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BOOK OF ABSTRACTS

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Impact of endocrine disruptors on MODY3 iPSCs differentiation toward SC-islets – potential diabetogens

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Introduction: It is known that HNF1A-MODY patients exhibit variable penetrance of a diabetic phenotype. Unlike for T1D and T2D, an environmental factor is yet to be discussed in the development of a diabetic phenotype associated with a mutation in HNF1A. One potential environmental factor is chemical exposure, which are linked to progression and severity of a diabetic phenotype, especially for T2D. We therefor wanted to assess if iPSCs could be used as a model to examine the effect of environmental changes on development and function on pancreatic islet cells.

Materials and methods: To reach this goal we differentiated both non carrier (NC) and HNF1A-MODY iPSCs toward SC-islets following a seven-stage protocol. The SC-islets were exposed to a mix of endocrine disrupting chemicals (EDC)s for 14 days during differentiation from the endocrine precursor stage at a human relevant dose.

Results: Our HNF1A-MODY cell line has a reduced insulin-cell content and function, followed by an increase in glucagon cells. The SC-islets had higher percentage of glucagon cells and reduced glucose tolerance. Interestingly, pathway analysis showed that low dose exposure to EDC mix during development of SC-islets did alter cell viability and survival signals, as well as reduced protein production and oxidative stress responses. On the other hand, the exposure did not affect hormone producing cell fate acquisition, nor the glucose tolerance of the HNF1A-MODY DC-islets.

Conclusion: Our data suggest that low dose exposure of EDC mix is likely to have adverse effect on SC-islets function in the long run.

Optimization of a surface functionalization of 3D printed polycaprolactone scaffolds with nanohydroxyapatite particles for bone tissue engineering

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Introduction: Polycaprolactone (PCL), a biocompatible thermoplastic, is recognized as a promising material for the biofabrication of scaffolds for bone regeneration. This material's good mechanical properties and low melting point are strong assets to engineer defect/patient-specific bone scaffolds using 3D printing technologies [1]. However, among other limiting factors, its lack of osteoconductive properties results in limited bone regeneration and restricts its clinical applications [2]. Hydroxyapatite and in particular nano-hydroxyapatite (nHA), due to its high similarity with natural bone apatite and its high osteoconductive properties, has a high potential as a functionalizing agent [3].

As a result, to tackle the lack of osteoconductivity of 3D printed scaffolds made of Medical Grade (MedG) PCL, their surfaces were functionalized with a nHA particles coating. The present work aimed to optimize the functionalization method by evaluating parameters such as nHA re-suspension solution, nHA concentration, and sodium hydroxide (NaOH) etching.

Methods: Surface coating of 3D printed MedG PCL scaffolds was assessed by scanning electron microscopy (SEM). Furthermore, the deposition of nHA was assessed by Alizarin Red S staining. The size of nHA re-suspended in ethanol (100% v/v) was confirmed by dynamic light scattering (DLS).

Results and Conclusion: The performed work demonstrated the ability to functionalize 3D printed MedG PCL scaffolds with nHA particles. A stable nHA coating of scaffolds with appropriate coating conditions in ethanol (100% v/v) was reached at concentrations of 1mg/mL or 5 mg/mL, following NaOH etching of scaffolds.

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Limbal Stem Cell Deficiency in Rat Models: A Foundation for Therapeutic Studies

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Introduction: Limbal stem cell deficiency (LSCD) poses significant challenges due to impaired corneal wound healing and ingrowth of blood vessels, often leading to vision loss. Current treatment options are limited, necessitating urgent improvements. Cell-based therapies are emerging as promising treatment options.

Purpose: To establish two LSCD models in Lewis rats and assess the viability of subconjunctivally injected mesenchymal stem cells (MSCs) in the damaged cornea.

Method: LSCD was induced via alkali burn or intrastromal suture placement. Vascularization levels were assessed using CD31 antibody immunohistochemistry on flat-mounted corneas. MSCs were pre-stained with DAPI before injection, and their viability was evaluated.

Result: In the alkali burn model, significant vascularization around the entire limbus was observed three days post-injury. In the suture model, sectorial vascularization developed within five days. Subconjunctival injection of MSCs in the alkali burn model resulted in complete cell migration over the entire cornea within four days.

Conclusion: Two models of LSCD have been successfully established, alongside protocols for subconjunctival injection of MSCs. Post-injection, MSCs not only remain viable but also exhibit directed migration toward the site of corneal injury. These models will be used for further research on treating LSCD with MSCs.

Proteomic analysis of human gingival fibroblast secretomes from healthy and periodontitis patients

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Objectives: Gingival fibroblasts (GF) play an important role in periodontal health and disease. GF regulate the local microenvironment through paracrine mechanisms via the secretion of bioactive molecules and vesicles (secretomes). The objective of this study was to compare the proteomic profiles of GF-secretomes derived from of healthy and periodontitis patients.

Methods: GF were isolated from gingival biopsies of periodontitis patients during flap-surgery (n=6) and from healthy donor-controls (n=6). Conditioned media of passage 2-3 healthy (h-GFS) and periodontitis GF (p-GFS) were obtained following 48 h serum-free culture and subjected to label-free mass spectrometry and multiplex immunoassay. Bioinformatics was performed to determine global profiles, differentially expressed proteins (DEP), and gene ontology (GO) enrichment of biological processes (BP), molecular functions (MF) and cellular components (CC).

Results: Proteomic analysis revealed a total of 2164 proteins in h-GFS and 2153 in p-GFS; 1706 proteins were common-, while 111 (h-GFS) and 127 proteins (p-GFS) were exclusive. These included several growth factors, cytokines/chemokines, and extracellular-matrix proteins important for wound-healing. Quantitative analysis revealed 93 DEP in h-GFS. GO analysis revealed significant enrichment of several CC, MF, and BP in both groups; a majority of the enriched proteins were related to cell metabolism and function. Expression of selected proteins relevant for wound-healing was confirmed by a multiplex assay.

Conclusions: Global proteomic profiles of GF-secretomes from healthy and periodontitis tissues were largely similar, including proteins important for wound-healing. Given the relative ease of harvesting during surgery, GF from periodontitis patients may represent a promising source of secretomes for regenerative therapies.

Partial insulin-producing β-cell ablation induces compensatory proliferation in mouse pancreatic islets

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Introduction: Regeneration is the ability of organisms to repair and replace damaged or lost tissue. The murine pancreas exhibits unexpected plasticity, deploying a wide range of responses following insulin-secreting β-cell destruction. The regenerative program depends on type of insult, age at injury, and extent of β-cell loss. Briefly, two main regeneration mechanisms were described: transdifferentiation after total β-cell loss, and proliferation of surviving β-cells following extensive β-cell damage. Both occur naturally in response to diverse stressors but are limited and inefficient, especially in adults.

Methods and methods: We investigated the islet regeneration following partial β-cell ablation by utilizing two mouse models: a rapid transgenic model (RIP-DTR) and a slow chemical model (streptozotocin).

Results: Induction of a moderate yet non-metabolically disruptive β-cell loss in both models revealed a similar compensatory proliferative signature, albeit with varying timing of the proliferative response. The increased proliferative response was not sufficient to rescue the lost β-cell population in both models. Further exposure to a metabolic stressor, high fat diet, increased β-cell proliferation at the cost of β-cell function in our rapid ablation model. Yet the slower model failed to respond to high-fat diet in similar fashion, indicating a differential response to metabolic stress.

Conclusion: Overall, our findings highlight the complex interplay between β-cell loss and stress in regulating the innate regenerative capacity of the pancreatic islet. Understanding the processes underlying the β-cell regulation found in these models could help advance research towards harnessing the innate β-cell proliferative potential for an improved diabetes treatment.