NEWSLETTER 50

WINTER 2017

ELLEGAARD
GÖTTINGEN MINIPIGS

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Clean pigs for clear results

Dear Reader

Time is flying, and not only is Christmas and New Year approaching, but also a milestone for Ellegaard Göttingen Minipigs, as the present Newsletter is our 50th jubilee Newsletter after years of publishing Newsletters with lots of scientific publications, news from Ellegaard Göttingen Minipigs and our global partners; Marshall BioResources in the US, Oriental Yeast Company in Japan and Woojung BSC in Korea plus the exchange of knowledge and experience from Göttingen Minipigs users.

We have over the last months expanded our provided services in our Research Barrier, and are now able to provide you Göttingen Minipigs with several types of surgically implanted catheters, vascular access ports and not least telemetry devices in addition to providing animal model preparation services.

Being committed to ensuring that the Göttingen Minipig remains the globally leading minipig, we have lots of focus on supporting the development of transgenic Göttingen Minipigs and offering contract breeding of transgenic Göttingen Minipigs in our Research Barrier. Our aim is to promote the 3rd party development and characterization of various strains of transgenic Göttingen Minipigs and in partnership with stakeholders to give our customers access to these within the coming years.

I am also very pleased to inform you that Ellegaard Göttingen Minipigs Research Foundation has awarded Steven Van Cruchten and his research team at the University of Antwerp, Belgium, the 2nd grant for their research project "Construction and activity of recombinant cytochrome P450 enzymes as an in vitro system for reaction phenotyping of drug candidates in the Göttingen Minipig", which you can read much more about in this Newsletter.

Finally, I would like to thank all of you for your business, for taking part in many exciting collaborations and not least for lots of good chats and exchange of knowledge at various scientific meeting and conferences.

Happy Holidays and happy reading …

Lars Friis Mikkelsen
CEO
Ellegaard Göttingen Minipigs

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Our company page on LinkedIn will keep you updated with useful and interesting information regarding our company and the Göttingen Minipig!

Göttingen Minipigs pre-implanted with DSI PhysioTel Digital Telemetry Implants

By Henrik Duelund Pedersen[1], Stefano Gaburro[2]
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[2] Data Sciences International, St. Paul, MN, USA

Introduction

In collaboration with Data Sciences International (DSI), Ellegaard Göttingen Minipigs A/S now offers Minipigs pre-implanted with PhysioTel Digital™ telemetry implants. The procedure has been established for implant placement in both the neck and the flank. The picture in Figure 1 was taken in the operating theater in the new Research Barrier at Ellegaard Göttingen Minipigs during one of the operations. Heather Bogie from DSI visited for one week, during which she trained Adrian Zeltner from Ellegaard Göttingen Minipigs in the procedures.

Figure 1: Adrian Zeltner from Ellegaard Göttingen Minipigs operates one of the Minipigs; supervised by Heather Bogie from Data Sciences International

Methods

In 4 Göttingen Minipigs (2 males and 2 females) aged 4 months, and weighing 6-10 kg, M-11 series Physiotel Digital implants were placed in the flank, with the ECG electrodes placed on the chest, and the blood pressure catheter placed in the femoral artery (extending into the aorta). This setting was chosen to mimic a typical toxicology study. In Figure 2, the implant can be seen in the flank of the Minipig on the top picture.

In 4 Göttingen Minipigs (2 males and 2 females) aged 8 months, and weighing 16-20 kg, L-11 series Physiotel Digital implants were placed in the neck, with one of the ECG electrodes placed on the chest and the other one placed in the external jugular vein. The blood pressure catheter was placed in the external carotid artery in these Minipigs (extending into the aorta). This setting was chosen to mimic a typical Safety Pharmacology set-up intended for re-using the Minipigs for several studies. In Figure 2, the circle drawn on the bottom picture illustrates the approximate placement of the implant, but the implant itself is difficult to see, as it was placed beneath skin and muscle, in a deep pocket extending from an incision made behind the ear.

All Göttingen Minipigs were housed in groups of 3 (two operated plus one extra companion) according to age and sex; had access to tap water always; were fed according to in-house standards with regular Minipig diet (Mini-Pig Expanded, Special Diets Services, Witham, UK); had artificial light between 6 am and 6 pm; and were housed in pens with heated floors. Most of the recordings were made with two transceivers placed approximately 1.8 m above the middle of the pen, except for one of the weekly recordings, which was made with only one transceiver (Figure 3). Examples of signals typically obtained can been seen in Figure 4.

The Minipigs were followed for 2 months after surgery, and average values were calculated for a full 24-hour period on 7

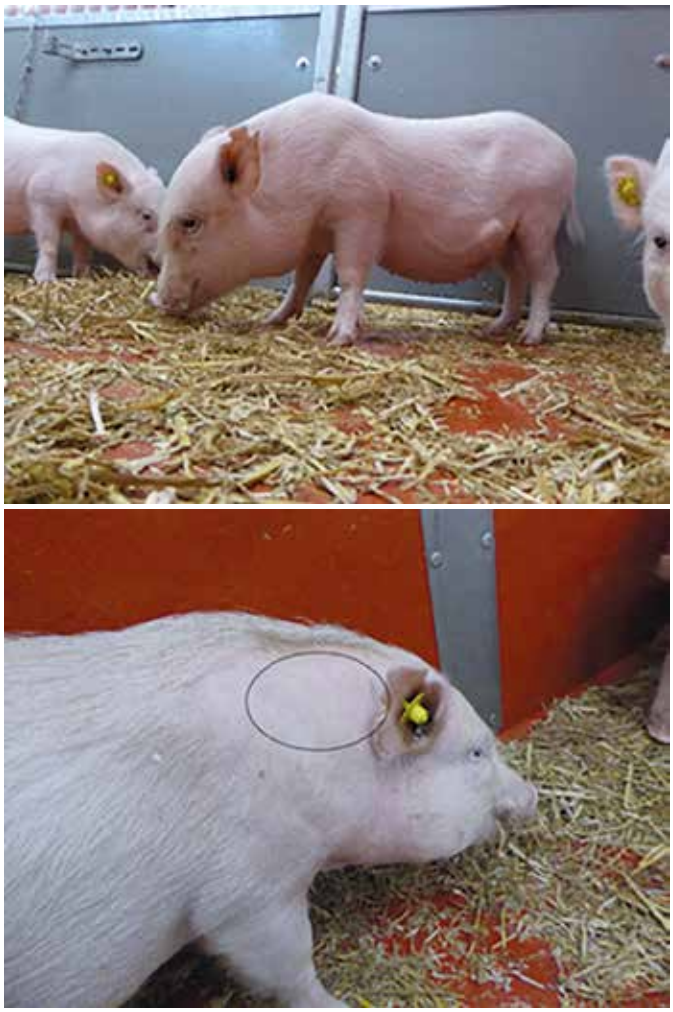

Figure 2: Göttingen Minipigs fitted with telemetry implants. In the small Minipig in the top picture, the implant can be readily seen in the flank. In the larger Minipig in the bottom picture the implant is placed in a deep pocket behind the ear (marked by the circle) and is not readily seen

Figure 3: One transceiver placed directly above each pen was sufficient to obtain high quality signals

weekends during that period. In addition, the response to pharmacological treatments known to have marked effects on blood pressure, heart rate and QT interval were tested on a Monday 5 to 6 weeks following surgery. Specifically, on the day of dosing, all 8 Minipigs were followed from 6 am to 2 pm, and at 7:30 am they were all dosed i.m. with 0.04 mg/kg medetomidine, and at 10:00 am with 0.04 mg/kg medetomidine plus 0.02 mg/kg atropine. During the 8-hour period, heart rate, blood pressure and various ECG parameters were extracted. The QT intervals were subsequently corrected for heart rate influence by means of Fridericia's formula, as has previously been used in a similar study[1].

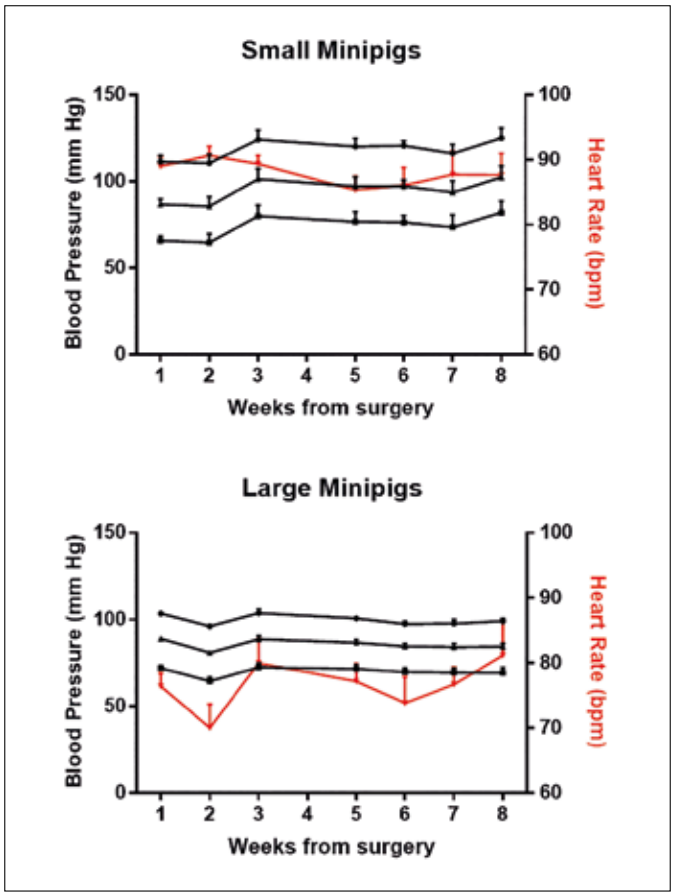

Figure 5: Weekly 24-hour recordings of blood pressure (systolic, mean and diastolic) and heart rate in the small Göttingen Minipigs (top graph) and larger ones (bottom graph). Error bars indicate mean ± SEM

Results and discussion

High quality ECG and blood pressure signals were obtained throughout the 2-month period. As can be seen in Figure 5, the pulse pressure, i.e. the difference between the systolic and the diastolic pressure, was constant in the entire period, indicating that there was no weakening of the signal, which might be seen if there is growth of fibrotic tissue on the pressure catheter. Also, it appears from both graphs in Figure 5 that the variation in the blood pressure data was small, whereas the variation in the heart rate data – as expected – was larger.

Also, the percentage bad data was the same in the last 24-hour period as it was during the first one (6% versus 3% on the blood pressure signal and 0.5% versus 0.3% on the ECG signal – both non-significant on a paired t-test). One transceiver recorded the signal just as well as two, as indicated by the fact that the percentage bad data was the same in the 24-hour period recorded with one transceiver versus an average of the percentage bad data obtained with two transceivers the weekends before and after (7% versus 6% on the blood pressure signal and 0.6% versus 0.7% on the ECG signal – both non-significant on a paired t-test).

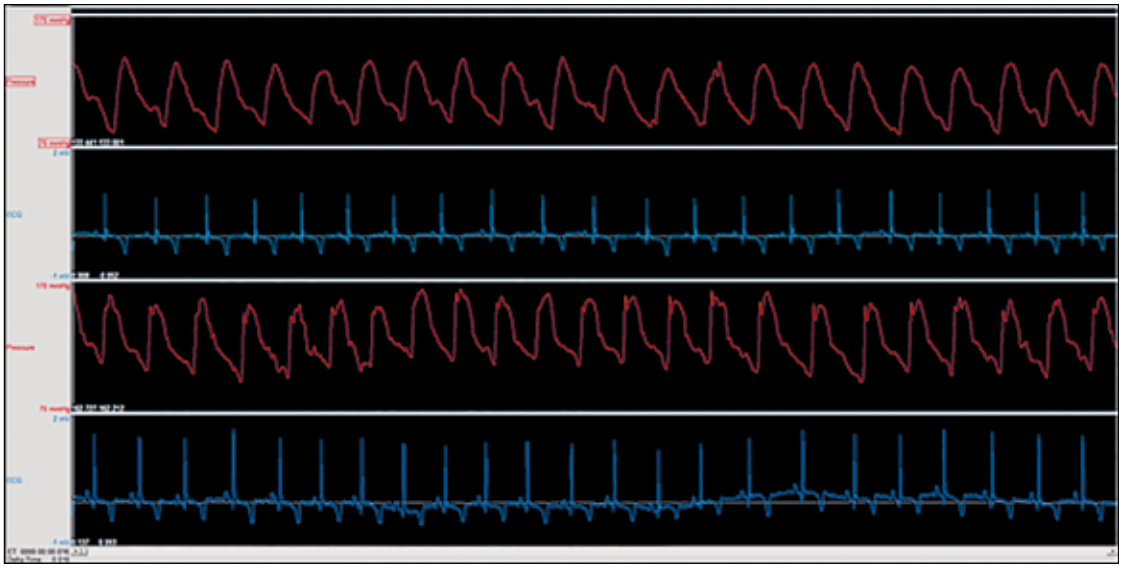

Figure 4:
Examples of blood pressure and ECG recordings from freely moving Göttingen Minipigs acquired with Ponemah DSI® software

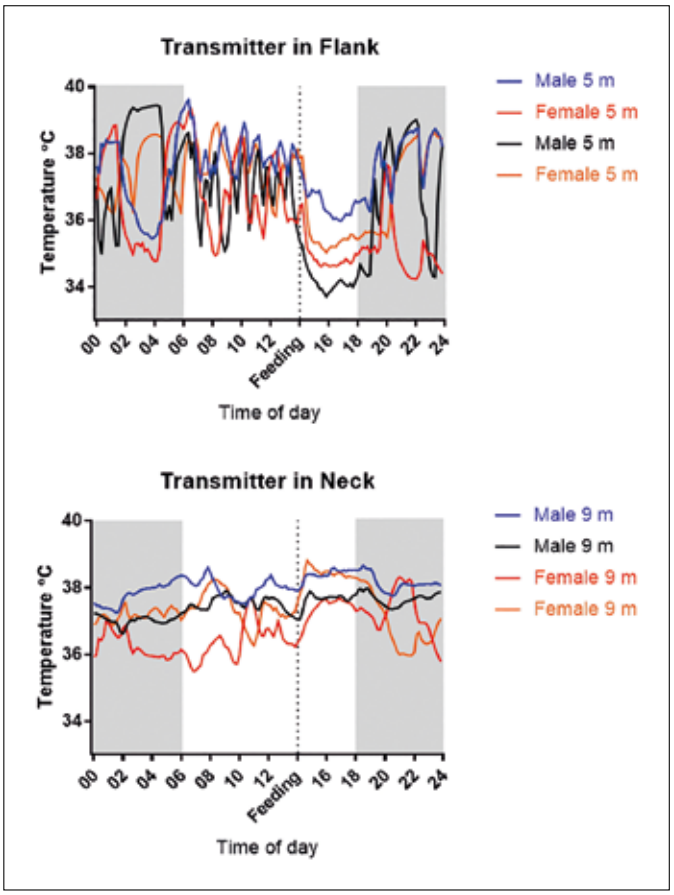

Figure 6: Temperatures recorded by the implants over a typical 24-hour period in small Göttingen Minipigs (5 months of age when operated) and in larger ones (9 months of age when operated). The superficial placement of the implants right beneath the skin led to more fluctuating temperatures being recorded for the small Minipigs (and one of the larger ones – the red line). The grey areas indicate periods without artificial light in the stable. For further details, please refer to the text

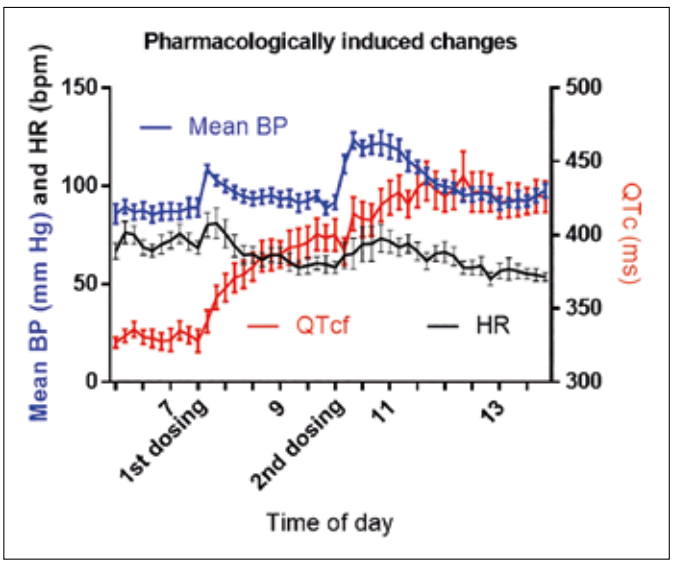

Figure 7: Pharmacological effects on mean blood pressure (BP), heart rate (HR) and QT-interval (corrected for heart rate) of medetomidine given at 7:30 am and medetomidine plus atropine given at 10:00 am. As can be seen, such pharmacological effects are readily picked up by this method. Error bars indicate Mean ± SEM

Temperature data were recorded with both types of implants throughout the study. As can be seen from Figure 6, the temperature was very sensitive to the temperature of the surroundings when recorded by an implant placed just under the relatively thin skin in the flank. Specifically, it appears that during deep sleep (at approximately 2 to 4 am) the temperature was high in two animals and low in two others – likely because the two former Minipigs slept with the implant-side facing down on the heated floor. Also, it can be seen from Figure 6, that there is a stable temperature for several hours following feeding (which was done by spreading the feed amongst the bedding on the floor). This likely reflects that Minipigs fed in this fashion are actively walking around foraging for several hours after feeding – an activity which is also known to influence the cardiovascular parameters in that period[2]. The temperatures recorded from implants placed in the neck were much more constant, as can be seen from the bottom graph in Figure 6. Except from one of the Minipigs (red line in the figure), these Minipigs had the implant placed in a deep pocket – below skin, subcutaneous fat and muscle.

Pharmacological effects of medetomidine and atropine on mean blood pressure and QT interval (individually corrected for heart rate) can be seen in Figure 7. As expected, the compounds caused marked changes that were readily picked up by the system.

Conclusion

Based on the results described here, we conclude that Ellegaard Göttingen Minipigs now can deliver Göttingen Minipigs pre-implanted with PhysioTel Digital™ telemetry implants.

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Minipig Research Forum

THE 12TH MINIPIG RESEARCH FORUM

16-18 MAY 2018 IN BARCELONA, SPAIN

The MRF meeting offers exiting presentations on the use of minipigs in research and drug development

THIS YEAR'S MAIN TOPICS ARE

- **Species selection**
- **Analytical methods & biomarkers**
- **Imaging & devices**
- **Disease models**

3 WORKSHOPS

- **Analytical methods**
- **Species selection**
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The registration fee is EUR 350 including get-together-evening, lunch on Thursday and Friday, coffee breaks, social event and gala dinner

WELCOMING LECTURE AND GET-TOGETHER-EVENING

Wednesday 16 May 2018

Prior to the actual start of the MRF, we would like to invite you to a welcoming lecture and get-together-evening. We will host an evening buffet with food and drinks from 06:00-10:00 pm. Registration opens at 05:00 pm.

We do hope to see as many of you as possible, so please keep this in mind when booking your flight. Welcoming lecture and get-together-evening will be at the same place as the venue.

For further information regarding the MRF 2018 registration, meeting details, preliminary programme, venue and accommodation, please contact us at contact@minipigresearchforum.org or visit www.minipigresearchforum.org

Update from North America

Marshall BioResources joined Ellegaard Göttingen Minipigs in sponsoring a seminar regarding minipig welfare at the 68th National AALAS Meeting, on October 18th 2017 in Austin, Texas, US. The seminar, "Tools for Developing a Stimulating and Cooperative Environment for Laboratory Minipigs," included four separate talks that covered minipig behavior, enrichment, socialization, acclimation and training from the perspectives of both research and breeding facilities. The seminar provided specific suggestions for enhancing a minipig behavioral management and enrichment program in the laboratory, taking into consideration that minipigs are an incredibly intelligent and easily motivated species. Attention to the natural social hierarchy, opportunities to satisfy rooting behaviors, individual variation between animals, and study parameters are all important factors when developing a program. Enrichment possibilities that stimulate all senses, including scent, sound and sight can be as important as tactile enrichment, and there are many ways to provide creative and stimulating enrichment for the minipigs. Study procedures can be a source of stress, however, minipigs learn very easily using positive reinforcement, therefore, acclimation and training can help reduce stress levels and improve animal welfare. The presentations are available through AALAS at www.aalas.org, or please contact either infous@marshallbio.com or ellegaard@minipigs.dk for more information on minipig behavioral management and enrichment.

Marshall is also preparing for our third Göttingen Minipig Symposium (GMS) in North America, to be held in mid-2018. We are currently soliciting topics and participants, therefore, if you have topics you would like to suggest, or if you have an interest in participating in the program please contact us at infous@marshallbio.com.

We are happy to report that availability of Göttingen Minipigs remains very good in North America. Please contact us at infous@marshallbio.com if you have a need for minipigs, training, or general information about Göttingen Minipigs. We are now also able to offer a wide range of pre-shipment services and conditioning.

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Testing dermal wound care products in the Göttingen Minipig

By Andrew Makin, Anette Blak Grossi, Trine Starostka
CiToxLAB Scantox, Ejby, Denmark

Why wound healing, and why minipigs?

The wound-care market is huge and there are countless dermal wound care products available. These range from plasters and bandages, through sutures, and to active pharmaceuticals presented as creams, gels, etc., and many things in between. Wound care products can be classified in various ways, and each product type and classification type requires a particular testing and approval procedure. For instance, many products are classified as medical devices and should be tested according to the requirements of ISO 10993, whilst others are pharmaceuticals and need to be tested through procedures according to the ICH requirements. Lastly, there are combination products which are devices that also contain a pharmaceutical component, and which should be tested according to a mixture of relevant guidelines.

Standard safety and biocompatibility testing procedures both for devices and for pharmaceuticals involve use of healthy (i.e. normal) animals as per the requirements of the guidelines. However, prior to human use, it is also normal to test the efficacy of the products in animal models. For wound care products, this is commonly done in appropriate models such as Göttingen Minipigs, where we have standard models designed to investigate efficacy. More and more often CiToxLAB is also including an element of wound healing in standard safety studies of pharmaceutical products that are intended to be used in human dermal wounds. The designs of the safety studies and efficacy models are very different from each other. Göttingen Minipigs are the preferred species for wound healing studies due to the well documented similarities between their skin and human skin. These similarities include epidermal and dermal thickness, cell types, cell turnover, presence of rete ridges and adherence to the underlying structures. Figure 1 shows typical Minipig skin.

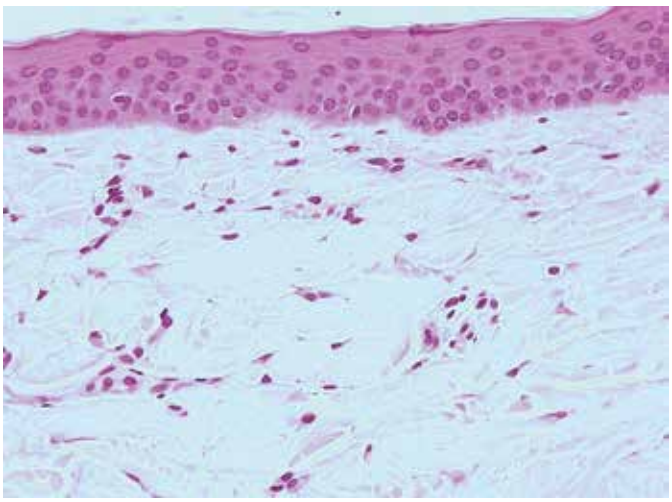


Figure 1, normal Minipig skin

The Göttingen Minipig is very useful due to its size, the lack of dark pigment in the skin, its health status and availability of background data.

In Göttingen Minipigs, the wound healing process is very similar to that of humans; involving inflammation, proliferation, contraction, re-epithelialisation and remodelling.

Rats and mice may also be used as models of wound healing, but in these species, the processes tend to be slightly different and the rate of healing is faster, however they are still useful, especially for screening purposes.

Minipig wound healing models

(a) Efficacy studies

Efficacy studies are most often performed on medical devices and are the link between biocompatibility studies and the clinical studies where the functionality of the product is tested, normally against an already-approved product or device (where possible). In principle, we have 2 models that we most often use. In all cases the wounds themselves are created under surgical conditions by trained staff under the direction of veterinarians, and using appropriate anaesthesia and analgesia.

- Split-thickness wounds, which are made by removal of the epidermis and upper dermis. They are square, created by use of a dermatome and they heal by re-epithelialisation in the course of around 7 days. Figures 2 and 3 show the creation of a split-thickness wound using a dermatome, and the wounds themselves.

- Full-thickness wounds, which are made using a circular knife (similar to a biopsy punch) which removes epidermis, dermis and subcutis. Healing is by granulation followed by re-epithelialisation and takes approximately 21 days.

In addition to these two models, we have others that are less often used such as incisional wounds, burn wounds and necrotic wounds.

Typically, the wound type chosen will reflect the human clinical situation and we find that most often, particularly for our efficacy studies, the majority of studies use the full-thickness wound.

Efficacy studies use only a small number of animals, typically 3, with up to 8 wounds per animal. In order to have sufficient space for the wounds (to avoid the various treatments interfering with each other) the animals for these studies need to be reasonably large, and normally they would be ca 30 kg. Normally we use female animals, and the reason for this is that anatomically,

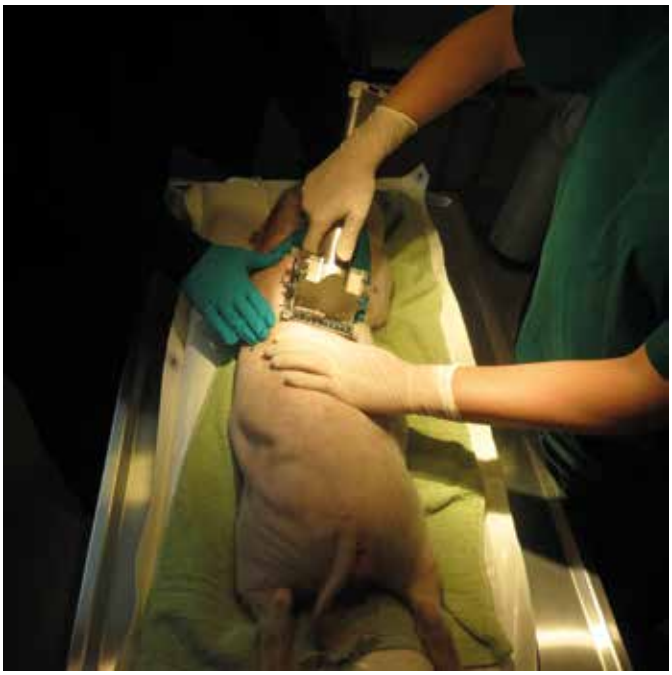


Figure 2, creating a split-thickness wound on a minipig

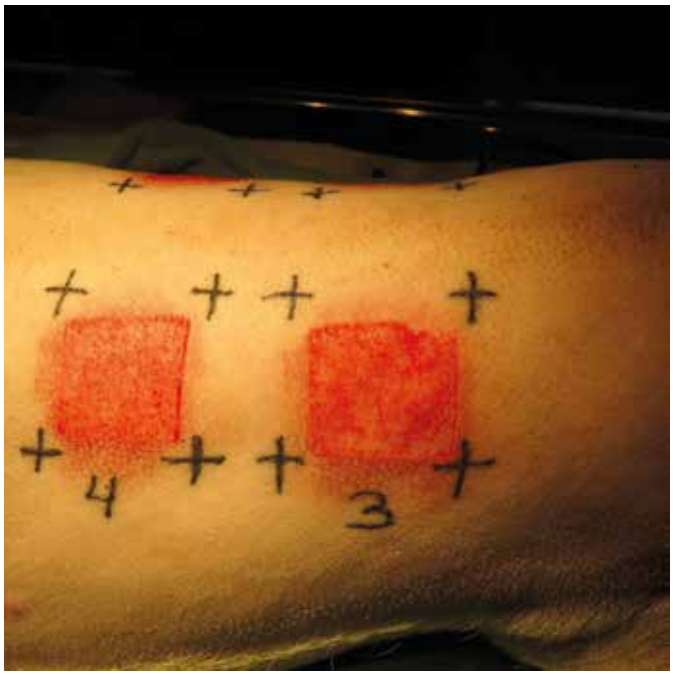


Figure 3, Split-thickness wounds

they are easier to bandage after the surgery. Each animal would have a variety of treatments expressed (e.g. the test material or device, a reference material or device and control) so that each different treatment is represented multiple times and inter-individual variability is minimised. Evaluations performed include haemorrhage, wound secretion, inflammation, necrosis, granulation/hypergranulation and re-epithelialisation. We also perform planimetric evaluations where we measure the total wound area, area of granulation tissue, area of re-epithelialised tissue and the area of necrotic tissue; these evaluations are computerised. Finally, we make histopathological evaluations of the wounds to include rete ridge formation, cellularity and foreign body reaction with special staining for collagen deposits and angiogenesis as optional endpoints.

For certain wound types, other endpoints could also be considered; for instance, for incisional wounds, tensile strength measurements could be included to gauge the efficiency of the wound healing process.

(b) Safety studies

As previously mentioned, we are seeing an increasing interest in a combination of the wound healing study with a full standard safety study. It is most normal that for safety studies, even for dermally applied products intended for use on humans with broken, damaged or compromised skin, the animals are normal and with intact skin. However, it is clear that when pharmaceuticals come into contact with breached skin, the likelihood of systemic exposure is high, and it is necessary to test for the effects of that. Most often this would be done by administration of the compound by a route that gives systemic exposure (e.g. subcutaneous dosing) in one or more studies specifically designed for this purpose.

Use of a combination of wound healing and safety in the same study gives some interesting challenges, but is certainly a real possibility. The main challenges to be overcome are the number of animals involved (instead of the 3 that we would use in a standard efficacy study, we will need to use standard toxicology group sizes of 3 males and 3 females per group or more). This is because each animal will only have one treatment (control, comparators and treatments will each be used in their own group of animals). Additionally, in order to make the space for 8 wounds in an efficacy study we require animals in the range of 25 kg or more. With animals of the size used in a typical safety study (ca 10 kg at study start) we cannot make so many wounds on an animal. Furthermore, when dosing into the wounds (which are typically in an area of 2 to 4 cm2) the total dose administered will be relatively low compared to what could be achieved by other routes, and this should be taken into consideration. Finally, once the wounds are healed, we cannot repeat the wounding, so in studies of longer duration it is normal to go over to another route of administration that maximises systemic exposure.

The advantage of this study design in the context of safety studies is that we can obtain information that we cannot in efficacy studies e.g. clinical pathology, electrocardiography and so on.

In conclusion, the minipig, and in particular the Göttingen Minipig, is ideal for studies to model the wound healing process. Efficacy studies have for many years been a standard procedure for CiToxLAB. In recent years, we have seen an increasing interest in, and demand for, a combination of wound healing with regulatory safety studies.

First grant awarded by the Ellegaard Göttingen Minipigs Research Foundation

Construction and activity of recombinant cytochrome P450 enzymes as an *in vitro* system for reaction phenotyping of drug candidates in the Göttingen Minipig

By Steven Van Cruchten[1], Miriam Ayuso[1], Alain Labro[2], Neil Parrott[3]
[1] Department of Veterinary Sciences, University of Antwerp, Belgium
[2] Department of Biomedical Sciences, University of Antwerp, Belgium
[3] Modelling & Simulation, Roche Pharma Research and Early Development, Switzerland

In Newsletter 47 (Winter 2016) it was announced that the Scientific Board of the Ellegaard Göttingen Minipigs Research Foundation awarded our project "Construction and activity of recombinant cytochrome P450 enzymes as an in vitro system for reaction phenotyping of drug candidates in the Göttingen Minipig" with a price of totally EUR 100,000 for a period of 2 years. In the following paragraphs, we will give a brief description of the project and the current status.

Most pharmaceutical companies perform already early on during drug development *in vitro* drug metabolism studies using either subcellular (e.g. liver microsomes, S9 fraction) or cellular/tissue systems (e. g. hepatocytes, liver slices) of several species, including man. These data are used for proper species selection and species comparison in preclinical safety studies and can be introduced as well in a physiologically based pharmacokinetic (PBPK) model to predict drug clearance *in vivo*. PBPK models have been established and applied for the most commonly used non-clinical species (mice, rat, dog), and also for the Göttingen Minipig efforts are ongoing to establish such a model[1-2]. Although several enzymes can be involved in drug clearance, the cytochrome P450 (CYP) enzymes, and in particular the CYP1-3 families, are one of the most important groups of enzymes. Within these families, several subfamilies with distinct isoenzymes exist and species differences regarding CYP abundance, activity and specificity occur, which may influence drug clearance among species. Also for the Göttingen Minipig, several CYP isoforms have been identified, such as CYP3A22 and CYP3A29[3]. However, in contrast to the other commonly used non-clinical species, less is known about the substrate affinity and specificity of Göttingen Minipig CYP isoforms. This is important information since these features determine drug clearance and as such drug exposure.

Steven Van Cruchten, Abbi Van Tilborg and Miriam Ayuso in the lab of Alain Labro.

Miriam places PCR tubes containing DNA and DNA-polymerase, together with some other co-factors, into the thermocycler to perform a PCR. After the reaction is completed, the gene of interest will have been copied several million times.

To unravel these differences, recombinant CYPs are a useful tool and they are commercially available for man, mouse, rat, dog and monkey, allowing a more precise species comparison than subcellular/cellular systems and thus better species selection. However, for the Göttingen Minipig no such enzymes are on the market.

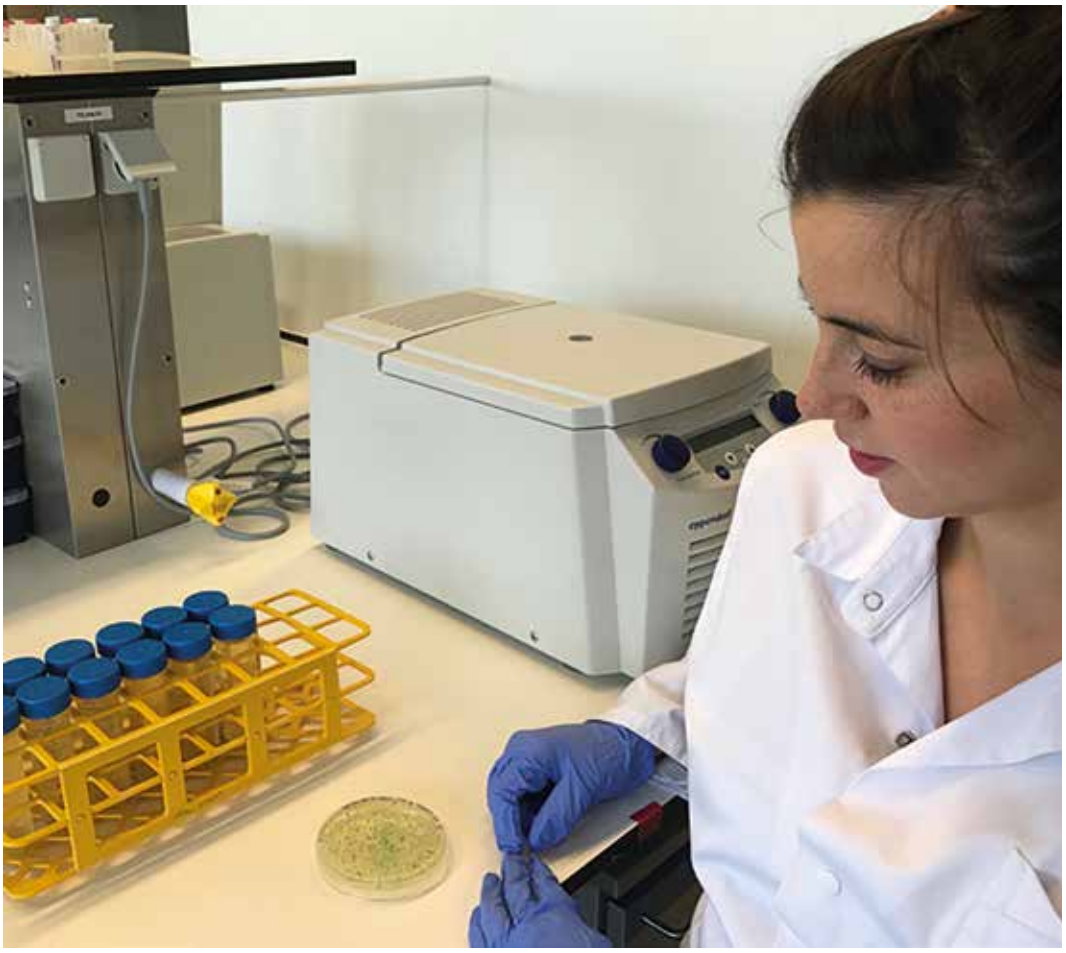
The aims of our project are first to construct recombinant CYP1A2, CYP2A19, CYP2C33, CYP2C42, CYP2C49, CYP2D25, CYP3A22, CYP3A29, CYP3A39 and CYP3A46 proteins. Secondly, to assess whether the Göttingen Minipig recombinant enzymes are active by using fluorogenic compounds, followed by further characterisation using tool compounds that have been characterised in man and minipig[4]. Thirdly, to determine pharmacokinetic parameters of drugs in the recombinant system and compare them with those in minipig liver microsomes. Finally, these data will be related to *in vivo* drug clearance data in Göttingen Minipigs.

We expect clear differences in substrate affinity between the different recombinant CYP isoenzymes. Furthermore, the pharmacokinetic parameters in the recombinant system and the minipig liver microsomes will help to understand the relative contribution of the different CYP isoforms in the metabolism of the tool compounds. Finally, the comparison of the *in vitro* data with the *in vivo* PK data will help to further build the Göttingen Minipig PBPK model.

The subfamily CYP3A is the most important one involved in drug metabolism in terms of clinically used human drugs, and therefore we started the recombinant work with the minipig CYP3A22, CYP3A29, CYP3A39 and CYP3A46. As NADPH P450 reductase and Cytochrome b5 are necessary in the *in vitro* system, we also included those enzymes.

So far, we have successfully amplified and cloned CYP3A22, CYP3A29, CYP3A46 and cytochrome b5 from Göttingen Minipig. For activity assessment of these CYPs, we are currently using the pig NADPH P450 reductase.

Different clones from each enzyme (obtained after different PCR reactions) were compared to identify differences in the nucleotide sequence due to amplification (PCR) errors. The clones showing the highest similarity with the reference sequence and no amplification errors (obtained from CYP3A22 and CYP3A29) were selected and ligated into an expression vector. CYP3A46 and cytochrome b5 clones presented non-synonymous amino acid changes and deletions that needed correction prior to expression, as these may result in an inactive protein. These corrections are currently being performed by PCR (the CYP3A46



Abbi is ready to select bacterial colonies from an agar plate. The TOPO-TA cloning system allows for white/blue screening, a method for selection of bacteria containing the successfully ligated insert, which can be easily identified by its white coloration from the unsuccessful blue ones.

sequence has been successfully corrected and is now ready to be ligated into the expression vector and two more mutation needs to be done in cytochrome b5).

The protocol for expression of CYP3A22 and CYP3A29 is now optimized. The expression vector was modified by adding a bacterial signal peptide. This is known to increase the expression of heterologous proteins in *E. Coli* and to facilitate the correct folding and inclusion into the cellular membranes of CYP proteins.

Both CYP3A22 and CYP3A29 have been successfully inserted into this new vector and expression will be carried out as the next step. This will then be followed by the other CYP enzymes.

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Services in the Research Barrier at Ellegaard Göttingen Minipigs

By Henrik Duelund Pedersen, Adrian Zeltner
Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark

The Research Barrier at Ellegaard Göttingen Minipigs opened last summer, and has since been used for a variety of different activities. Here, we would like to share the many opportunities this barrier opens to our customers.

Surgically prepared animal models

The large operating theater in the Research Barrier has two surgical stations, allowing a multitude of surgical activities. It is, for instance, possible for us to deliver Göttingen Minipigs pre-implanted with catheters, vascular access ports, telemetry transmitters (see the article elsewhere in this Newsletter) or other devices. We can also provide neutered animals, i.e. castrated males or ovariectomized females. A room leading up to the operating theater is dedicated to preparing the animals for surgery; another room allows surgeons to prepare; and there is also a dedicated recovery room with heated floor and oxygen supply. The operations can be performed together with costumer staff and/or experts specialized in certain techniques – as illustrated by the picture below.

Training and feasibility studies

The state-of-the-art anesthesia equipment in the operating theater is used for anesthesia courses, as described together with other courses offered at Ellegaard Göttingen Minipigs in Newsletter 49. Also, the facilities have been used for several feasibility studies focused on new catheters, new devices, innovative diagnostic techniques, and novel animal models. The animals used in the various studies in the Research Barrier come from one of our two breeding barriers, and the barrier conditions and health status is kept the same throughout.

Diet induced models

The pens in the Research Barrier are designed to house all sizes of Göttingen Minipigs, allowing us to prepare diet-induced obese animals. Also, other diet-induced models can be prepared; such as animals with atherosclerosis or non-alcoholic steatohepatitis (NASH). In Göttingen Minipigs with atherosclerosis, the ultrasound equipment in the Research Barrier allows us to monitor, semi-quantitatively, the build-up of plaque in the abdominal aorta. Regarding NASH, we participate in the Innovative Medicines Initiative 2 project LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis; www.litmus-project.eu), having the task to develop and characterize a Göttingen Minipig model of the disease. The picture above shows diet-induced obese Göttingen Minipigs housed in the Research Barrier.

Breeding of transgenic Göttingen Minipigs

Ellegaard Göttingen Minipigs has an agreement with the University of Göttingen enabling us to develop and commercialize transgenic Göttingen Minipigs, and to offer these rights to third parties. We already participate in several collaborations aimed at establishing transgenic models, and are interested in even more. Our key role in such collaborations will typically be to breed the transgenic animals in the Research Barrier.

The above gives a flavor of the many opportunities given by the Research Barrier, and if you feel that we in any way might be of assistance, please contact us. We believe in science and collaboration.

New scientific publications on the Göttingen Minipig

Ellegaard Göttingen Minipigs A/S gives high priority to collaborative projects that aim to better characterize and validate the Göttingen Minipig as a translational animal model and which facilitate and refine the use of the minipig in research projects and safety testing. Please contact us if you have an idea for such a collaborative project. Below is a list of a few recent articles on the Göttingen Minipig.

- Corte GM, Plendl J, Hünigen H, Richardson KC, Gemeinhardt O, Niehues SM. **Refining experimental dental implant testing in the Göttingen Minipig using 3D computed tomography-A morphometric study of the mandibular canal.** *PLoS One.* 2017 Sep 14;12(9):e0184889. doi: 10.1371/journal.pone.0184889. eCollection 2017.

- Overgaard NH, Frøsig TM, Jakobsen JT, Buus S, Andersen MH, Jungersen G. **Low antigen dose formulated in CAF09 adjuvant Favours a cytotoxic T-cell response following intraperitoneal immunization in Göttingen minipigs.** *Vaccine*. 2017 Oct 9;35(42):5629-5636. doi: 10.1016/j.vaccine.2017.08.057. Epub 2017 Sep 5.

- Yamamoto S, Karashima M, Sano N, Fukushi C, Tohyama K, Arai Y, Hirabayashi H, Amano N. **Utility of Göttingen minipigs for Prediction of Human Pharmacokinetic Profiles After Dermal Drug Application.** *Pharm Res.* 2017 Nov;34(11):2415-2424. doi: 10.1007/s11095-017-2247-7. Epub 2017 Aug 21.

- Otto S, Pautke C, Martin Jurado O, Nehrbass D, Stoddart MJ, Ehrenfeld M, Zeiter S. **Further development of the MRONJ minipig large animal model.** *J Craniomaxillofac Surg.* 2017 Sep;45(9):1503-1514. doi: 10.1016/j.jcms.2017.07.002. Epub 2017 Jul 18.

- Roldán JC, Klünter T, Schulz P, Deisinger U, Diez C, Waiss W, Kirschneck C, Reichert TE, Detsch R. **Bone Morphogenetic Protein-7 Enhances Degradation of Osteoinductive Bioceramic Implants in an Ectopic Model.** *Plast Reconstr Surg Glob Open.* 2017 Jun 22;5(6):e1375. doi: 10.1097/GOX.0000000000001375. eCollection 2017 Jun.

- Staunstrup NH, Stenderup K, Mortensen S, Primo MN, Rosada C, Steiniche T, Liu Y, Li R, Schmidt M, Purup S, Dagnæs-Hansen F, Schrøder LD, Svensson L, Petersen TK, Callesen H, Bolund L, Mikkelsen JG. **Psoriasiform skin disease in transgenic pigs with high-copy ectopic expression of human integrins α2 and β1**. *Dis Model Mech*. 2017 Jul 1;10(7):869-880. doi: 10.1242/dmm.028662.

- Jansson K, Dreckmann K, Sommer W, Avsar M, Salman J, Siemeni T, Knöfel AK, Pauksch L, Gottlieb J, Frühauf J, Werner M, Jonigk D, Strüber M, Haverich A, Warnecke G. Splenocyte **Infusion and Whole-Body Irradiation for Induction of Peripheral Tolerance in Porcine Lung Transplantation: Modifications of the Preconditioning Regime for Improved Clinical Feasibility.** *Transplant Direct.* 2017 Jun 6;3(7):e170. doi: 10.1097/TXD.0000000000000689. eCollection 2017 Jul.

- Callesen MM, Árnadóttir SS, Lyskjaer I, Ørntoft MW, Høyer S, Dagnaes-Hansen F, Liu Y, Li R, Callesen H, Rasmussen MH, Berthelsen MF, Thomsen MK, Schweiger PJ, Jensen KB, Laurberg S, Ørntoft TF, Elverløv-Jakobsen JE, Andersen CL. **A genetically inducible porcine model of intestinal cancer.** *Mol Oncol*. 2017 Nov;11(11):1616-1629. doi: 10.1002/1878-0261.12136. Epub 2017 Oct 10.

- Berthelsen MF, Callesen MM, Østergaard TS, Liu Y, Li R, Callesen H, Dagnæs-Hansen F, Hamilton-Dutoit S, Jakobsen JE, Thomsen MK. **Pancreas specific expression of oncogenes in a porcine model.** *Transgenic Res.* 2017 Jun 29. doi: 10.1007/s11248-017-0031-4. [Epub ahead of print]

Meeting calendar 2018

Name	Date	Location
ToxExpo and SOT Annual Meeting	11 - 15 March	San Antonio, Texas, USA
IAT Congress	20 - 23 March	Northern England
Scand-LAS	26 - 28 April	Kristiansand, Norway
Minipig Research Forum	16 - 18 May	Barcelona, Spain
AFSTAL	13 - 15 June	Reims, France
EUROTOX	2 - 5 September	Brussels, Belgium
ESTP	11 - 14 September	Copenhagen, Denmark
GV-SOLAS	12 - 14 September	Munich, Germany
SPS	30 September - 3 October	Washington, DC, USA
ACT	4 - 7 November	Palm Beach, Florida, USA
Bio-Europe	5 - 7 November	Copenhagen, Denmark / Malmö, Sweden
AFLAS	19 - 21 November	Bangalore, India

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