Cell Transplantation and Tissue Engineering

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Cell Transplantation

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Research Area; Cell Transplantation
The rapidly emerging achievements in cell transplantation and stem cell-based treatments have the potential of providing new therapeutic options for several illnesses. As such, Islet transplantation represents a research model for other cellular treatments. The Research Group for Cell transplantation is part of the Nordic Network for Clinical Islet Transplantation (NNCIT) and works in close collaboration with Uppsala University. During the last years, extensive work has been carried out to refine established methods for islet isolation and to develop new strategies for cell-based engineering technology to improve the long-term outcome of islet transplantation as treatment for type 1 diabetes. The group has leaded the work with the aim to create a “Center for Cell Replacement in Diabetes” at Oslo University Hospital. The intention is to develop a multidisciplinary center composed of experts within basic research and clinicians with a unique and comprehensive knowledge and expertise in diabetes, cells and stem cells biology, pharmacokinetics and transplantation. The strength in this organization is that the people involved are a mix of full time researchers and active clinicians who quickly can transfer new knowledge from the laboratory into the clinic, which is mandatory in modern medical science.

Aims
The group’s overall aim is develop and improve islet isolation, engraftment and clinical outcome after islet transplantation for treatment of patients with diabetes.

Background
Type 1 diabetes (T1D) is an autoimmune disease caused by destruction of the alpha- and beta cells in the pancreas. Prevention of T1D is hampered by the fact that the actual mechanism of cell destruction is largely unknown. Standard of care in T1D is life-long therapy with exogenous insulin. In spite of optimal insulin therapy (including digital insulin pump devices and subcutaneous glucose sensors), periods of hyperglycemia induce serious vascular- and neurological complications, such as accelerated arteriosclerosis, kidney failure and impaired vision. There is currently no cure for diabetes, and exogenous insulin administration, either as injection or via a subcutaneous pump, is the selected treatment for most patients suffering from the disease. However, it is apparent from numerous clinical studies that insulin does not offer the effective glucose homeostasis required to prevent the deadly long-term complications of the disease, and better treatment options are highly warranted. As many as 25% of these patients have impaired awareness for hypoglycemia and some develop life threatening brittle diabetes, with complete unawareness (lack of symptoms) for severe hypoglycemia. The estimated prevalence of unawareness among T1D patients is 2-3%, i.e. 500-1000 patients in Norway suffer from this condition. These patients have constant concerns regarding their blood glucose levels with frequent day and night measurements, working disability and severe quality of life impairment. Hypoglycemia is a significant cause of cardiovascular events and death among persons with diabetes, and the mortality rate of brittle diabetes patients (dead-in-bed syndrome) is markedly increased compared to the normal population.

It is well documented that beta cell replacement therapy, either as whole organ pancreas transplantation or as islet transplantation may cure or stabilize T1D. More than 1000 patients worldwide have received islet transplantation since.
the breakthrough in 2000. Islet transplantation is highly successful in improving glycemic control and reducing life-threatening episodes of hypoglycemia,. Up to 80% of the patients are temporarily free from exogenous insulin after transplantation (most often with islets from multiple donors), but the long-term efficacy is too low and further refinement of the treatment is highly needed.

Current Research activity

Clinical islet transplantation
Islet transplantation is currently standard treatment of care in selected patients with T1D, refunded through the DRG system.

This year we established a GMP human cell processing facility for large-scale production of human islets to be used in clinical islet transplantation at the Department for Cellular Therapy (Radiumhospitalet) in close collaboration with Professor Gunnar Kvalheim (Figure 2).

Experimental Cell Transplantation

1. Investigate the intracellular immunosuppressive concentrations in human islets and the regulation thereof.

Following islet transplantation the recipients are in need of lifelong immunosuppression. Known diabetogenic side effects of immunosuppressive therapy are particularly deleterious in the situation of a reduced beta cell mass (like in islet transplantation), possibly contributing to the historically poor success rate of human islet allografts. The islets engrafted in the liver are exposed to relatively high concentrations of immunosuppressive drugs, even when systemic drug levels are carefully controlled for. The high liver concentrations may be toxic to the islets and could impair revascularization and proliferation, and are most likely not required to prevent acute transplant rejection. Studies to determine the right dosages and optimal combinations of available immunosuppressants for optimal islet survival are needed.
Together with the i2mc research group, headed by Prof. Stein Bergan, we are investigating the pharmacokinetics, pharmacodynamics and pharmacogenomics of the immunosuppressive drugs in human islets in vitro. The potential direct toxic (e.g. diabetogenic) effects of immunosuppressants on islets during engraftment may influence upon their survival and function. Protective mechanisms like efflux transporters (e.g. p-glycoprotein) and drug-metabolizing enzymes may counteract the damaging effects of immunosuppressants on the transplanted islets. Preliminary data from our group (in preparation for publication) indicate that there is significant expression of some of these transporters and enzymes in the islets, and also that the intra-islet concentrations of immunosuppressants are significantly influenced by concomitant drugs.

2. Adult stem cells promote islet survival
Mesenchymal stem cells (MSCs) are multipotent stromal cells found in many different types of tissue. The MSCs show immunomodulatory and regenerative properties. MSCs from bone marrow have been documented to reduce inflammation and promote islet survival both in vitro and in vivo. The use of intravenous administration of MSCs in animal experiments has also proven to have an anti-diabetic effect in T1D. It has been suggested that MSCs transdifferentiate into pancreatic islets. But recently it was reported that the most likely effect of MSCs in diabetes is due to their immune-modulatory effect, restoring Th1/Th2 balance and modifying the pancreatic microenvironment. Most of the work has been done on bone marrow derived MSCs. However, the frequency of MSCs in bone marrow is reported as low. Adipose tissue yield 100-500 times more cells than bone marrow, and generates less pain and morbidity for the donor. The stromal vascular fraction (SVF) can be cultured and expanded in vitro to adipose stem cells (mesenchymal cells). In a pre-clinical setting we will explore whether ex vivo expanded MSCs from fat and bone marrow and fresh ADRCs can improve islet transplantation. In addition, in vitro and in vivo co-culturing experiments will be established.

Experimental imaging of beta cells and MSCs
Non-invasively monitoring of changes in islet mass following islet transplantation is important to provide new insight into factors regulating post transplant function. For in vivo surveillance cells can be labeled with protein. In house, we have mouse strains that express a beta cell-specific reporter (luciferase) which allows for bioluminescence imaging of the beta cell mass (Figure 3). In a set of experiments we will combine GFP labeling of MSCs with luciferase positive beta cells for non-invasive imaging of the fate of the co-culture using IVIS Spectrum (already in use in our labs).

3. Develop a transplantation model to evaluate the potency of human islet grafts in vivo.
In collaboration with AstraZeneca AB research team and Uppsala University, we have established a double islet transplantation model to be able to investigate the impact of new compounds on the expansion of beta cell mass and improvement of function (Fig.4). Briefly, two islet transplantations are performed in the same animal. First, mouse islets are transplanted under the left kidney capsule to restore hyperglycemia, the second transplantation is performed after a recovery time of 2-4 weeks. A suboptimal dose of human islets are transplanted under the right kidney capsule and the grafts are allowed for a recovery period for up to 3 weeks, the first graft is removed by left sided nephrectomy which allows for followed islet function and possible proliferation in the second graft in response to different interventions in vivo. Exendin-4 has the potential to increase beta-cell mass either by stimulating proliferation or inhibiting apoptosis. We study the effects of Exendin-4 on metabolic parameters of aged human islets in vivo and report that Exendin-4 could play an important role in the first period after islet transplantation where normal levels of blood glucose are desirable to reduce glucose toxicity to the islets (manuscript in preparation).

![Figure 3. Bioluminescence in B6 Albino mice transplanted with synergistically MIP-luc islets under kidney capsule. Representative image of transplanted MIP-luc islets before (baseline) and day 3 and 7 after induction of diabetes with alloxan treatment (correspondent blood glucose levels shown below).](image-url)
4. Beta cell regeneration and stem cells
Expanding the beta cell mass would greatly improve opportunities for cell-based therapy in diabetes. The fact that the beta cell mass in a person is capable of expanding in response to insulin resistance, obesity, pregnancy and trauma is the background for our research focus on conversion of non-beta cells within the pancreas to beta-like cells. The adult pancreas consists of both exocrine and endocrine parts with different functions. The acinar, ductal and pancreatic progenitor cells of the exocrine pancreas have been suggested as source for conversion into beta-like cells or to prevent hyperglycemia in diabetic animals. In addition, it is believed that other cells belonging to the endocrine part of the pancreas can be transformed into beta-like cells, especially when the transformation occurs in the native microenvironment. Among the endocrine cells, the glucagon-producing alpha cells seem to be the potential source. In human T1D, random beta cells are found scattered around the pancreas. Whether this is a result of regenerating new beta cells in the adult pancreas or persistence of existing cells that have escaped from autoimmune reaction, is unknown. In this study we will investigate different approaches to induce human islets proliferation and survival, and explore the mechanisms involved by in vitro models of whole islets or dispersed islets (Fig.5).

5. Role of NLRP3 inflammasome in type 1 diabetes.
This project is performed in collaboration with Dr. Arne Yndestad and Dr. Trine Ranheim, Institute for Internal Medicine OUH. Recently publications indicate a role of the NLPR3 inflammasome in type 2 diabetes. The inflammasome is part of the innate immune system and by assembling the protein during activation promotes the maturation of the pro-inflammatory cytokines IL-1beta and IL-18. This project seeks to unravel the role of the NLRP3 inflammasome in islet transplantation. Pancreatic islets are susceptible to hypoxia-induced dysfunction. Central to the injurious effects of hypoxia in many cell types, is the involvement of inflammasomes like NLRP3. The NL-RP3-ASC inflammasome complex is involved in the activation of caspase-1, interleukin-1 (IL)-1 and IL-18 secretion, and has been implicated in type 2 diabetes. Whether hypoxia could activate NLRP3 in islets is not established. In this study, we aimed to investigate whether ablation of the NLRP3 complex (NLPR3-/- or ASC-/-) could alleviate the negative impact of hypoxia on islets. Our preliminary results showed that ablation of the NLRP3 inflammasome in islets significantly improves hypoxia-induced cell death, and reduces the mRNA expression of HIF-1 and VEGF. This suggests that the NLRP3 inflammasome conveys the deleterious effect of hypoxia in islets.

Scientific Collaborators Cell transplantation
- Professor Olle Korsgren, Uppsala University Hospital, Sweden
- Professor Peter Stock and Ass. Professor Qizhi Tang, University of California, San Francisco, USA
- Professor Gunnar Tufveson Uppsala University Hospital, Sweden
- Professor Peetra Magnusson, Uppsala University Hospital, Sweden
- Advanced Center for Translational REgenerative Medicine (ACTREM), Karolinska Institutet, Sweden
- Professor Tomas Totterman, Uppsala University
Background

Tissue Engineering combines research within cellular biology and cellular transplantation with material- and engineering science to develop biologic substitutes. The goal is to restore and maintain normal organ function that has been damaged due to disease, trauma, and cancer therapy or by other causes.

Tissue engineering is a multi-disciplinary field of research that has seen intense developments in recent years. It involves in vitro seeding and attachment of cells onto a scaffold. After proliferation and migration, reinforced by growth factors, cells differentiate into the specific tissue. Scaffold technology is also developing fast and is made up of synthetic and/or degradable biomaterials. Tissue engineering utilizes living cells as engineering materials.

Recently, there has been a great trend towards the use of adult mesenchymal stem cells from bone marrow or fat and iPSCs. These cells can differentiate into a variety of tissue types also including endocrine and nerves.

Tissue engineered tracheal replacement

For the first time, a patient, in 2011, was given a new trachea made by TE technique using a synthetic scaffold seeded with his own stem cells at Karolinska University Hospital in Huddinge, Stockholm, by Professor Paolo Macchiarini. Professor Macchiarini led an international team including colleagues from UK and USA who provided the tracheal scaffold and a specifically designed bioreactor used to seed the scaffold with the patient’s own stem cells. The TE-project group has an ongoing collaboration with Advanced Center for Translational Regenerative Medicine at the Karolinska Institute (ACTREM).

Organ and tissue bioengineering

Proof-of-principle studies of tissue engineered bioartificial heart, liver, pancreas, including kidney have been established.

Organization and research plan

At Oslo University Hospital the Division of Cancer Medicine, Surgery and Transplantation and Division of Cardiovascular and Pulmonary Diseases decided, in 2011, to initiate a joint project in tissue engineering with the aim to provide patients with treatment solutions based on regenerative medicine and bioengineering techniques. Professor Aksel Foss, Transplantation Surgery, accepted to lead the project. Initial phase of the project was estimated to last for 2-3 years and included establishment of the research group on tissue engineering and international network cooperation. Recently, all activities on regenerative medicine and tissue engineering at OUH and UiO were...
joined in Oslo Regenerative Medicine Initiative ORMI, (Figure 6) and the initiative has been selected as Focused Area of Research at OUH 2014-2018. A formal collaboration with Karolinska Institute has been established. A joint initiative is preparing an EU Horizon 2020 proposal (Program PHC 16 – 2015: Tools and technologies for advanced therapies on Bioengineered Human Kidney Transplantation (NEOrgan consortium) to advance cell- and organ-based technologies into human clinical bioengineered kidney transplantation.) The consortium partners consist of ORMI, Karolinska Institute, The Ottlab, Harvard Medical School and the industrial partners AstraZeneca - Center for CardioMetabolic Research and Regenerative Medicine -, Organ Recovery Systems and Corline Systems AB (participating SME). ORMI will be the Principle Investigator of the project.

Scientific Staff Tissue Engineering (Oslo Regenerative Medicine Initiative (ORMI)):

- Prof. Aksel Foss (leader) Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation
- Prof. Lars Engebretsen, Department of Orthopaedic Surgery, Division of Surgery and Clinical Neuroscience (Research area: Cartilage tissue engineering)
- Prof. Morten C. Moe, Center for Eye Research, Department of Ophthalmology, Division of Surgery and Clinical Neuroscience (Research area: Stem cells and tissue engineering in the treatment of ocular disorders)
- Prof. Joel C. Glover, Norwegian Center for Stem Cell Research, OUH/University of Oslo (Research area: Human somatic and pluripotent stem cell differentiation, characterization and genetic manipulation)
- Prof. Iver A. Langmoen, Department for Neurosurgery, Division of Surgery and Clinical Neuroscience. Stem cells in the adult human brain (Research area: Stem cells in brain cancer. Vilhelm Magnus Laboratory for Neurosurgical Research)
- Prof. Gunnar Kvalheim, Department of Cellular Therapy, Division of Cancer Medicine, Surgery and Transplantation (Research area: Adipose tissue stem cells in tissue repair)
- Dr. Kim A. Tønseth, Department of Plastic and Reconstructive Surgery, Division of Surgery and Clinical Neuroscience (Research area: Regenerative medicine in plastic surgery)
- Prof. Stefan Krauss, SFI-CAST Biomedical Innovation Center, University of Oslo (Research area: Chemical biology in regenerative medicine)
- Dr. Hanne Scholz, Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation (Research area: Cell replacement and tissue engineering)
- Dr. Jan E. Brinchman, Norwegian Center for Stem Cell Research and Department of Immunology (Ex vivo), Division of Diagnostics and Intervention (Research area: Cellular therapy)
- Prof. Jørgen J. Jørgensen Department of Vascular Diseases (Oslo Vascular Centre), Division of Cardiovascular and Pulmonary Diseases (Research area: Tissue-engineered allogenic vein valves in treatment of chronic venous insufficiency)
- Prof. S. Petter Lyngstadaas, Faculty of Dentistry, University of Oslo (Research area: Scaffold technology and biomaterials)
- Dr. Einar Martin Andahl, Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation and the Biotechnology Center of Oslo (Research area: Transplantation surgery, experimental immunology and tissue engineering)

Work Packages (WP) (short version) timeframe 2014-2020

WP1: Kidney decellularization/recellularization
WP1a: Decellularization of kidney scaffold
WP1b: Repopulation with endothelial and epithelial cells matured to functional kidney constructs in vitro for subsequent transplantation
- Small and large animal models
- Semi-xeno models (pig kidney architecture – human recellularization)
WP2: Stem cell technologies
   WP2a: Improving culture of stem cells for directed differentiation and up-scaling
   WP2b: Integrating novel mRNA technologies into regenerative medicine
   WP2c: Cell culture (differentiation, expansion and up-scaling) technologies (nephrospheres, kidney organoids)

WP3: In situ clinical stem cell kidney repair
WP4: Transplantation of human bioengineered kidney
WP5: Regulatory compliance, administration of the consortium, training, exchange of personnel.

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- Prof. Harald Ott, The Ottlab Massachusetts General Hospital, Harvard Medical School, Boston, USA
- AstraZeneca (Center for Cardio Metabolic Research and Regenerative Medicine)
- Organ Recovery Systems
- Corline Systems AB (participating SME)