TISSUE AND ORGAN ENGINEERING IN T1D, ANTONIO CITRO

State-of-art and future development/perspectives of the research area at international level (max 2000 characters):

Tissue and organ engineering program at the DRI has the ambition to develop innovative technologies to tackle two major international needs:
i) the beta cell replacement strategy
ii) the generation of ex vivo 3D platform for studying vascularized islet biology in both health and disease.
As of now, the shortage of organ donors, an unsatisfactory tissue engraftment, the immune reaction against the graft and the lack of an ex vivo model to recapitulate the vascularized endocrine pancreas are the major limitations in both fields. To overcome these limitations the urgent needs are the identification of:
- innovative technologies to allow endocrine engraftment ex vivo to recreate the endocrine vascularized niche
- unlimited source of insulin-producing cells (i.e. neonatal porcine islet cell cluster (NPIs) or iPSC derived beta cells (iβ)) able to escape the immune recognition.
At the international level, two main technologies have been identified to recapitulate the endocrine vascularized niche: a) organ decell/recellularization and b) 3D-bioprinting technology. In both cases, worldwide groups are customizing their technologies to match the minimum requirement for clinical translation. At the DRI, we have successfully developed a Vascularized islet Organ (VIO) based on the combination of native organ ECM (lung) repopulated with endothelial and endocrine cells. VIO is now used as a template to foster the knowledge i) in the generation of biological scaffold for the beta cell replacement, ii) in the use of alternative sources of beta cells (i.e. NPIs and iβ) with biological scaffolds and iii) in native organ engineering to translate what learned in the 3D bioprinting technologies. In all the ongoing projects, we are selecting the best product combination (ECM and cells) to match a future clinical translation. This will place the Tissue and organ engineering program in strong competition within the international research.

Actual lines of research (as is) of the Diabetes Research Institute (max 2000 characters):

The Pancreas Bioengineering team has ongoing different lines of research aimed to generate Vascularized Endocrine Constructs for T1D based on: i) decellularized lung organ (rat and pig) architecture (GR-2018 and JDRF), ii) ECM gel from decellularized human placenta (Vanguard EU-H2020 - Prof. Piemonti) and iii) pancreas ECM ink for 3D bioprinting technology (FID).
Those lines aim to engineer ex vivo the native endocrine niche based on the assembly of different endocrine cells (i.e. NPIs, iβ and human islets) within the vascularized native organ ECM environment.
In order to achieve those aims we will use: i) organ decellularization to obtain native organs ECM, ii) different sources of human patient endothelial cells (Blood Outgrowing Endothelial cells – BOEC or iPSC derived endothelial cells) and iii) different sources of endocrine cells WT (human islets, iβ and NPIs) or genetically modified (GM) (i.e. NPIs) able to escape the immune recognition (iβ in collaboration with Dr. Sordi - stem cell research area; human islet in collaboration with Dr. Nano - Human islet research area).
The generated constructs will be used i) as a beta cell replacement strategy in preclinical small (mouse - short term goal) and large (pig-long term goal) diabetes animal models and ii) as a tool of endocrine niche ex vivo to study in real-time treatments efficacy, metabolic, endothelial and immunological features in endocrine disorders.

Strengths of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

i) Novelty of the engineering process based on multiple competitive approaches: organ engineering and 3D bioprinting technology.
ii) Generation of strategic devices to answer multiple questions: beta cell replacement and ex vivo 3D platform for studying islet biology in both health and disease.
iii) The generation of possible products for different areas out of the diabetes area (i.e. bio-inks from different organs to adopt 3D bioprinting approach in different diseases)
iv) Preclinical models for beta cell replacement (i.e. intrahepatic, kidney capsule, deviceless and subcutaneous islet implantation
v) Connection with international/national teams to achieve different goals: pig organs (for large organ decellularization) and NPIs (WT and GM) for engraftment and immune escape purpose, ECM functionalization, endothelial isolation, 3D-bioprinting technology
vi) Strong experience in organ engineering (decellularization machine) and bioreactor system (customization/assembly)
vii) Starting a program in 3D bio-printing: i) future partnership with an international company leader in 3D bioprinting technology and expert in beta cell replacement and ii) connection with national chemical biologist and bioengineer to develop dedicated ink for 3D bio-printing machine
viii) Multi-disciplinary team based on chemical biologists, biotechnologists and bio-engineers
ix) Bio-DRI – the DRI biobank is a strong value for the DRI Tissue and Organ engineering program due to the possibility to collect samples related to current/future projects
x) the DRI Pancreatic Islet Process Facility (PIP) is a strong value for the DRI Tissue and Organ engineering program. Active collaboration for the use of human isolated islets (GR2018 and Vanguard h2020).
xi) active collaboration with DRI-stem cell research area (Dr. Sordi) for the use of in house iβ for the beta cell replacement area of the project.
xii) The DRI tissue and organ engineering program is well recognized by IPITA, EPITA and SID scientific community.

Weaknesses of the research area (as is) of the Diabetes Research Institute (max 2000 characters):

The tissue and organ engineering program require a step forward in different areas in order to meet the international requirements for future product clinical translation.

Scientific Weakness:
i) Potential issues driven by the generation of a device based on different components (ECM and cells) that will require an independent GMP validation
ii) Modulation of the cell/device implantation. The project will need a strong collaboration with immunologists
iii) Lack of strong visibility/connections in the bio-engineering scientific community (i.e. Termis congress)

Road to clinical application weakness:

i) Clinical area - limited connections with clinical side for sample collection and active discussion to translate different strategies in future application
ii) Lack of GMP facility to generate biological samples in respect of the FDA/AIFA rules for clinical translation (i.e. iPSC derived products, endothelial cells, decellularized product etc..)
iii) Lack of connections with national/international companies that have experience in scaffold large scale production and GMP factory for scaffold/device production

Administrative weakness: Lack of “Facilitators”: lack of an administrative network embedded with EU/US entities to discuss clinical translation requirements for different ATMP’s beta cell replacement products

Short-medium term OSR/UniSR goals (0-18 months): milestones and deliverables (max 1000 characters):

M1: validation of the best vascularized endocrine construct with both organ engineering and 3D bioprinting technologies
D1: define the best 3D bio-printed scaffold architecture compared to native organ structure.
D2: set up a protocol for production of decellularized porcine lung scaffold
D3: define the best endocrine and endothelial cell combination for vascular and endocrine function
M2: demonstration of scale-up ability: from preclinical to clinical application.
D1: scale-up bioreactor for large organ scaffold culture ex vivo
D2: define the therapeutic endocrine mass to treat human T1D
M3: identification of the best platform in modeling the vascularized islet biology in health and disease in respect of M1 results.
D1: study the ability of the scaffold in mimicking leukocytes cell trafficking/reaction ex vivo

Medium term OSR/UniSR goals (18-36 months): milestones and deliverables (max 1000 characters):

M1: Evaluation of the selected scaffold ex vivo immune escape ability in the presence of GM-stealth endocrine cells and autologous endothelial cells
D1: assessment of the scaffold ability in escaping the activated immune reaction in the presence of stealth endocrine/endothelial cells ex vivo
M2: Demonstration of the selected scaffold in vivo function in immunodeficient diabetic mouse model
D1: study the scaffold engraftment and glycemia correction
M3: Demonstration of the selected scaffold in vivo immune escape ability in humanized mouse model
D1: validation of scaffold engraftment and immune escape ability

Long term OSR/UniSR goals (36-60 months): milestones and deliverables (max 1000 characters):

M1: demonstration of selected scaffold function in immune-competent diabetic large animal model (pig)
D1: define the scaffold engraftment and function
M2: validation of the selected scaffold based on regulatory requirements (safety and potency) for future phase 1 clinical trial
D1: pre-clinically validated scaffold ATMP for clinical studies

Investments of the Diabetes Research Institute (e.g. personnel, space, technology) to achieve the short-medium-long term goals (max 2000 characters):

The standard strategic activity in the next future will be based on i) large (pig) and small (rat) organ model surgery/handling, ii) large (pig) and small (rat) organ decellularization in scaled automatized decellularization machine, iii) large (pig) and small (rat) organ culture in scaled bioreactor systems and iv) the 3D-printing program activation with the acquisition of dedicated 3d-bioprinters (likely 2).
To meet the Tissue and organ engineering program needs, the team require:
- a lab equipped with 5 benches: 3 for organ small/large organ automatized decellularization, bioreactor assembly/testing and 2 for 3D-Bioprinting purpose.
- 2 postdocs (1 already available), 2 PhD students (2 already available), 2 fellow (1 available) and 1 technician dedicated to preclinical experimental duties (set up relevant preclinical models (i.e. humanized mice, NOD mice etc..), daily mouse handling, mouse islet isolation/transplantation, surgery, follow up and sampling).
- Proportional office spaces, equipped with pc, compared to the team expansion
- Administrative facilitators: Grant manager, Administrative for clinical ATMP translation
- A DRI agreement with histological OSR service to accelerate the project's evolution or, alternatively, the generation of an equipped histological DRI area
Technology: a DRI dedicated Confocal/IF microscope