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Introduction: Replacing loss of cardiac cells after ischemic heart injuries using a tridimensional biocompatible scaffolds for cell delivery could be assimilated to a microenvironment mimicking culture device directly in contact with the targeted area. Our main work was to modify those scaffolds properties to improve cardiac commitment of human embryonic stem cells (hESC).

Experimental Procedures: We modified scaffolds physical and chemical properties to support human embryonic stem cells (hESCs) differentiation toward the cardiac lineage, by incorporating gelatin and Fucoidan, and analyzed hESC cardiac commitment markers expression by qPCR and histological analysis.

Results: Fucoidan scaffolds ability to locally concentrate and slowly release TGF-β and TNF-α was confirmed by Luminex technology. They support significantly higher expression of the early step of embryonic cardiac differentiation markers: NKX2.5 ($p<0.05$), MEF2C ($p<0.01$), and GATA4 ($p<0.01$). We also found that Fucoidan scaffolds supported the late stage of embryonic cardiac differentiation marked by ANF expression ($p<0.001$). Moreover, thin connecting smooth muscle cells filaments enabled maintenance of beating areas for up to 6 months.

Conclusion Perspectives: Porous scaffolds are a promising method to improve cells delivery to damaged myocardium. Absence of mechanical stress in the soft hydrogel impaired sarcomere formation, as confirmed by cardiac muscle myosin MYH6 and sarcomeric α-actinin analysis. As a consequence, further scaffolds chemical stiffening or combination with mechanical stress, could enhance sarcomere formation at terminal stages of differentiation (TEA. 2014 doi: 10.1089)

OR067

Differences in collateral-dependent muscle perfusion may explain efficacy variation in clinical angiogenic gene therapy trials

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Background: Angiogenic gene therapy aims to improve ischemic tissue oxygenation by opening up the capillary bed and supporting collateral growth in chronically ischemic conditions where endogenous vascular repair mechanisms are compromized. However, to date only a few patients have benefited from the clinical trials of the novel treatment.

Methods and aims: We initiated a clinical phase I-II, placebo controlled, single blind angiogenic gene therapy trial in chronic lower limb ischemia to test the safety and angiogenic potential of the mature form of vascular endothelial growth factor D in an adenoviral vector (AdVEGF-DdNdC). 30 patients undergoing peripheral bypass surgery will receive i.m. injections of 3x10e10 or 3x10e11vp of AdVEGF-DdNdC or NaCl as control. Importantly, the study utilises multimodality imaging (including MRI/S, FDG-PET contrast enhanced ultrasound and fotoacustic imaging) to objectively assess the effects of the investigational therapy and to reveal possible sources of variation in patient responses.

Results: With the lower dose group of patients now completed, the AdVEGF-DdNdC gene therapy was well tolerated and improved relative tissue perfusion with all treated patients one week after gene transfer. However, significant variation in the baseline level of collateral-dependent microvascular perfusion was observed among patients negatively affecting the potential efficacy of AdVEGF-DdNdC and increasing variation in the final results.

Conclusions: The baseline level of collateral-dependent microvascular perfusion varies considerably among patients with limb ischemia and seems to negatively affect the results of angiogenic gene therapy. Patients with initially low or low post operative microvascular perfusion may best benefit from angiogenic therapies.

OR068

Modulation of neuroinflammatory responses in the cuprizone mouse model following transplantation of mesenchymal stem cells genetically engineered to secrete IL13 coincides with the appearance of multiple alternatively activated macrophage and microglia phenotypes

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Forced alternative activation of neuroinflammatory responses is expected to become a new therapeutic approach for a variety of central nervous system (CNS) disorders in which pro-inflammatory microglia and/or macrophage responses contribute to the neuropathology. With this multidisciplinary study, we propose a novel neuroimmune-modulating approach involving intracerebral transplantation of mesenchymal stem cells (MSC) genetically engineered to secrete interleukin 13 (IL13). In the first part of this study we investigated whether grafting of IL13-producing MSC in the corpus callosum can alter the course of cuprizone (CPZ)-induced CNS inflammation and demyelination. Using non-invasive T2-weighted magnetic resonance imaging we here demonstrate that grafting of IL13-producing MSC displays a significantly superior protection against CNS inflammation and demyelination as compared to grafting of non-engineered MSC. Further experiments in eGFP+bone marrow chimeric mice and in the CX3CR1+/eGFPCCR2+/RFP transgenic mouse model, both used to histologically discriminate between brain-resident microglia and CNS-infiltrating bone marrow derived macrophages, we demonstrate that grafting of IL13-producing MSC results in a significant recruitment of peripheral macrophages into the CNS parenchyma which, by the action of IL13, become forced into multiple alternatively activated phenotypes as demonstrated by differential expression patterns of MHCII and/or Arginase-1. Moreover, the appearance of these alternatively activated macrophage phenotypes following grafting of IL13-producing MSC

coincides with the observed microglial quiescence in the CPZ model. Concluding, with this study we suggest that controlled and localized introduction of alternatively activated macrophages at the onset of neuroinflammatory responses has the potential to exert a major immunomodulatory effect on pathology-associated microglial immune responses.

OR069

Extensive comparison of HEK293 transfection process and Baculovirus expression system to manufacture an AAV8-based treatment for Crigler-Najjar syndrome

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1: GENETHON

An AAV8 vector expressing the human UDP-glucuronosyltransferase 1 polypeptide A1 (UGT1A1) has been developed for the treatment of Crigler-Najjar syndrome. To enter phase I/II clinical phase, a robust and GMP-compliant manufacturing process is needed to produce the therapeutic vector in sufficient quantity and quality. Two production platforms have been evaluated to produce the recombinant AAV8 in stirred tank bioreactors: on one hand, the standard triple transfection process was adapted to HEK293 cells growing in suspension; on the other hand, the dual Baculovirus expression system in Sf9 cells was tested. In both cases, the AAV8 vector was produced in 2L and 10L scale bioreactors and was then purified by immunoaffinity chromatography, concentrated and formulated using tangential flow filtration. The resulting final products have been thoroughly analyzed using a wide set of analytical tools to characterize the product purity, integrity and efficacy. Both processes displayed comparable productivity and generated similar ratios of full/empty capsids. However, the results revealed that the HEK293 transfection process was the most efficient to produce AAV8-UGT1A1 vector with the foreseen target profile, particularly with regards to genome integrity, capsid proteins content and infectivity. As expected, this vector profile correlated with the highest level of transgene expression in vitro and with the best efficacy to decrease bilirubinemia in a rodent model of Crigler-Najjar syndrome. Based on these results, the triple transfection process is being scaled up in disposable bioreactors in order to produce clinical grade AAV8-UGT1A1 vector.

OR070

iCELLis® fixed-bed technology provides an efficient scalable system for viral vector production

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Large scale vector manufacturing has proven to be challenging. The iCELLis® 500 provides disposable fixed-bed system with perfusion capability and with automated control of stirring, temperature, pH and dissolved oxygen. The cultivation area varies between 66 and 500 m2. It is ideal to satisfy demand for phase III / commercial requirements. In iCELLis® Nano, the cultivation area 0.53-4 m2 is ideal for small batch production and process development purposes. We have used iCELLis Nano for the production of different kind of viruses, such as adenovirus, lentivirus and AAV. HEK293 and 293T cell culture parameters were optimized in adherent mode and also suspension cells in serum-free medium were tested. Adenovirus infection and harvest by chemical lysis inside the bioreactor was proven to be efficient. Lentivirus and AAV production by calcium phosphate mediated plasmid transfection was optimized. Lentivirus harvest was performed by perfusion, whereas AAV harvest was done by lysis technique. We have also tested baculovirus mediated lentivirus production in iCELLis®. The scalability of the system was demonstrated by manufacturing adenovirus using iCELLis® 500 100m2 bioreactor with the process parameters defined in a small scale. High yield productivity was achieved in a large scale system. The iCELLis® bioreactors supported cell growth, although an uneven distribution of the cells was observed, mostly in iCELLis® Nano 4m2 bioreactor. To conclude, iCELLis® equipment has shown us an efficient way to manufacture large batches of different kinds of gene therapy products suitable for large preclinical animal models and up to phase III trial and beyond.

OR071

Human cardiac stem cells for allogeneic cell therapies: integrating bioprocess development and "omics" characterization tools

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Stem cells therapies stand as a promising strategy for cardiac repair as the adult heart homes a population of resident cardiac stem cells (hCSC) capable of regenerate contractile myocardium upon activation [1]. Decoding the intricate cellular pathways, growth-factors and receptors involved in this process is also of major relevance to understand cardiac repair and improve clinical intervention. With this work we aim at establishing a robust and scalable bioprocess for hCSC expansion to support allogeneic cell therapies but also to perform the proteomic profiling of hCSCs receptome and secretome to provide new insights about the molecules and pathways involved in cardiac repair events. Significantly higher expansion cell yields were obtained in stirred tank bioreactors when compared to static cultures while cells remained phenotypically and functionally similar, concerning cell viability, metabolism, GF secretion and differentiation potential. A high-throughput proteomics workflow was implemented, enabling identification of challenging proteins such as GF and receptors, which are relatively low abundant and hydrophobic membrane proteins, respectively. hCSC receptome analyses lead to the identification of more than 3000 proteins, several hundred with numerous transmembrane domains (e.g. Connexin-43), among which more