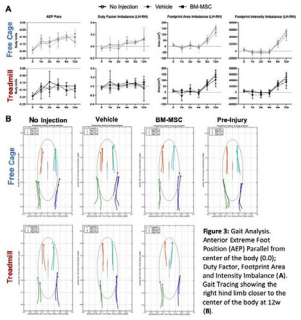

Figure 3: Gait Analysis. Anterior Extreme Foot Position (AEP) Parallel from center of the body (0,0); Duty Factor, Footprint Area and Intensity Imbalance (A). Gait Tracing showing the right hind limb closer to the center of the body at 12w (B).

9

Engineered nasal cartilage for the repair of osteoarthritic knee cartilage defects

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Background & Aim Osteoarthritis (OA) is the most prevalent joint disorder causing pain and disability predominantly in the ageing population but also in young individuals. Current treatments are limited to alleviate symptoms, e.g. with anti-inflammatory drugs, until at the end stage of the disease the degenerated joint is replaced by a prosthetic implant. In this study we hypothesize that degenerative cartilage defects can be treated using Nasal chondrocyte-based Tissue Engineered Cartilage (N-TEC), as previously applied for the repair of focal articular cartilage lesions. In particular, we tested (i) the response of N-TECs to inflammatory factors, (ii) their capacity to modulate the inflammatory profile of OA joint cells in vitro as well as (iii) their survival in - and integration with OA tissues in vivo. Based on these results we then (iv) assessed the performance of N-TEC upon implantation in OA knee cartilage lesions of human patients.

Methods, Results & Conclusion When N-TEC was exposed *in vitro* to inflammatory stimuli, as those found in OA joints (IL-1β/TNFα/IL-6 cytokines or factors secreted by OA synoviocytes) we demonstrate that it maintained its cartilaginous properties (i). Moreover, the secretome of N-TEC positively influenced the inflammatory profile of cells from OA joints, i.a. by reducing the expression of IL-6 and TNFα (ii). *In vivo* in an ectopic mouse model reproducing a human osteochondral OA tissue environment as well as in chronified articular cartilage defects in sheep, we further demonstrated cell survival and engraftment of N-TEC with the surrounding OA tissues (iii). Finally, we tested clinically the implantation of autologous N-TEC in two patients with advanced OA (Kellgren and Lawrence grade 3 and 4), who were otherwise considered for unicondylar knee arthroplasty. Patients reported reduced pain as well as improved joint function and life quality 14 months after surgery. In addition, a radiologically observed increase in joint space, indicating safety and feasibility of the treatment (iv). Together, our findings indicate that N-TEC can directly contribute to cartilage repair in OA joints. A phase II clinical trial is now required to assess efficacy in a larger cohort of OA patients.

10

Probing MSC and Tumor Cell Secretome Locally via Dynamic Sampling Platform (DSP)

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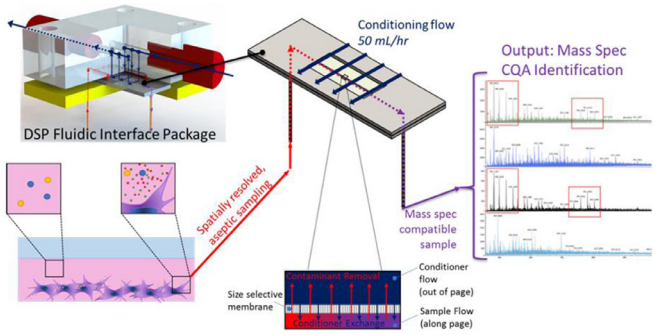
Background & Aim Within any cell culture, especially on the larger scale of commercial bioreactors, there is considerable heterogeneity that directly impacts the final cell product. This variation results in part from the local shear forces experienced by cells as well as the biochemical microenvironment, e.g., metabolic products and secreted protein factors that can act in a paracrine fashion to modify nearby cells. The ability to measure these critical quality attribute (CQA) biomarkers with high spatial and temporal resolution is foundational to the development of real-time quality monitoring for cell manufacturing. Standard analytical methods quantify neither the local heterogeneity nor the transient nature of cell growth. The Dynamic Sampling Platform (DSP, figure 1) overcomes both barriers by enabling local sampling of the secretome for rapid, high fidelity, and label free CQA identification via inline electrospray ionization mass spectrometry (ESI-MS). Real-time monitoring of the microenvironment with DSP will help predict cell health, viability, and potency, thus enabling the Chemistry, Manufacturing and Controls (CMC) standards for the scale-up and scale-out of cell-based therapies.

Methods, Results & Conclusion DSP with ESI-MS sensing enables continuous collection, treatment, and direct infusion of ultra-small samples taken directly from cell culture bioreactors for ESI-MS detection of the cell secretome. DSP-nanoESI-MS is comprised of three elements: 1) a non-invasive sampling interface, which has demonstrated the ability to continuously sample from 2D cell cultures aseptically; 2) a microfabricated mass exchanger for sample treatment which simultaneously removes compounds detrimental to MS analysis, e.g., inorganic salts, and introduces compounds that enhance MS sensitivity; and 3) a spray port for direct infusion nano-ESI-MS analysis resulting in identification/fingerprinting of biomolecules in cell cultures.

DSP has been applied to multiple live cell cultures to identify differences in the secretome associated with cell state. Analysis of wild type versus interferon gamma primed mesenchymal stromal cells (MSCs) as well as OS-17 and 143-B tumor cell types reveal an enriched ESI-MS signature associated with the different cell types. Localized sampling by DSP is shown to be critical in order to capture higher concentration biomarkers near the cell membrane vs bulk samples that are masked by excessive abundance of biochemical background within the media.

The Dynamic Sampling Platform (DSP) samples directly from a cell culture with high spatial resolution to capture critical quality attribute (CQA) biomolecules which indicate the instantaneous cell state and growth trajectory. Rapid, inline sample treatment using the microfabricated mass exchanger removes contaminants, such as inorganic salts, and infuses chemicals that enhance the sensitivity of ESI-MS analysis. Larger CQA biomarkers are selectively retained in the sample channel by the size selective nanomembrane, while smaller contaminants and enhancers transfer across the membrane freely. Real-time electrospray ionization mass spectrometry (ESI-MS) provides detection and identification of broad range of CQA biomolecules with no a priori knowledge of chemical composition.



11

Engineering of NK activating receptor ligands enhances immune compatibility of MHC-I$^{-/-}$ iPSC-derived β cells for cell therapy of type 1 diabetes

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Background & Aim Induced pluripotent stem cells (iPSCs) represent a renewable source of pancreatic β cells and an ideal platform for production of genetically engineered cell therapies for type I diabetes (T1D). Nevertheless, autologous cell engraftment is strongly limited by autoimmune response in T1D, whereas transplantation of allogeneic iPSC-derived β cells, as well as donor islets, results in progressive CD8$^+$ T cell-mediated allograft rejection. Although proposed cell therapy with MHC-I$^{-/-}$ iPSC-derived β cells may result in prevention of CD8$^+$ T cell-mediated immune response, it could trigger missing-self recognition of natural killer (NK) cells, nullifying this strategy. In light of this, we aimed to inhibit NK lysis by modulation of NK activating ligands to enhance transplant compatibility of MHC-I$^{-/-}$ iPSC derivatives.

Methods, Results & Conclusion Methods - Differentiation of iPSCs into β cells was achieved by in vitro protocol mimicking pancreas development. Expression of NK ligands during β cell differentiation was assayed by flow cytometry, IF and qRT-PCR. Gene editing was performed by using CRISPR/Cas9 system. Immunogenicity tests were set up by culturing target cells with CD8$^+$ T or NK cells, at different target-effector ratios. NK activation markers were assayed by flow cytometry.

Results - Performing cytotoxicity tests on wild type and MHC-I$^{-/-}$ cell lines revealed that MHC-I$^{-/-}$ cells efficienlty escape CD8$^+$, but not NK lysis. We found that iPSC-derived β cells express high levels of B7H3 and moderate levels of DNAM-1-ligand CD155, whereas NKG2D-ligands MIC-A, MIC-B and RAET1E were significantly down-regulated on plasma membrane of terminally differentiated β cells. Supposing that such surface ligands could be involved in the amplification of NK activating signals following loss of self on target cells, we generated MHC-I-deprived B7H3$^{-/-}$, CD155$^{-/-}$ and B7H3$^{-/-}$/CD155$^{-/-}$ iPSCs. All three iPSC lines correctly differentiated into functional pancreatic β cells and interestingly appeared to significantly reduce NK lysis compared to MHC-I$^{-/-}$ ones. We also confirmed this dampened response by evaluating levels of NK activation markers, such as CD107a, TNFα and IFNγ.

Conclusion - Our study suggests NK activating receptor ligands as target to make graft invisible to immune recognition, offering new perspectives for using iPSC-derived β cells as next generation cell source for T1D treatment.

12

Enhancing the therapeutic potential of mesenchymal stem cell-based therapy via CRISPR/Cas9-based genome editing

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Background & Aim Mesenchymal stem cell (MSC) based therapy have been shown promising efficacy and safety in treating various diseases. However, transplanted MSCs last only short-term which may require frequent repeated administration. One of the potential reasons behind low viability of MSCs upon transplantation is un-balanced oxygen tension between culture condition and biological tissues. For example, bone marrow or umbilical cord which are considered promising sources of MSCs were shown to have low oxygen tension. MSCs grown under hypoxia condition has been shown to have increased survival and potency upon transplantation. One of the key factors driving these positive effects of hypoxia conditioning in MSCs suggested is HIF1a and we hypothesized that increasing HIF1a level would increase the therapeutic potential of MSCs. In this study, we employed CRISPR/Cas9-based non-viral gene editing to enhance HIF1a level to increase the survival and potency in a mouse model of Alzheimer's disease.

Methods, Results & Conclusion CRISPR/Cas9 was designed to target genes involved in regulation of human *HIF1A* to enhance *HIF1A* level. Using Wharton's jelly-derived human MSCs, selected CRISPR/Cas9 components were treated in ribonucleoprotein complexes. Viability and growth factor release upon gene editing of different genes involved in HIF1a regulation were tested in the context of sub-lethal hypoxia and oxidative stress. Furthermore, survival and potency of MSCs upon gene editing were tested via intra-hippocampal injection into 5XFAD AD mouse model.

Several genes involved in HIF1a regulation were successfully edited without alterations in viability and tri-lineage differentiation potentials and particularly, we found that *HIF1AN* gene edited MSCs showed significantly increased viability under sub-lethal hypoxic and oxidative stress condition. *HIF1AN* gene edited MSCs also showed better survival upon transplantation into hippocampus of 5XFAD mice when compared to control MSCs. More importantly, *HIF1AN* gene edited MSC transplanted 5XFAD mice showed less Aβ burden when compared to control MSC transplanted 5XFAD mice.

We established a simple CRISPR/Cas9-based method to edit genome of human MSCs without compromising their physiology. Specifically, we mimicked hypoxia pre-conditioning of MSCs via targeted editing of *HIF1AN* which showed improved therapeutic potential of MSCs. Therefore, targeted gene editing of *HIF1AN* of MSCs prior to transplantation may hold promise to enhance the therapeutic potential of MSC-based therapy.

13

Fast and serious: comparative study of the impact of accelerated procedure designations on market access for advance therapy medicinal products

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Background & Aim Advance therapy medicinal products (ATMPs) represent promising therapy for many untreatable or life-threatening diseases. To guaranty rapid accessibility to these therapies, Health agencies from United State (FDA), Europa (EMA) and Japan (MHLW) have established accelerated procedures through, respectively, breakthrough (BT) or regenerative medicine advance therapies (RMAT), Priority medicines (PRIME) and *Sakigake* (ST) designations. These designations, granted on the base of preliminary data of safety and effectiveness in unmet or life-threatening indications, permit to