Ellegaard
GÖTTINGEN MINIPIGS A/S

Newsletter 35
SPRING 2011

Clean pigs for clear results

DEAR READER,

The year 2011 is just a few months old and while I am looking forward with great anticipation to the rest of the year I also would like to highlight some of our achievements in 2010.

In 2010, we experienced increasing interest from the pharmaceutical industry, which has laid the groundwork for new and strong collaborations. New companies have been added to the family of enthusiastic minipig technicians and scientists, and the collaboration with our existing customers has been strengthened. We value our customers highly and wish to establish and maintain close partnerships, because we can all benefit from one another's knowledge. We are always available for guidance and whatever support our customers may need, and we find it very interesting when our customers wish to share their thoughts and experiences with us. The market keeps changing and, as well as we can support our customers with our knowledge and experience, we also find ourselves in an infinite learning process.
Several of our customers and other contacts send us articles to be included in our newsletter, which we greatly appreciate because it enables us to let minipig users share experiences with one another.

The tendencies from 2010 and the first few months of 2011 have been very positive and we look forward to the rest of the year with great expectations. In 2010, the Rethink report was published as a special issue of Journal of Pharmacological and Toxicological Methods (Volume 62, Issue 3). The Rethink report confirms what we have always known: that the Minipig is an excellent non-rodent model for non-clinical safety testing. The publishing of the Rethink report has brought the Göttingen Minipig into focus throughout Europe and in many other parts of the world.

The global demand for Göttingen Minipigs has been increasing in recent years. To meet this demand, we have signed an agreement with Oriental Yeast Co., Ltd, to be the exclusive breeder and distributor of Göttingen Minipigs in Japan. We are very pleased with this agreement and look forward to continuing our rewarding collaboration with Oriental Yeast Co., Ltd. Marshall BioResources is doing a great job breeding and selling Göttingen Minipigs to the North American market. We appreciate our close partnership with both companies.

Health, quality and animal welfare are some of our top priorities, and we always strive to create the best possible environment and conditions for the minipigs. Our facilities have been AAALAC accredited since 1998 and, in late 2010, our facilities were reaccredited after being reviewed by two AAALAC visitors.

2010 was also the year where His Royal Highness the Prince Consort visited Ellegaard Göttingen Minipigs. During their annual summer tour around Denmark, Her Majesty Queen Margrethe II of Denmark and His Royal Highness the Prince Consort visited our area and, as a part of their programme, a visit to Ellegaard was scheduled for the Prince Consort. Here the Prince Consort saw our minipigs through the viewing windows and our production process was explained to him. He was genuinely interested in our minipigs and their welfare. The visit by His Royal Highness the Prince Consort was a great experience for us at Ellegaard and a strong recognition of our company and our employees for their dedicated work.

CONCLUSIONS
from the
Rethink report!

Oriental Yeast Co., Ltd – new exclusive breeder of Göttingen Minipigs in Japan

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For Göttingen Minipigs in Japan please contact Oriental Yeast Co., Ltd. - fbi@oyc.co.jp

In our newsletter last autumn, we focused on the use of the Göttingen Minipig in Cardiovascular Safety Pharmacology. Subsequently, we have received more articles on this topic which we have chosen to print in this newsletter. Our veterinarian has been involved in the project behind the article on wireless monitoring for animal wellbeing. We highly value our project partnerships and collaborations, so that we can investigate how to refine and continuously improve the conditions and procedures for minipigs and the people working with them. In this newsletter, our laboratory technician Adrian Zeltner also shares his experiences with implanting a Central Venous Catheter (CVC). Knowledge and documentation are the codes for success in the biomedical industry, and we are constantly seeking opportunities to create and collect minipig data and information that everyone can benefit from. Please contact us if you need information or if we can be of assistance in any way.

Sincerely,
Jens Ellegaard
CEO, Ellegaard Göttingen Minipigs A/S

Oriental Yeast Co., Ltd - exclusive breeder of Göttingen Minipigs in Japan

In December 2010 Ellegaard and Oriental Yeast Co., Ltd (OYC) part of the Nisshin Seifun Group of Companies signed a license agreement granting OYC the exclusive rights to breed and distribute Ellegaard Göttingen Minipigs in Japan. "In OYC we have found a license partner who is very well established and recognized in the Japanese market", says Jens Ellegaard, CEO – and he continues: "During the process of getting to know each other OYC has been visiting us and we have been visiting OYC. At these meetings it has become obvious that both companies share the right company culture and values needed for a long standing business relationship. We have felt a strong enthusiasm from OYC and its dedicated employees to learn all about our Göttingen Minipigs and our way of selling them. We clearly feel that OYC is committed to establishing the market for Göttingen Minipigs in Japan as quickly as possible and we highly appreciate this".

Currently, the Japanese market for Göttingen Minipigs is supported by export from Denmark to Japan. Meanwhile a barrier breeding facility for Göttingen Minipigs is being built by OYC. When it is completed, a breeding colony will be exported from Denmark. Breeding – still under the supervision of Göttingen University to ensure global uniformity – will then take place in Japan for the Japanese customers.

"This agreement with OYC is one step further towards globalisation of the Göttingen Minipig, and we welcome OYC in the exclusive group of Göttingen Minipig breeders. We are looking very much forward to continuing our good collaboration with OYC", concludes Jens Ellegaard.

HIS ROYAL HIGHNESS THE PRINCE CONSORT AT ELLEGAARD GÖTTINGEN MINIPIGS

During Her Majesty Queen Margrethe II of Denmark and His Royal Highness the Prince Consort's annual summer tour around Denmark, the Royal Yacht Dannebrog visited our area and as a part of their programme a visit for the Prince Consort was scheduled at Ellegaard. Here we proudly showed him our minipigs through the viewing windows and our production facilities. He showed a clear interest in our minipigs and their welfare. It was interesting for us all to meet the Prince Consort and a great recognition to our team and company.

His Royal Highness the Prince Consort observes our Göttingen Minipigs through the viewing windows.

We showed His Royal Highness the Prince Consort our facilities and explained how we develop, breed, house and transport the Minipigs. He asked several questions during the tour around our facilities

Macro- and micromorphometric studies of the vascular structures from the Göttingen Minipig

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3: Charité-University Medicine Berlin, Department of Radiology, Berlin, Germany

ABSTRACT

Porcine models have become increasingly popular in cardiovascular research, because physiological and anatomical features of the cardiovascular system from pigs including the coronary vascular system and the coronary collateral vessels are comparable to humans. The standard farm pig rapidly increases in body weight and size, potentially confounding serial measurements of cardiac function and morphology. In contrast Göttingen Minipigs have a characteristic growth curve that avoids the dramatic increase in weight in adulthood seen in farm pigs. The Göttingen Minipig is especially suitable for long-term studies because of its inherent small size and ease of handling, even at full maturity, which is reached at 2 y of age compared with 3 y for domestic pigs. However, there is still a need on detailed information about the macro- and microvascular characteristics of the vascular system from the Göttingen Minipigs.

The study was aimed to describe the macro- and microvascular characteristics of adult Göttingen Minipigs (n=18) by use of CT-imaging and histology over a time period of 4 month starting from 16 month of age up to 20 months. The animals showed no clinical symptoms of disease and were kept in-house at a light/dark rhythm of 12:12 under defined climatic conditions. The experiments were licensed by the regional authorities for health and social affairs (LaGeSo), Berlin. The study included the measurement of the length and of the luminal diameter of arteries and veins from the neck, thorax, abdomen, and limbs which are frequently used in experiments with pigs. In addition microscopical and histological parameters (luminal vessel diameter, thickness of the tunica externa, tunica media, and tunica interna) were studied on hematoxylin-eosin-stained sections of the blood vessel.

Key words: minipigs, vasculature, vessel wall

INTRODUCTION

Porcine models have become increasingly popular in cardiovascular research, because physiological and anatomical features of the cardiovascular system of pigs (including the coronary vascular system and the coronary collateral vessels) are quite comparable to humans [1, 2, 3]. The standard farm pig rapidly increases in body weight and size, potentially confounding serial measurements of cardiac function and morphology. In contrast, Göttingen Minipigs have a unique growth characteristic that avoids the dramatic weight and size increase seen in farm pigs even when they reach the adulthood [4, 5]. The Göttingen Minipig was developed in the early 1960s at the Institute of Animal Breeding and Genetics (University of Göttingen, Germany) to reduce space requirements and housing costs for preclinical porcine studies [6]. They were created by crossbreeding the Minnesota Minipig with Vietnamese potbelly and German Landrace pigs [7]. The Göttingen Minipig is especially suitable for long-term studies because of its easy handling and inherent small size even at its full maturity, which is reached in 2 years while it takes 3 years for domestic pigs [8]. However, more detailed information about the macro- and microvascular characteristics of the Göttingen Minipigs has to be elucidated to prove its scientific usefulness.

MATERIALS and METHODS

Animals, housing and care

In-vivo experiments approved by the regional office for health and social welfare (LaGeSo) of Berlin were performed at the Charité University Clinic, Campus Virchow (Berlin, Germany), Department of Experimental Medicine (certified by ISO 9001) using adult female pigs (n=18) from the Göttingen Minipig (Ellegaard, Denmark). At the beginning of the test the animals were 17 months old and had a body weight of 26.3 ± 1.8 kg. The animals were cared according to the guidelines of the European societies of laboratory animal sciences. Animals were housed as a group of 6, in an environmentally controlled room (12/12 light/dark-rhythm, 15-24 °C, 55±10 % relative humidity).

Anaesthesia

Prior to CT-Imaging and tissue sampling anaesthesia was performed. One night before anaesthesia the animals were fastened, but had free access to water. The minipigs were premedicated by intramuscular (i.m.) injection of 0.5 ml atropine (Atropinum sulfuricum, 1mg/ml, Eifelfango,

Germany) and anesthetized by i.m. injection of ketamine (i.m., 27 mg/kg, UrsotaminTM, 100 mg/ml, Serumwerk Bernberg, Germany), xylazine (i.m., 3.5 mg/kg, RompunTM TS, 20 mg/ml, Bayer Vital, Germany) and 3 ml azaperone (i.m., Stresnil, 40 mg/ml, Janssen Animal Health, Germany). Additionally an electrolyte solution (Ionosteril, Fresenius, Germany, 1000 ml) was continuously infused intravenously (i.v.).

Computed tomography

The vascular structures of the pigs were studied by computed tomography (CT) analysis at an age of 17, 19 and 21 month according to table 1. The CT scan was done in prone position on a 64-slice scanner (LightSpeed 64 ; GE Medical Systems, Milwaukee, IL, USA). The scan protocol used contrast-enhancement with automatic intravenous injection of 80 ml nonionic iodinated contrast medium (XenetiX 350, Guerbet GmbH, Germany 350 mg/ml iodine) in each pig. The scan parameters were standardized (voltage 120 kV, maximal 500 mA with automatic mA-optimization at a noise index of 15, mean mA 490; collimated slice thickness: 64×0.625 mm; total detector width: 55 mm; rotation speed: 0.4 s; table feed per rotation: 55 mm) so the scan speed was approximately 3 s for 30 cm scan length in the z-axis. For volumetric assessment 1.25 mm images were reconstructed without overlapping. Basic image analysis was performed using Advantage Windows 4.2 (GE Medical Systems, Milwaukee, IL, USA) and AccuLite (AccuImage Diagnostics Corporation, South San Francisco, CA, USA).

Histology

Histological examination was done on 5 representative arteries (elastic arteries: Aorta thoracica, Aorta abdominalis, Arteria carotis communis; muscular arteries: A. femoralis, A. renalis) and 5 representative veins (Vena cava cranialis, V. cava caudalis, V. jugularis externa, V. renalis, V. femoralis). After embedding into paraffin, cross sections of these blood vessels with a thickness of 5 µm were made and stained using hematoxylin-eosin. Five slides per sample were prepared and each slide was evaluated at five different fields of view. The thickness of the tunica media and the thickness of the lamina endothelialis were determined using a transmitted light microscope (AxioSkope 2, Zeiss, Germany) and the imaging software AxioVision (Zeiss, Germany).

Statistics

Data are reported as mean value ± standard deviation for continuous variables or as selective frequency for categorical variables and analyzed by Student's t-test or the Chi square test. A p value of less than 0.05 was considered significant.

RESULTS

Body weight

The body weight increased from 26.3 ± 1.8 kg to 33.8 ± 4.7 kg (+ 28.5 %, during the time period between 17 and 21 month of age).

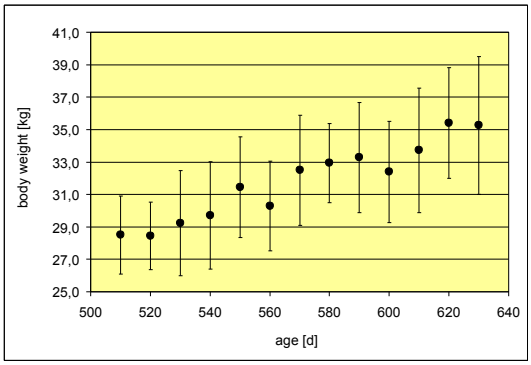

Figure 1: *Body weight of Göttingen Minipigs in the age range of 17 to 21 month; (mean value ± standard deviation, n = 18)*

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CT analysis

Ct analysis revealed that the luminal diameter and the length of the arteries and veins did not change during the time period between 17 and 21 month of age ($p<0.05$, figures 2).

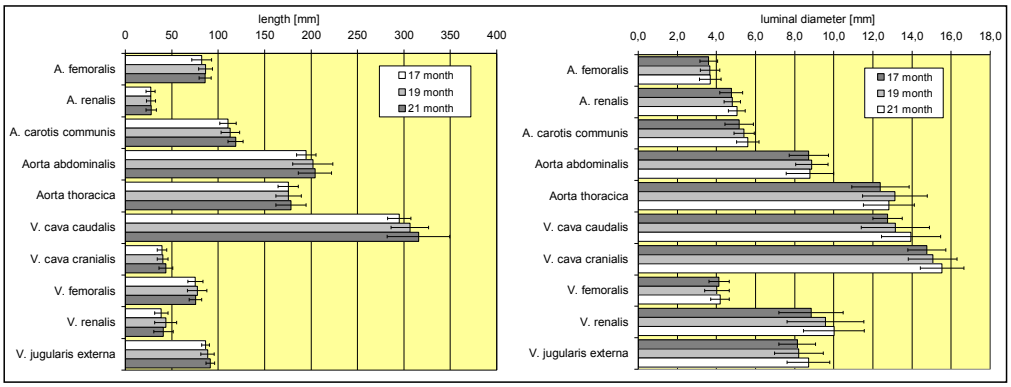

Figure 2: *Luminal diameter and length of veins and arteries of Göttingen Minipigs (during the time period between 17 and 21 month of age); CT-based data; (mean value ± standard deviation, n = 18)*

Histology

Figure 3 shows the thickness of the tunica media and of the lamina endothelialis from arteries and veins of the animals at an age 21 month. The thickness of the lamina endothelialis showed no significant difference between the veins and arteries.

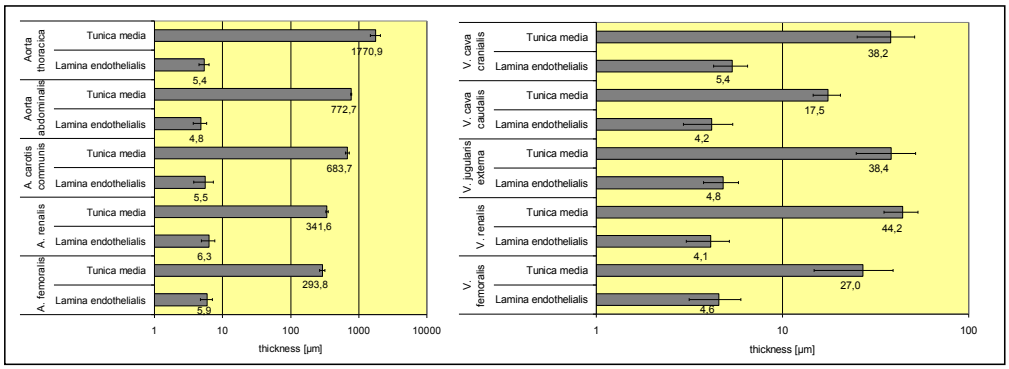

Figure 3: *Thickness of the tunica media and the lamina endothelialis of arteries and veins of 21 month old Göttingen Minipig; data based on histological examination; (mean value ± standard deviation, n = 3)*

DISCUSSION

In order to investigate the properties of implantable biomaterials a biological model is essential before establishing a clinical trial [9]. Therefore, the selection of the appropriate animal model is a major part of experimental work.

The study showed that the vasculature - luminal diameter and the length of the arteries and veins analyzed - remained constant over time in young adult Göttingen Minipigs despite of a significant increase of the body weight ($p<0.05$) over 4 months (from the 17. to the 21. month). This is in good agreement with recently published data showing that the diameters of the iliac bifurcation arteries of minipigs (Yucatan) did not change with increasing body weight [10]. In summary the results showed that the adult Göttingen Minipig proved to be a suited animal model for in-vivo studies which require long-term stable vascular macro- and micromorphometric parameters, such as implantation of stents, vein cuffs, heart or venous valves and vascular prostheses. The histomorphometric parameters (thickness of the tunica media and the lamina endothelialis) can provide helpful baseline data for developing implants.

Wireless Monitoring for Animal Wellbeing

ECG, HR and acceleration in minipig: a feasibility study

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3. Holst Centre / imec, www.holstcentre.com

GENERAL

1. Wireless sensor nodes and Animal 3Rs

Recent progress by researchers at imec and Holst Centre comprises the realization of ultra-low power wireless body area networks (WBAN) used for communication among sensor nodes operating on, in or around a man's body to monitor vital body parameters and movements (EEG, ECG, EMG, skin conductance, Skin temperature, and more) *[1]*. In biomedical research the application of these wireless sensor nodes -so far- was restricted to human individuals. We argued that smart application of such wireless sensor nodes in animals could contribute considerably to the reduction and refinement of (mandatory) animal use in biomedical research. Animal use for biomedical research is under intense societal debate which -to some extent- is in conflict with the *mandatory* use of animals for research to protect man against undesired effects of new drugs. With current societal interest in replacement, reduction and refinement of animal experimentation (Animal 3Rs), authorities, industry and regulatory agencies work together to accomplish reduced animal use in (regulatory) safety testing.

2. Minipig and Safety pharmacology

Legislation to protect man from adverse effects of drugs, chemicals and food ingredients *demands* the conduct of animal studies which are outlined in regulatory test guidelines. At some point in the past, rodents were chosen for this kind of research. However, predictivity of the rodent model for man appeared not always sufficient and so, regulatory authorities demand that safety evaluation studies during development of a new drug are *additionally* carried out in a *non-rodent* model, mostly dog or non-human primate, or -since recently- minipig. Anatomical, biochemical, physiological and genetic similarities to man, and available knowledge on e.g. diseases and immunology, warrant the minipig a potent model for safety pharmacology testing *[2]*. This model can contribute to Animal 3Rs for *better information* will be obtained with the minipig model when it comes to predictivity and translation of animal results to man, ultimately leading to reduction in animal use.

The use of minipigs could *further* contribute to the refinement and reduction of animal testing (3Rs) when the testing *per se* is non-invasive and animal friendly. Thinking of Holst Centre and imec ultra-low power wireless body area networks and safety pharmacology testing the ideal situation would be that the three main organ systems (cardiovascular, respiratory and nervous systems) *with* accompanying behaviour (locomotion, location, body posture) are addressed *simultaneously* and measured *longitudinally/ repeatedly* in an *animal-friendly* way by smart combinations of multiple *wireless* sensor nodes. Such measurements will increase the (statistical) power -multiple physiological parameters and organ systems addressed simultaneously, and repeated measurements within one and the same individual- and so, fewer animals will be needed.

FEASIBILITY STUDY: ECG, HEART RATE, ACCELERATION WIRELESS MONITORING IN MINIPIG

1. Introduction

Cardiovascular safety pharmacology in freely moving minipigs

Especially for cardiovascular safety pharmacology research the minipig was proven to be an attractive model *[3]*. Moreover, the consideration of data improvement has led to successful measurements in freely moving minipigs using 'non-invasive' telemetry applications, either with pre-implanted or jacketed transponders. For the first approach surgery and anaesthesia is required which may affect study outcome; for the second pre-training of the animal to wear and accept the jacket without stress.

Here we explored –as a first example– the applicability of the Holst Centre wireless ECG Necklace sensor node and X, Y and Z-acceleration node in minipig as the test subject, focussing especially on animal (dis)comfort when wearing the sensor nodes, and on quality and usefulness of the signals.

2. Methods *[Figures 1A-E]*

Principle of the ECG necklace and application on minipig

The ECG necklace *[4]* is easy to use and characterized by a low power consumption ensuring 7 days autonomy.

It contains imec's proprietary ultra-low power analogue readout ASIC (Application-Specific Integrated Circuit), and relies on a low power commercial radio and microprocessor platform. A wavelet-based heart beat detection algorithm is embedded in the processor that ensures the accurate computation of the instantaneous heart rate, even under high level of noise. The microcontroller controls the wireless transmission of the ECG data to a receiving base station connected to a computer within a range of 10m. An optional non-volatile memory module enables continuous data logging for applications in case the receiving computer is not in the neighbourhood. This was also used in the feasibility study reported here.

To attach the sensor to the minipig, the pig's skin was shaved around the left fore paw / heart region and cleaned with antisceptic solution. Electrode patches were sticked to the skin, one left-sided below the heart; the other right-sided above the heart. Sensor leads were attached to the electrodes. The sensor itself was clamped into a pre-prepared 'pocket' of an elastic belt attached around the pig's belly. Belt and electrodes were covered by a commercially available bandage. Both fore-paws perforate the bandage allowing the animal to freely move around, whereas belt and other equipment stay in place. Care was taken to register and note down the orientation of the sensor on the pig, allowing translation of acceleration signals of the X, Y and Z axes.

3. Results of the feasibility study *[Figures 2A-E]*

The ECG signal turned out well (the signal quality was comparable with a wired solution) and so did the signal produced by the X, Y, Z-acceleration node. Changes in direction of the sensor nodes resulting from changes in the pig's body orientation -like turning around during walking while standing on 4 paws- were observable as an interchange of the X, Y, and Z-axis signals. Intense movements of the minipig were visible by complimentary changes in the X, Y, and Z signals as well as an increase in Heart Rate (HR).

4. Discussion / conclusions

The results of the feasibility study indicate that the Holst Centre and imec wireless sensor technology could perfectly fit-in to contribute to the principles of animal 3Rs: the minipig accepted wearing the sensor (*refinement*), and the signals of ECG, Heart rate and acceleration were acquired and analysed without any problem and with good results. Continuous and repeated, *simultaneous* monitoring with *multiple* sensor nodes addressing multiple organ systems seems to be within reach and so, more information can be obtained from fewer animals (*reduction*). Since the minipig is considered a very predictive model to extrapolate results to man, it is the ideal model to further explore the relevance of the Holst Centre and imec wireless sensor technology for safety evaluation studies; all of this in the context of animal 3Rs.

PROSPECTS

The promising results on ECG/HR/acceleration in minipig encourage further exploration of the Holst Centre and imec ultra-low power wireless sensor technology for monitoring of physiological characteristics of minipig in an animal-friendly way (*refinement*). For safety pharmacology, an *integrative* system of sensor nodes addressing *simultaneously* the cardiovascular, respiratory and nervous systems *with* accompanying behaviour is a primary goal. This will lead to animal *reduction* since more information is obtained from an individual animal, saving also time and costs. Such an integrative system for multimodal physiological assessments will find broader application in biomedical animal research (e.g. juvenile toxicology, disease models, *in vivo* imaging and mechanisms).
Miniaturization of the sensors –another goal- is a challenge by itself, but a prerequisite for non-invasive application in small animals like rats and mice.

In the *longer-term*, the data base on toxicological information on minipig will expand and –slowly but surely– information in rodents will not add new information to the data in the non-rodent species examined with such a highly sophisticated, animal-friendly, information increasing and cost reducing *integrative test system* for simultaneous, multimodal physiological assessments. Rodent studies may become needless. In this context, it should be borne in mind that the development of *regulatory* requirements (outlined in testing guidelines) always depends on the state of the science and the state of the art of test methodology, and hence it may be possible in the future to replace the currently required tests on single endpoints in different species by a limited test battery in a single species that at least has the same –but most likely better– predictivity of effects and from which the results can readily and reliably be translated to humans.

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INFORMATION ON COLLABORATIVE INSTITUTIONS

1. Holst Centre

Holst Centre (*www.holstcentre.com*) is an independent open-innovation R&D centre that develops generic technologies for Wireless Autonomous Sensors and for Flexible Electronics. A key feature of Holst Centre is its partnership model with industry and academia in shared roadmaps and programs. Holst Centre was founded by imec (*www.imec.be*) and TNO (*www.tno.nl*) and is located on High Tech Campus Eindhoven, The Netherlands.

2. TNO Innovation for life

TNO is the largest Dutch public-private R&D organization. TNO has over 75 years of experience in generating knowledge and turning it into practical applications, contributing to the innovative power in the Netherlands and abroad. TNO's seven research themes are Healthy Living, Industrial Innovation, Safety, Energy, Mobility, Built Environment and Information Society. In 2005, TNO assigned one of its business units to coordinate its activities in Holst Centre.

3. imec

imec performs world-leading research in nanoelectronics and leverages its scientific knowledge with the innovative power of its global partnerships in ICT, healthcare and energy. imec delivers industry-relevant technology solutions. In a unique high-tech environment, its international top talent is committed to providing the building blocks for a better life in a sustainable society. imec is headquartered in Leuven, Belgium, and has offices in Belgium, the Netherlands, Taiwan, US, China and Japan.

In 2005, imec setup a legal entity 'imec-nl' to coordinate its activities in Holst Centre. imec offers several interactive programs, which give companies and institutes the opportunity to directly access imec's core expertise in leading-edge technology. Only by joining forces, it is possible to tackle tomorrow's technological challenges and to share the rising R&D costs.

LEGENDS TO THE FIGURES

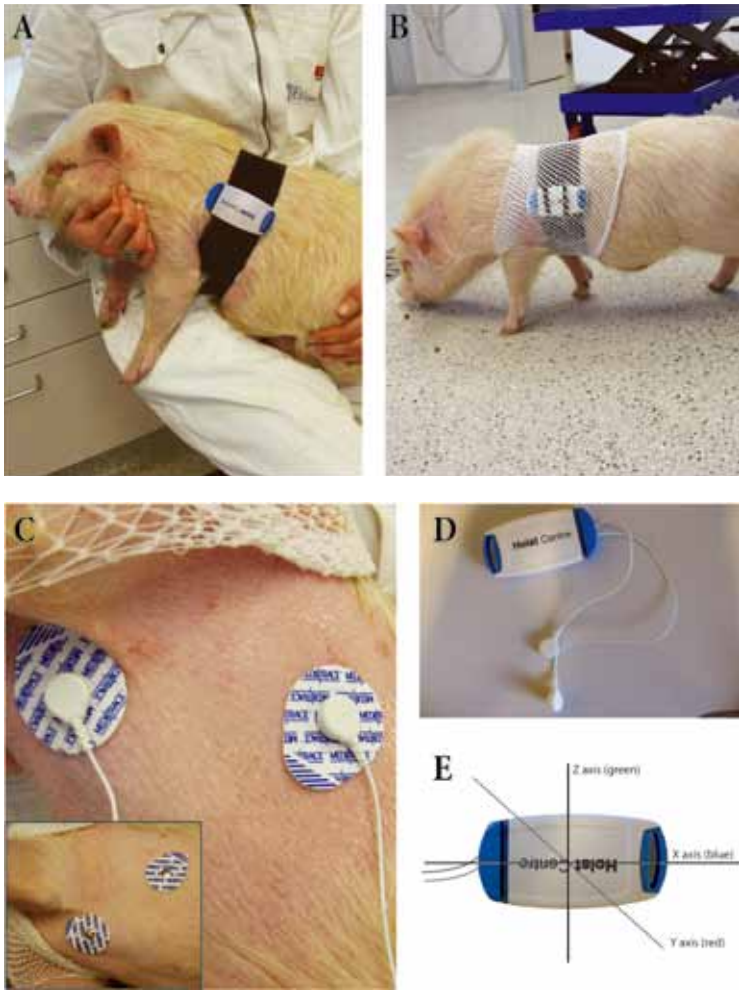

Figure 1*. Minipig test subject (male; age: 5-6 Months; body weight 11.1 kg). Figs.1A, 1B) The Holst Centre ECG necklace was clamped in the pre-prepared 'pocket' of an elastic belt worn around the pig's belly. The orientation of the ECG necklace is noted down to be able to interpret acceleration changes in the X, Y and Z axes, since acceleration information is acquired simultaneously with the ECG. [Notice that in Fig.1A, the 'pocket' on the belt is 'deleted' (Photoshop) for the sake of illustration]. Fig.1C) Attachment of the sensor leads to the Meditrace ECG electrodes [cf. insertion], which are attached to the pig's shaved and cleaned skin. Fig.1D) Image of the HOLST Centre sensor. Fig.1E) The Holst Centre sensor node with the orientation of the different axes relative to the sensor [compare Figure 1A].*

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Soroe Landevej 302, DK-4261 Dalmose

Thank you

PLEASE NOTE: Ellegaard Göttingen Minipigs A/S will not release or give away a subscriber's e-mail address, name or any other information provided.

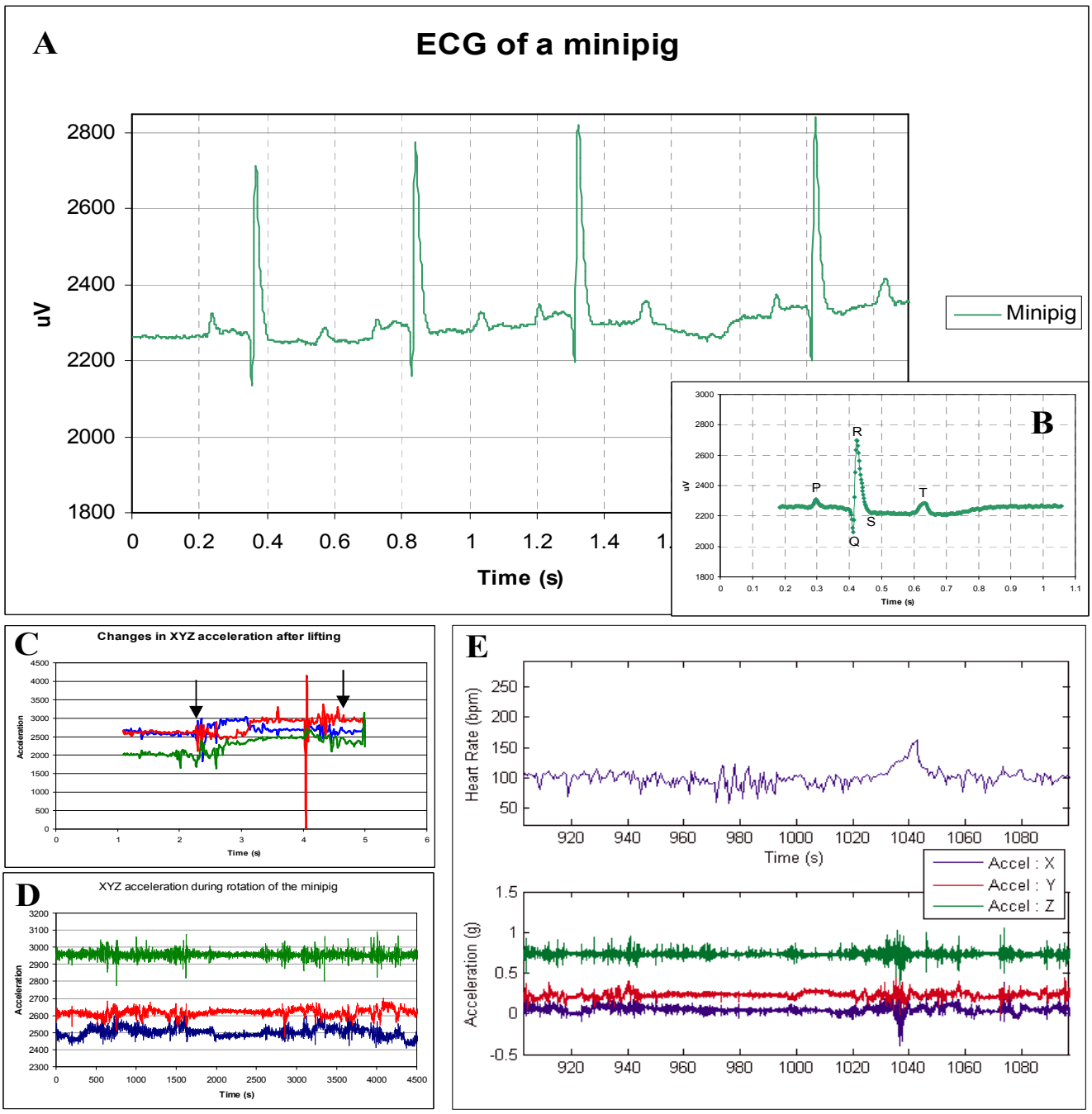

Figure 2. *Minipig test subject (male; age: 5-6 Months; body weight 11.1 kg).*
Fig.2A) Multiple ECG complexes demonstrating a clear signal, acquired with the ECG necklace [Fig.1A]. Gain and filter setting were optimized for use in man and might be slightly adapted to acquire optimal recordings of the minipig ECG. Fig.2B) ECG complex with characteristic waves and peaks (P, Q, R, S, T) measured with Holst Centre wireless ECG Necklace sensor node. Fig.2C) Changes in XYZ acceleration (arrows) during lifting of the animal. [Compare sensor orientation of Fig. 1B (horizontal) and 1A (under slight change of angle), and Fig. 1C]. Fig.2D) Acceleration of the minipig during moving. Notice that at around 1500 seconds the animal rotates around its own axis while standing on 4 paws, resulting particularly in changes in the x-axis (blue curve); same behaviour and accompanying changes in x-axis can be observed at 2200 and 3200 seconds. Fig.2E) Notice similarities between Acceleration (lower panel) and Heart Rate patterns (upper panel).

Catheter Workshop for easy blood sampling

Intravenous access for injections and blood sampling of the Göttingen Minipig has always been known as a bit of a challenge. Large blood vessels like the jugular vein are not visible and are situated deep in the tissue; superficial veins are often small like the ear vein or are not practical to access, like on the medial side of the thigh. The saphenous vein could be an alternative.
Especially serial blood sampling can be stressful to both pigs and personnel. Blood sampling is often performed by placing the pig in a V-trough to collect blood from the jugular vein or precaval sinus. This procedure requires trained personnel to keep the stress level of the pig to a minimum. But even with well trained technicians and pigs there is only a certain amount of samples that can be taken in a given time to maintain good animal welfare with this method. If frequent samples have to be taken from the pig over a short period of time, catheterisation is an option.

Long term access
For long term access, surgically implanted catheters are indicated, either tunnelled percutaneously to the exterior or fully implanted systems with a Vascular Access Port (VAP). These procedures are well described in literature and can be learned and trained in one of our surgery courses. Please refer to our website www.minipigs.dk for the next dates.
An implanted catheter may also be connected to an automated or remote blood sampling system to obtain samples without having to handle the pig.

Short term access
For short term access a Central Venous Catheter (CVC) percutaneously inserted using the Seldinger technique is a valid choice. The advantage of these methods is that the pig does not have to undergo surgery; general anaesthesia however is necessary. It is a quick procedure and the catheter can remain open for up to 14 days or more.
There are some risks and challenges involved with that procedure. In an earlier newsletter we published an article describing the use of the ARROW® brand catheter. In the meantime we have tested other brands and variation of the method and as a result created a guide on the use of CVC. You can obtain a copy by writing to ellegaard@minipigs.dk. We have also tested peripherally inserted midline catheters; initial results look promising but more work needs to be done.

Catheter Workshop
As there is a big interest among our customers to facilitate venous access and improve animal welfare we have created a Catheter Workshop. It is a one day event where you have the chance to try different catheterization methods and sites. The focus is on practical hands on experience; you will receive literature and documentation which is recommended to study before or after the workshop. Content will be adjusted to participant's need. For further information, dates and prices please contact us.
If you have experience with catheters in Minipigs and are happy to share it with other Minipig users you are most welcome to send your comments to az@minipigs.dk.

Overview of catheters

Short term 1- 14 days	Long term Up to 12 months	
Percutaniously implanted Seldinger Methode	surgically implanted	
Easy to remove	Surgical removal of catheter	
Minimal invasive	Invasive	
Risk of displacement	Securely tied with ligatures to vessel	
Patency of vessel maintained	Patency of vessel lost	
Secured by ext. suture	Externalised, secured by cuff	Totally implanted with VAP
Risk of infection	Risk of infection	Reduced risk of infection
Painless access	Painless access	Painful access
Single housing	Single housing	Group housing possible

Ellegaard Göttingen Minipigs DVDs available:

- **The Göttingen Minipig – Handling and dosing.**
  This DVD is available in German as well as French and English. A unique tool for those who work with the Göttingen Minipig.
  Price each € 90 excl. shipping and handling.

- **The Göttingen Minipig – Histology**
  This DVD allows users to familiarize themselves with the normal histology of the Göttingen Minipig.
  Price each € 65 excl. shipping and handling.

Please contact us for further information at ellegaard@minipigs.dk or phone +45 5818 5818.

EDUCATIONAL PACKAGE FOR MINIPIG USERS

We value our customers as close collaborators and we focus on supporting you as much as we can. We want to make it easy for both new and less experienced customers to adapt to the use of Minipigs in research and we also think that many of our experienced customers will benefit from an educational package for Minipig users. This is why we have prepared an educational package with 10 topics. The educational package covers the following topics:

- Toxicology
- Histopathology
- DMPK
- Safety Pharmacology
- Immunology/Immunotox
- Surgery
- Handling & Dosing
- Training of Minipigs
- Animal Welfare
- Economics, Regulatory Acceptance

Toxicology and histopathology are complete and we are in the process of finalizing the other topics. Histopathology, toxicology, DMPK and Immunology/Immunotox have been prepared by external experts which provides our customers with their objective and practical perspective. All topics will be continuously updated.

Please contact us if you are interested in receiving part of or the full educational package or if you would like further information.

NEW ARTICLES ABOUT MINIPIGS

A soft-tissue coupling for wound closure.
Melvin AJ, Melvin DB, Kitzmiller WJ, Fath KR, Biddinger PW, Juncosa-Melvin N.
J Biomed Mater Res B Appl Biomater. 2011 Feb 16.

Cervical multilevel intraspinal stem cell therapy: assessment of surgical risks in Gottingen minipigs.
Raore B, Federici T, Taub J, Wu MC, Riley J, Franz CK, Kliem MA, Snyder B, Feldman EL, Johe K, Boulis NM.
Spine (Phila Pa 1976). 2011 Feb 1;36(3):E164-71.

Enlargement of Catheter Ablation Lesions in Infant Hearts with Cryothermal Versus Radiofrequency Energy: An Animal Study.
Khairy P, Guerra PG, Rivard L, Tanguay JF, Landry E, Guertin MC, Macle L, Thibault B, Tardif JC, Talajic M, Roy D, Dubuc M.
Circ Arrhythm Electrophysiol. 2011 Jan 21.

Derivative and Parathyroid Hormone on Bone Formation in Standardized Osseous Defects. An Experimental Study in Minipigs.
Jensen SS, Bo C, Bornstein MM, Bosshardt DD, Buser D. Effect of Enamel Matrix
J Periodontol. 2011 Jan 10.

Changes in Brain Activity After a Diet-Induced Obesity.
Val-Laillet D, Layec S, Guérin S, Meurice P, Malbert CH.
Obesity (Silver Spring). 2011 Jan 6.

Autologous stem cell therapy maintains vertebral blood flow and contrast diffusion through the endplate in experimental IDD.
Bendtsen M, Bünger CE, Zou X, Foldager C, Jøregensen HS.
Spine (Phila Pa 1976). 2010 Dec 29.

Long-term delivery of nerve growth factor by encapsulated cell biodelivery in the Göttingen minipig basal forebrain.
Fjord-Larsen L, Kusk P, Tornøe J, Juliusson B, Torp M, Bjarkam CR, Nielsen MS, Handberg A, Sørensen JC, Wahlberg LU.
Mol Ther. 2010 Dec;18(12):2164-72.

Contribution of animal models to the understanding of the metabolic syndrome: a systematic overview.
Varga O, Harangi M, Olsson IA, Hansen AK.
Obes Rev. 2010 Nov;11(11):792-807.

Lateral augmentation of the mandible in minipigs with a synthetic nanostructured hydroxyapatite block.
Kirchhoff M, Lenz S, Henkel KO, Frerich B, Holzhüter G, Radefeldt S, Gerber T.
J Biomed Mater Res B Appl Biomater. 2011 Feb;96(2):342-50.

A computed tomography scan application to evaluate adiposity in a minipig model of human obesity.
Val-Laillet D, Blat S, Louveau I, Malbert CH.
Br J Nutr. 2010 Dec;104(11):1719-28.

Evaluation of fenestrated and non-fenestrated biologic grafts in a porcine model of mature ventral incisional hernia repair.
Jenkins ED, Melman L, Deeken CR, Greco SC, Frisella MM, Matthews BD.
Hernia. 2010 Dec;14(6):599-610.

A novel device for islet transplantation providing immune protection and oxygen supply.
Ludwig B, Zimerman B, Steffen A, Yavriants K, Azarov D, Reichel A, Vardi P, German T, Shabtay N, Rotem A, Evron Y, Neufeld T, Mimon S, Ludwig S, Brendel MD, Bornstein SR, Barkai U.
Horm Metab Res. 2010 Dec;42(13):918-22.

Heparin- and basic fibroblast growth factor-incorporated stent: a new promising method for myocardial revascularization.
Liu XC, Zhao J, Wang Y, Liu TJ, Lü F, He GW.
J Surg Res. 2010 Dec;164(2):204-13.

Slower eating rate is independent to gastric emptying in obese minipigs.
Val-Laillet D, Guérin S, Malbert CH.
Physiol Behav. 2010 Nov 2;101(4):462-8.

A minipig model of maxillary distraction osteogenesis.
Papadaki ME, Troulis MJ, Glowacki J, Kaban LB.
J Oral Maxillofac Surg. 2010 Nov;68(11):2783-91.

Platelet-rich plasma on calcium phosphate granules promotes metaphyseal bone healing in mini-pigs.
Jungbluth P, Wild M, Grassmann JP, Ar E, Sager M, Herten M, Jäger M, Becker J, Windolf J, Hakimi M.
J Orthop Res. 2010 Nov;28(11):1448-55.

The Rethink report

November 2010 the Rethink report was printed as a special edition of Journal of Pharmacological and Toxicological Methods (Volume 62, Issue 3). The conclusions of the Rethink project confirm what we have always known: That the minipig is a very suitable non-rodent model for non-clinical safety testing.

The Rethink report consists of the following articles:

- Editorial
- Minipigs as models for the toxicity testing of new medicines and chemicals: an impact assessment
- Ethical implications of using the minipig in regulatory toxicology studies
- Welfare of the minipig with special reference to use in regulatory toxicology studies
- Regulatory acceptability of the minipig in the development of pharmaceuticals, chemicals and other products
- The utility of the minipig as an animal model in regulatory toxicology
- Genetic management of the Göttingen Minipig population
- The minipig as a platform for new technologies in toxicology
- The RETHINK project on minipigs in the toxicity testing of new medicines and chemicals: Conclusions and recommendations

Here are some of the conclusions from the Rethink report:

The minipig is an important model for drug discovery, safety pharmacology investigation and drug safety studies in general; it is bred to high quality standards, is well understood, its readouts are reproducible, and it is readily available.

The minipig is readily accepted by regulatory authorities, for investigations concerning a range of different product types.

Rethink Editorial
Curtis

Overall, this review leads us to believe that the minipig might be a better non-rodent toxicology model than the dog.

All these characteristics favor the use of the minipig for cardiovascular assessments in toxicological and pharmacological studies, in particular in comparison to dog

Rethink article: The utility of the minipig as an animal model in regulatory toxicology
Bode, Clausing, Gervais, Loegsted, Luft, Nogues, Sims

These factors all support the idea that the minipig is well placed to meet the challenges of the emerging technologies and the toxicology of the future; it also seems likely that the minipig can be an advantageous model for the testing of biotechnology products.

Rethink article: The minipig as a platform for new technologies in toxicology
Forster, Ancian, Fredholm, Simianer, Whitelaw

The Rethink report is available online: http://www.rethink-eu.dk

MEETING CALENDAR

NAME	DATE	PLACE
SOT ToxExpo	6-10 March	Washington, D.C.
BTS	27-30 March	Durham, UK
AFSTAL	25-27 May	Paris, France
SCAND-LAS	25-28 May	Copenhagen, Denmark
Swine in Biomedical Research Conference	17-19 July	Chicago, Illinois

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For Göttingen Minipigs in Japan please contact Oriental Yeast Co., Ltd. - fbi@oyc.co.jp