Conclusion: Cartilage or chondrocytes from transgenic pigs might be a promising tissue/cell source to overcome the lack of effective therapies for cartilage repair, because cartilage from TG and GGTA1KO/TG animals presented normal histomorphologic features and was protected from early humoral rejection. Our results indicate tissue-specific features of transgene expression. Nevertheless, in our approach, CD55 and CD59 expression reduced complement activation, even in presence of a-Gal. Further studies will focus on incorporated transgenes A20 and HO-1, their biological activity in cartilage context and role in cellular rejection.

330.13 (and poster P.211) | A novel ex vivo differentiation technique using pig bone marrow for production of human platelet-like cells

Shingo Fujiyama[1]; Nobuyasu Hori[1]; Toshiyuki Sato[1]; Shin Enosawa[2]; Mitsuru Murata[3]; Eiji Kobayashi[4]

[1]Central Research Laboratory, Sysmex Corporation, Hyogo, Japan; [2]Division of Advanced Medical Sciences, National Center for Child Health and Development, Tokyo, Japan; [3]Laboratory Medicine, School of Medicine Keio University, Tokyo, Japan; [4]Organ Fabrication, School of Medicine Keio University, Tokyo, Japan

Background: Platelet transfusion is required for the treatment of various conditions such as cancer, thrombocytopenia, and platelet dysfunction. However, there will be donor shortage in the future, as it is donor-dependent. Therefore, many in vitro platelet production methods are being developed to create a new source of platelets for transfusion. Nevertheless, efficiency of in vitro platelet production is low compared with that of in vivo, since some key factors are being lacked for platelet production. We developed the ex vivo platelet production system in which promote platelet production by using pig biological environment. The purpose of this study is to evaluate the efficiency of platelet production using the ex vivo system as compared to in vivo and in vitro systems.

Methods: Human umbilical cord blood-derived $CD34^+$ hematopoietic stem cells were differentiated into megakaryocytes and used for platelet production. The megakaryocytes were transferred into the thighbone harvested from livestock pig and then produced platelets for 3 h. In the in vivo system, the megakaryocytes were transferred into thighbone of anesthetized pig and produced platelets for 3 h. As a control, platelets were produced in the in vitro differentiation culture system. The number of produced platelets by ex vivo and in vivo system were counted by immunostaining of bone marrow tissue sections, and the number of platelets produced by in vitro system was measured by flow cytometry. In addition, platelets produced by ex vivo system was collected by perfusion and function assessed by flow cytometry.

Results: 4.41×10^3, 3.79×10^3 and 1.24×10^2 of CD61+ platelet-like cells were produced from 1×10^3 megakaryocytes by ex vivo, in vivo and in vitro systems, respectively. The produced platelet-like cells by ex vivo and in vitro expressed CD62P functional molecule stimulated by thrombin. Therefore, efficiency of ex vivo $CD61^+$ platelet-like cells production was comparable to in vivo production method, and superior to that in in vitro production method.

Conclusion: These results show that the ex vivo production method using xenogeneic bone marrow can be a novel technique for mass production of platelet-like cells.

330.14 (and poster P.203) | Validation of safety and function of a new macroencapsulation device in small and large animal models

Jordan Magisson[1]; Aladin Sassi[1]; Aram Kobalyan[1]; Charles-Thibault Burcez[1]; Kfir Molakandov[2]; Alon M. Levy[2]; Pierre Gianello[3]; Richard Bouaoun[1]; Séverine Sigrist[1]

[1]Defymed, Strasbourg, France; [2]Kadimastem Ltd, Ness-Zionna, Israel; [3]Laboratory of Experimental Surgery, Université Catholique de Louvain, CHEX 5570, Brussels, Belgium

The number of donors required per recipient and the associated immunosuppressive regimen are the major limitations, of human pancreatic islet transplantation. Therefore, an alternative inexhaustible source of insulin-secreting cells is required, in order to allow this therapeutic approach for a large number of patients. Among others, pig islets and human stem cells (hSC) are being currently investigated. Regarding the immunosuppressive regimen, parallel efforts are put together on the development of a bioartificial pancreas that can encapsulate such cells. The concept of this medical device is to immune-isolate the encapsulated cells, whilst protecting the host from these cells. MailPan® is a bioartificial pancreas developed to treat type-1 diabetic patients. This macro-encapsulation device is designed to contain insulin-secreting cells of different origins (animal or human), based on two main features: (i) the use of semi-permeable membranes allowing the passage of glucose and insulin, but impermeable to the components of the immune system; (ii) "In" and "Out" implantable ports in order to allow prevacularization of the MailPan®, and its filling or emptying whenever the cells become exhausted.

To validate the immune-protection of the MailPan® and its biofunctionality, several animal models were used: (i) allogenic models where rat insulin secreting cells were injected in rats within the MailPan® and (ii) a xenogeneic model where human beta cells differentiated from human embryonic stem cells were injected in rats in a MailPan®. Moreover, biointegration of the MailPan® was assessed in pigs and primates, for up to 4 and 8 months of implantation, respectively.

Injection of allogeneic rat islets in the MailPan® did not result in a significant increase of a global inflammatory marker (plasmatic α2-Macroglobulin), and no antibody formation against encapsulated islets was detected after 1 month in the serum of recipients. In diabetic rats, MailPan® device filled with rat beta cells was able to normalize fasting glycaemia, and restore body weight gain. Moreover, MailPan® devices explanted from pigs and primates showed a very satisfying biointegration, as reflected by low or

no fibrosis and cell infiltration, with an optimal vascularization of surrounding tissues. Finally, human embryonic stem cells differentiated into insulin secreting cells rapidly displayed functionality, starting 6 days following injection in MailPan® implanted in rats, with a stimulation index ≥2, for more than 8 weeks without any sign of rejection.

This study demonstrated the compatibility of the MailPan® with several cell types in allogenic and xenogenic models with a prolonged survival and function of the cells. Further studies are currently ongoing to test the long-term efficacy of the combination.

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RAPID-FIRE ABSTRACTS SESSION 331 ON GENETIC ENGINEERING OF THE DONOR ANIMAL, TESTING THE FUNCTIONALITY OF GENETIC MODIFICATIONS AND SAFETY

331.1 (and poster P.230) | Progress of multi-genetically modified minipigs for xenotransplantation

Dengke Pan[1]; Xiangyang Xing[2]; Jiaxiang Du[2]; Minjie Du[2]; Qipin Xu[2]; Hongwei Luo[2]; Huibin Zhu[2]; Shaoping Deng[1]

[1]Organ Transplant and Clinical Immunology Translational Medicine Key Laboratory of Sichuan Province, Sichuan Academy of an Transplant Science & Sichuan Provincial People's Hospital, Chengdu, People's Republic of China; [2]Chengdu Clonorgan Biotechnology, Co., Ltd, Chengdu, People's Republic of China

Introduction: Due to the high similarities with humans, such as the anatomical and physiological characteristics, genetically modified pigs, especial of minipigs, have been considered as one of the potential donor animal sources for xenotransplantation. However, some problems, such as immunological rejection, including hyperacute rejection (HAR) and acute humoral xenograft rejection (AHXR), or coagulation dysfunction should be systematically smoothed before using genetically modified minipigs in xenotransplantation. Herein, our researches of multi-genetically modified minipigs were presented.

Methods: As a model, Chinese Bama minipigs were sequentially gene-modified as following. First, to prevent the antigen-antibody response between pigs and humans, gene editing technology was employed to knockout some key porcine genes, concerning the expression of sugar residues and antigens, naturally existing in humans, such as GGTA1, CMAH, and β4GalNT2. Next, human-complement and coagulation-control transgenes, such as hCD46, hCD55 and human thrombomodulin (hTBM), were inserted to deal with further humoral immune response and coagulation dysfunction. Moreover, regulatory factor expression of immune responses could inhibit the adaptive immune rejection.

Results: We have successfully set up an efficient strategy of genetically modified minipigs for xenotransplantation. Firstly, the highly-efficient method, combining TALEN mRNA and magnetic beads with somatic cell nuclear transfer (SCNT), was exploited to obtain GGTA1 knockout (GTKO) pigs. Secondly, on the basis of the GGTA1 knockout, considering the CMAH and β4GalNT2 antigens could cause post-αGal-related rejection, GGTA1/CMAH and GGTA1/βGalNT2 knockout pigs were further generated via CRISPR/Cas9. Thirdly, the engineered hCD46 minigene was reconstructed to generate transgenic minipigs with a high-level expression of hCD46. Fourthly, hCD55 cDNA was inserted into the porcine Rosa26 (pRosa26) locus via CRISPR/Cas9 to establish a new cell line, expressing hCD55 on the basis of a GGTA1KO minipig's ear fibroblast line, resulting in GGTA1KO/hCD55 pigs. Fifthly, considering the expression of exogenous hTBM in pig vascular endothelial cells could be helpful for overcoming the molecular incompatibilities, GGTA1KO/hCD46 minipigs were further developed to express hTBM cDNA under porcine TBM promoter. Sixthly, based on the normal reproductive capacity, observed in the above genetically modified minipigs, the cross-breeding technique was farther established between them.

Conclusion: A variety of genetically modified pigs, including GTKO, GTKO/hCD46, GTKO/hCD46/hTBM, GTKO/β4GalNT2KO, GTKO/CMAHKO and GTKO/hCD55, have been developed by SCNT. Furthermore, the cross-breeding technique could be used to obtain the multi-genetically modified donor pigs as expect for xenotransplantation.

331.2 (and poster P.205) | Localization of hCD47 expression in kidney grafts plays a key role for the development of post kidney xenotransplant proteinuria and systemic inflammatory responses through the CD47/SIRPα and/or CD47/TSP-1 pathways

Yuichi Ariyoshi[1]; Kazuhiro Takeuchi[1]; Thomas Pomposelli[1]; Lennan Boyd[1]; Ermance Estime[1]; Dilrukshi Ekanayake-Alper[1]; Hisashi Sahara[1]; Scott J. Arn[1]; Robert Hawley[1]; David Ayares[2]; Marc Lorber[3]; Megan Sykes[1]; David Sachs[1]; Richard Johnson[4]; Kazuhiko Yamada[1]

[1]Columbia Center for Translational Immunology, Columbia University Medical Center, New York, NY, United States; [2]Revivicor Inc, Blacksburg, VA, United States; [3]Lung BioTechnology PBC, Silver Spring, MD, United States; [4]Renal Diseases and Hypertension, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

Introduction: We have recently reported that both CD80 upregulation and the degradation of SMPDL-3b on glomerular podocytes are involved in the development of post kidney xenotransplant (XKTx) proteinuria in a pig-to-baboon model. In this study, we assessed (1) if the incompatibility of porcine CD47 and baboon macrophages (CD47/SIRPα pathway) was involved in the development of post XKTx proteinuria, and (2) if transgenic (Tg) expression of human CD47 (hCD47) caused negative effects via a CD47/TSP-1 pathway following pig-to-baboon K+VT Tx.