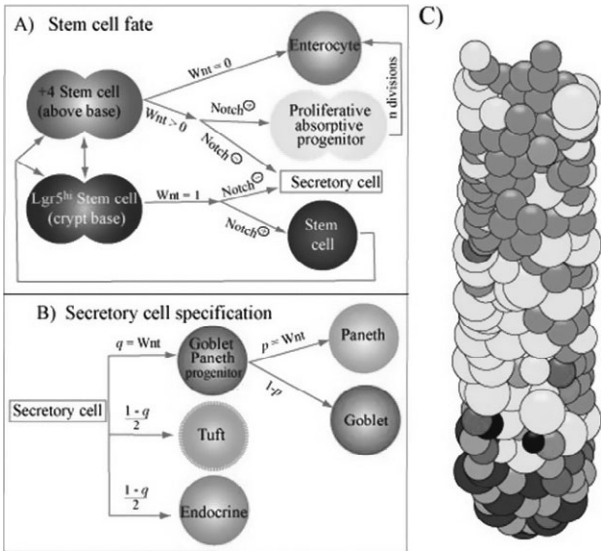

Fig. 1. Model for cellular differentiation and proliferation in the crypt of mouse small intestine.

epithelial cell generation in crypts, providing insight into the dynamics of cell differentiation and localisation, and the propagation of mutations. Using Chaste as a computational framework, and through continual development and refinement of models at different levels of special granularity, we aim to simulate crypt-villus epithelial dynamics in both healthy and diseased mucosa.

In combination with mathematical approaches, we have tracked and manipulated epithelial cell behaviour using a variety of in vivo murine models of healthy and altered epithelia, including inflammatory and pro/anti-proliferative settings. For example, using an LPS–induced model of epithelial damage, we observe intense apoptosis at villus tips and a significant reduction in villus height in the small intestine, with recovery of typical morphology within a few hours. Using in vivo and in vitro microscopic and histological techniques, we have traced epithelial cell proliferation, migration and shedding to better define the processes involved in villus regeneration.

Our ongoing work continues to combine experimental and mathematical approaches to define mechanisms which are essential to maintain lifelong health of the gastrointestinal tract and ultimately aims to identify strategies for intervention in intestinal disease.

Keywords: Intestine, modeling, Stem cells.

SUN-447
A neurofilament-derived peptide targets neural stem cells and alters their self-renewal capacity

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The neural stem cells (NSC) are characterized by their capacity to self-renew, to form neurospheres in culture, to proliferate, and to generate neurons, astrocytes and oligodendrocytes (multipotency). The characteristics of these cells provide new therapeutic approaches for the treatment of neurodegenerative disorders and

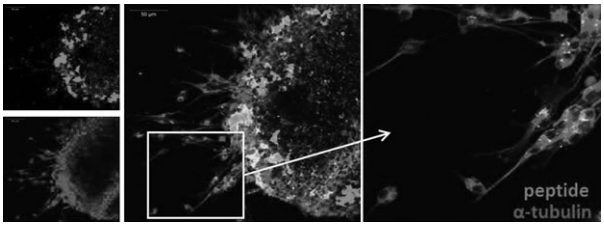

Fig. 1. Confocal microscopy of the peptide (in green) uptake in neural stem cells of new-born rats (stained with anti-tubulin, in red).

for the treatment of malignant glioma. The development of neural stem cell-based therapies may be beneficial to target these cells, increase their mobilization and stimulate neurogenesis for regenerative medicine and for the treatment of brain tumours.

We previously showed that a peptide corresponding to the tubulin-binding sequence located on neurofilament, alone or linked to nanoparticles, is able to target glioblastoma cells *in vitro* and *in vivo*. The selective and massive uptake of this peptide by glioblastoma cells occurs through endocytic pathways and is related to their high proliferative state, whereas a low level of internalization occurs for slow proliferative healthy cells (astrocytes, oligodendrocytes or neurons). Moreover, while the peptide is able to disrupt the microtubule network of glioblastoma cells and inhibit their proliferation, it has no major effect on the microtubule network from healthy cells. Finally, injected in rats bearing glioblastoma, the peptide reduces tumour development (Bocquet et al., 2009; Bergès et al., 2012; Balzeau et al., 2013; L-Chambaud et Eyer, 2013).

In this study we show that this peptide is able to translocate passively in neural stem cells *in vitro*. Moreover, the *in vitro* formation of neurospheres was not altered by the peptide whereas the self-renewal capacity of these cells was slightly reduced and associated with an increase of adherent cells and a decreased of NSC proliferation. Finally, when injected in the cerebrospinal fluid of rats the peptide targets adult neural stem cells *in vivo* without major detectable cytotoxicity. These results indicate that this peptide represents a new molecular tool to target neural stem cells in order to develop new strategies for regenerative medicine and treatment of brain tumours.

Keywords: neural stem cells, self-renewal, targeting peptide.

SUN-448
A novel method to identify and isolate pluripotent human stem cells and mouse epiblast stem cells using lipid body-associated retinyl ester fluorescence

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We describe a characteristic blue fluorescence exhibited by Human embryonic stem cells which helps in identification and isolation of human pluripotent stem cells. The blue fluorescence is easy to observe with basic epifluorescence microscopy. The intensity of blue fluorescence correlates with the expression of pluripotency markers such as OCT4, SOX2 and NANOG. We show that with the help of FACS, undifferentiated pluripotent stem cells can be easily isolated based on their blue fluorescent intensities. This blue fluorescence phenomenon appears early during reprogramming and arises from cytoplasmic lipid bodies by the sequestration of reinyl esters. These blue fluorescent lipid