TS023
Thermosensitive poly-caprolactone scaffolds for 3D differentiation of C2C12 cells and human adipose-derived stem cells

V Hruschka[1], A Saeed[2], R Cheikh al Ghanami[2], G Feichtinger[1], S Nürnberger[1], A Meinl[1], T Nau[1], H Redl[1], K Shakesheff[2] and S Wolbank[1]

[1]*Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Austrian Cluster for Tissue Regeneration, Vienna, Austria;* [2]*School of Pharmacy, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, United Kingdom*

For tissue engineering (TE) applications, the natural process of regeneration is imitated by using bioresorbable scaffolds that support cellular attachment, migration and proliferation, along with cells capable of differentiation upon exposure to inductive factors. Based on the idea of combining a fully degradable polymer (Poly(ϵ-caprolactone; PCL) with a thermoresponsive polymer (polyethylene glycol methacrylate, PEGMA) a scaffold was developed, which liquefies at 4 °C and solidifies at 37 °C. Adult stem cells, which can be obtained out of human fat tissue, have the ability to be cultured *in vitro* and the potential to differentiate into various lineages, including the osteogenic and adipogenic lineage. There are several advantages using adipose-derived stem cells (ASCs), for instance the possibility to isolate large amounts in an easy way or the possibility to use them in an autologous setting. The mouse C2C12 cell line also features stem cell characteristics and has the potential to differentiate towards the myocyte as well as osteocyte lineage. In this study, the novel thermoresponsive material PCL-PEGMA was combined with C2C12 cells or human ASCs to generate an expandable 3D construct for soft or bone TE, depending on the *in vitro* differentiation conditions. As a first step, biomaterial seeding for C2C12 cells as well as ASCs was optimized and their attachment, survival, distribution and persistence within the 3D material was characterized using viability assays, fluorescence microscopy and scanning electron microscopy. C2C12 cells as well as ASCs attached to the polymers were viable and evenly distributed in all scaffolds. For C2C12 differentiation, the cells were seeded 3D within the scaffold and stimulated with either osteogenic or myogenic differentiation medium for 7 days. The ASC-scaffold constructs were cultured in media supporting either adipogenic or osteogenic differentiation of ASCs for 21 days. 3D differentiation of the cells was examined using qRT-PCR for differentiation specific markers. Interestingely, C2C12 cells differentiated in the myogenic lineage as well as ASCs treated with adipogenic differentiation medium showed increased marker expression in 3D compared to 2D, suggesting that the thermoresponsive PCL-PEGMA scaffold qualifies for 3D differentiation. Conclusion: The thermoresponsive scaffold presented in this study is a suitable material for 3D soft tissue engineering. The properties of the material show limited potential to support osteogenic differentiation of C2C12 or ASCs. In contrast, the matrix allows the attachment of C2C12 cells as well as ASCs and was able to support 3D myogenic differentiation of C2C12 cells and 3D adipogenic differentiation of ASCs.

TS024
Intracellular delivery of methylprednisolone by dendrimer-based nanoparticles improves locomotor outcomes after spinal cord injury

SR Cerqueira[1,2,3], JM Oliveira[1,2], BL Silva[2,3], N Silva[1,2,3], JF Mano[1,2], N Sousa[2,3], AJ Salgado[2,3] and RL Reis[1,2]

[1]*3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Guimarães, Portugal;* [2]*Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal;* [3]*ICVS/3B's Associate Lab, PT Government Associated Lab, Braga, Portugal*

Spinal cord injuries (SCI) still remain a major challenge in current biomedical research. In spite of several advances in the understanding of its mechanisms there has not been an equal significant translation into the clinics. As a result, there is no effective treatment that can overcome the biochemical and cellular adverse reactions that lead to a chronic severely impaired condition. One of the first opportunities to minimize these drastic consequences is to control the secondary events that follow the trauma. We are proposing the local delivery of an anti-inflammatory corticosteroid - methylprednisolone (MP) – in an attempt to modulate the noxious effects of the inflammation in the acute SCI. A sustained delivery as the one provided by these nanoparticles (NP) can be highly advantageous, maximizing the drug's potency in the target site. Therefore, we synthesized MP-loaded NPs composed of an inner poly/(amido)amine (PAMAM) dendrimeric core and grafted with carboxymethylchitosan (CMCht). Chemical and biological characterization studies were carried out showing that the NPs are stable in acidic and neutral buffer solutions. Also, the viability of primary glial cultures was not compromised by the presence of 200 μg/mL of NP. In turn, an MP action in microglial cultures was observed in dosages above 1 mg/mL showing that MP is being released from the NPs inside the cells. The uptake profile of these NPs is time dependent and reaches its maximum 24 h after incubation with astrocytes, oligodendrocytes and microglia. In a preview of a possible therapeutic effect, the NPs were administered in hemisected spinal cord injured rats. To assess the efficacy of local injections around the lesion site the animals were sacrificed 3 h after surgery and frozen sections were observed. The fluorescently labeled-NPs were detected in the injury and in the surrounding spinal tissue indicating a successful delivery of the NP to the spinal tissue. The local injections were repeated in hemisected rats that were kept for 1 month, performing the BBB locomotory test weekly. Significant differences in the BBB test were found between the MP-loaded NPs injected rats and the sham group as well as the ones injected with MP, demonstrating a favorable action of the MP-NPs in the acute phase of the injury. This work revealed that sustained delivery of MP via a NP system can be highly beneficial in the management of the secondary injury that follows SCI improving the overall functional outcome of the injured animals.