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Göttingen Minipigs
MAGAZINE

ELLEGAARD
GÖTTINGEN MINIPIGS

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Dear reader

A lot has happened over the Summer. In May 2023, we finished celebrating our 30-year anniversary - a journey that, when looking back, we can proudly say have brought us safely down new paths and into the future.

Genetically altered minipig models is part of that future and an area in rapid development. In this edition of the Göttingen Minipigs Magazine, you will get the very first taste of the development of an entirely new breed: Göttingen Micropigs. Based on Göttingen Minipigs DNA, an even smaller breed has successfully been developed, and I am very excited finally being able to unveil this very promising work.

Another genetically altered Göttingen Minipig model, is the Humanized IgG Göttingen Minipigs. This magazine presents you with an article showcasing the hematology, coagulation, and clinical chemistry parameters of Humanized IgG Göttingen Minipigs. Data which are of course also available to our readers and customers (see p. 6).

In May, the Minipig Research Forum was conducted. It was a pleasure seeing both familiar and new faces taking part in this 3-day conference, sharing knowledge and networking across organisations and fields of expertise.

In this connection, I would like to highlight a new initiative, which actually came to be during the Minipig Research Forum: A new pig biomarker knowledge sharing group was established, with the purpose of sharing data and establishing qualified biomarker assays for minipigs across companies and academia.

This can potentially reduce time and resources spend in minipig studies, not to mention improve the utility if minipigs in biomedical research.

Last but not least, the conference high season is just around the corner, and I hope to see you out there.

Martin Windfeld Velin, CEO
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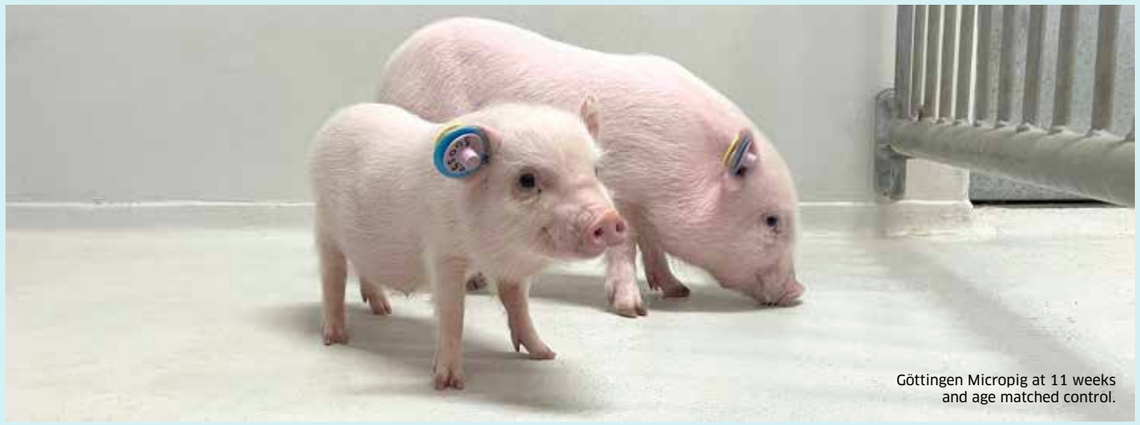
Growth Hormone Receptor Knockout Results in New Göttingen Micropigs

Through genetic alteration, the inactivation of the Growth Hormone Receptor (GHR) gene has resulted in the development of a novel porcine strain: Göttingen Micropigs. Through targeted genetic intervention, researchers have achieved growth retardation, resulting in the establishment of a distinct breed characterized by miniature proportions.

At the centre of this scientific endeavour lies the GHR gene, a key mediator in growth signalling. By using the CRISPR/Cas9 technology, scientists executed a gene knockout strategy by inserting a 7bps segment of genetic material into the gene. This resulted in loss of functionality and effectively disrupted the normal functioning of the GHR gene.

Initial physiological data for the Göttingen Micropigs confirmed the endocrine effect of this disruption by demonstrating significant changes in growth hormone (GH) and insulin-like growth factor 1 (IGF1) concentrations in the blood. Under normal physiological conditions, pituitary-derived GH stimulates hepatic production of IGF1, a key hormone in growth promotion in peripheral tissue. A lack of functional GHR diminishes IGF1 production from the liver, and as IGF1 production is reduced so is the IGF1-mediated feedback regulation of GH. Consequently, GH levels are continuously elevated. The low level of IGF1 and the high but non-functional GH action ultimately leads to growth retardation.



Göttingen Micropig at 11 weeks and age matched control.

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How Göttingen Micropigs has become a reality

The Growth Hormone Receptor Knockout is a genetic intervention, which results in the lineage of GHR-KO animals, characterised by a substantial deceleration in postnatal growth. The new Göttingen Micropig breed is distinguished by their markedly reduced size compared to their relatives, Göttingen Minipigs.

Göttingen Micropigs are expected to be commercially available in 2024-25.

In addition to the observed changes in GH and IGF1 levels, we expect the Göttingen Micropigs to have other distinctive physiological features as seen in humans with Laron Syndrome. Based on previous research of GHR KO on German Landrace background no deleterious effects on the cardiovascular, immune system or metabolism are indicated, and the genetic modification of GHR and resulting altered growth patterns is not expected to cause general health implications. The Göttingen Micropigs will be fully immunocompetent and have the same health status as the Göttingen Minipigs bred and housed at Ellegaard Göttingen Minipigs A/S.

Next step

Introducing the GHR-KO mutation in Göttingen Minipigs has proven a suitable approach in the attempt to develop an even smaller breed of minipigs. Next step will be to characterize the new Göttingen Micropigs under standardized conditions and to breed it to a genetically stable herd available for commercial biomedical purposes.

MORE INFORMATION

For more information about Göttingen Micropigs or other genetically altered Göttingen Minipigs, incl. considerations of using Göttingen Minipigs as a background strain for development of new models, please contact Ellegaard Göttingen Minipigs A/S at ellegaard@minipigs.dk.

Göttingen Micropig at 11 weeks and age matched control.

Hematology, coagulation and clinical chemistry data on Humanized IgG Göttingen Minipigs

By Andres Eskjær Jensen[1], Mikkel Lykke Jensen[2], Kirsten Rosenmay Jacobsen[1], Jes Tovborg Jensen[2]

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Therapeutic antibodies have become an important treatment modality in a variety of clinical disease areas such as cancer and autoimmune diseases. However, toxicological testing of human antibodies can be challenging as these results in a xeno-response in the animal models, resulting in rapid clearance of the drug and toxicities through formation of anti-drug-antibodies (ADA). This makes most animal models unsuitable to predict adverse outcomes in human patients. The transgenic Göttingen Minipigs carrying a mini repertoire of human Ig-γ1 heavy, and the human κ light chain genes show tolerance to multiple human recombinant antibodies (Flisikowska et al, 2022).

Photo 1
Humanized IgG Göttingen Minipigs.

Introduction

The humanized Göttingen Minipigs may provide a novel model – and an alternative or supplement to non-human primates - for safety testing of therapeutic antibodies.

In this article, the hematology, coagulation and clinical chemistry parameters of the Humanized Göttingen Minipigs will be presented. The characterization of blood parameters took place from April 2022 until April 2023.

Methods and Materials

Animals

A total of 57 animals were included in the characterization (30 males and 27 females). Animals were not followed over time but chosen based on availability to capture selected age groups, hence most animals appeared multiple times in the data set, but not necessarily at all timepoints. The age groups were within ±3 days of the age in weeks. The housing of the animals was in accordance with EU Directive 2010/63/ on the protection of animals used for scientific purposes in the Research Barrier of Ellegaard Göttingen Minipigs, Dalmose, Denmark. The Research Barrier is fully AAALAC accredited and in compliance to the Eighth Edition of the Guide for the Care and Use of Laboratory Animals (NRC 2011).

Procedures

Blood was collected by jugular vein puncture on non-fasted animals fixated in slings. Blood for hematology was collected in K3 EDTA tubes and for coagulation tests plasma was isolated from citrate stabilized blood. Clinical chemistry was measured on serum from blood collected in tubes with clotting activator.

Equipment

Blood samples were analyzed at Scantox A/S, Denmark. Hematology parameters were analyzed on a Yumizen H2500 hematology analyzer from Horiba Medical. Clinical chemistry samples were analyzed on a Cobas 6000 analyzer from Roche Diagnostics. Coagulation parameters were analyzed on an ACL 9000 analyzer from Instrumentation Laboratories. The equipment was fully validated to measure minipig derived samples.

Age (weeks)		White Blood Count (^9/L)	Red Blood Count (^12/L)	Haemoglobin (mmol/L)	Haematocrit (L/L)	Platelet Count (^9/L)	Lymphocytes (Relative) (%(UD))	Lymphocytes (Absolute) (^9/L)	Monocytes (Relative) (%(UD))	Monocytes (Absolute) (^9/L)	Neutrophils (Relative) (%(UD))	Neutrophils (Absolute) (^9/L)	Eosinophils (Relative) (%(UD))	Eosinophils (Absolute) (^9/L)	Basophils (Relative) (%(UD))	Basophils (Absolute) (^9/L)	Reticulocyte (Relative) (%(UD))	Reticulocyte Absolute (^12/L)
6	Female (n=7)	Mean ± SD	11,3 ± 4,6	6,6 ± 1,1	7,6 ± 0,9	0,33 ± 0,06	517 ± 147	81 ± 6	9,3 ± 4,2	0,9 ± 0,8	16,2 ± 5,3	1,73 ± 0,76	2,06 ± 1,17	0,2 ± 0,09	0,1 ± 0,082	0,011 ± 0,015	0,47 ± 0,13	0,034 ± 0,008
		95% CI	7,9 – 14,7	5,8 – 7,3	6,9 – 8,2	0,29 – 0,38	408 – 625	76 – 85	6,2 – 12,4	0,3 – 1,5	12,2 – 20,1	1,16 – 2,29	1,19 – 2,92	0,14 – 0,27	0,04 – 0,16	0,001 – 0,022	0,34 – 0,59	0,026 – 0,042
	Male (n=9)	Mean ± SD	11,4 ± 2,7	6,9 ± 0,6	7,4 ± 0,6	0,35 ± 0,02	553 ± 162	75 ± 10	8,6 ± 2,4	0,9 ± 0,56	22,8 ± 9,2	2,54 ± 1,04	1,63 ± 1,6	0,19 ± 0,22	0,067 ± 0,05	0,007 ± 0,005	1,23 ± 1,68	0,084 ± 0,109
		95% CI	9,6 – 13,1	6,5 – 7,3	7 – 7,8	0,34 – 0,37	447 – 659	68 – 81	7 – 10,2	0,53 – 1,27	16,8 – 28,8	1,86 – 3,21	0,59 – 2,68	0,04 – 0,33	0,034 – 0,099	0,003 – 0,01	–0,01 – 2,47	0,003 – 0,165
16	Female (n=8)	Mean ± SD	13,2 ± 0,8	8,3 ± 0,8	8,4 ± 0,5	0,41 ± 0,02	402 ± 50	75 ± 6	9,9 ± 0,8	0,91 ± 0,31	12,2 ± 1,8	1,73 ± 0,76	2,79 ± 2,21	0,29 ± 0,25	0,05 ± 0,076	0,005 ± 0,008	1,42 ± 0,47	0,108 ± 0,037
		95% CI	12,6 – 13,7	7,7 – 8,9	8 – 8,7	0,4 – 0,43	367 – 436	71 – 79	9,3 – 10,5	0,7 – 1,13	11,6 – 12,8	1,16 – 2,29	2,26 – 4,32	0,11 – 0,46	0,000 – 0,102	0 – 0,01	1,09 – 1,74	0,082 – 0,133
	Male (n=8)	Mean ± SD	11,5 ± 3,4	7,9 ± 0,3	7,9 ± 0,3	0,39 ± 0,01	476 ± 112	57 ± 20	6,1 ± 1	0,84 ± 0,34	41,2 ± 20,1	5,3 ± 3,82	0,63 ± 0,35	0,07 ± 0,03	0,075 ± 0,046	0,008 ± 0,005	0,71 ± 0,28	0,056 ± 0,021
		95% CI	9,2 – 13,9	7,7 – 8,1	7,7 – 8,1	0,38 – 0,4	398 – 554	43 – 71	5,4 – 6,8	0,6 – 1,07	27,3 – 55,2	2,65 – 7,94	0,38 – 0,87	0,04 – 0,09	0,043 – 0,107	0,004 – 0,011	0,51 – 0,9	0,041 – 0,07
20	Female (n=8)	Mean ± SD	11 ± 1	8,1 ± 0,5	8,6 ± 0,5	0,42 ± 0,02	400 ± 36	73 ± 7	8,1 ± 1,4	0,94 ± 0,17	23,7 ± 7,3	2,57 ± 0,64	2,45 ± 1,15	0,28 ± 0,15	0,075 ± 0,046	0,008 ± 0,005	0,39 ± 0,17	0,031 ± 0,014
		95% CI	10,3 – 11,7	7,7 – 8,4	8,2 – 8,9	0,4 – 0,43	375 – 425	68 – 78	7,1 – 9	0,82 – 1,05	18,7 – 28,8	2,13 – 3,02	1,65 – 3,25	0,18 – 0,38	0,043 – 0,107	0,004 – 0,011	0,27 – 0,5	0,021 – 0,041
	Male (n=8)	Mean ± SD	9,4 ± 2,5	8,3 ± 0,4	8,5 ± 0,4	0,42 ± 0,01	381 ± 130	63 ± 10	5,7 ± 1	1,24 ± 0,61	34,3 ± 10,6	3,41 ± 1,79	1,25 ± 0,77	0,11 ± 0,07	0,125 ± 0,089	0,011 ± 0,006	0,32 ± 0,14	0,027 ± 0,012
		95% CI	7,6 – 11,1	8 – 8,6	8,2 – 8,8	0,41 – 0,43	291 – 471	56 – 70	5 – 6,4	0,82 – 1,66	27 – 41,7	2,16 – 4,65	0,71 – 1,79	0,07 – 0,16	0,064 – 0,186	0,007 – 0,016	0,23 – 0,42	0,019 – 0,035
29	Female (n=8)	Mean ± SD	10,7 ± 1,5	8,1 ± 0,7	8,9 ± 0,7	0,43 ± 0,03	432 ± 98	74 ± 5	7,9 ± 1,4	0,46 ± 0,19	23,3 ± 6	2,46 ± 0,56	2,68 ± 1,05	0,3 ± 0,17	0,025 ± 0,046	0,003 ± 0,005	0,9 ± 0,6	0,074 ± 0,052
		95% CI	9,7 – 11,7	7,7 – 8,6	8,4 – 9,4	0,4 – 0,45	364 – 500	70 – 77	6,9 – 8,9	0,33 – 0,6	19,2 – 27,5	2,07 – 2,85	1,95 – 3,4	0,18 – 0,42	0,000 – 0,057	0,000 – 0,006	0,48 – 1,31	0,038 – 0,109
	Male (n=8)	Mean ± SD	8,3 ± 1,2	7,8 ± 0,6	8,4 ± 0,4	0,4 ± 0,02	419 ± 95	67 ± 11	5,4 ± 0,9	0,48 ± 0,29	31 ± 11,7	2,62 ± 1,16	1,94 ± 1,41	0,16 ± 0,11	0,038 ± 0,052	0,004 ± 0,005	1,19 ± 0,96	0,093 ± 0,078
		95% CI	7,4 – 9,1	7,4 – 8,2	8,2 – 8,7	0,39 – 0,42	353 – 484	59 – 74	4,8 – 6,1	0,28 – 0,67	22,9 – 39,1	1,81 – 3,42	0,96 – 2,91	0,08 – 0,24	0,002 – 0,073	0 – 0,007	0,53 – 1,86	0,039 – 0,147
42	Female (n=8)	Mean ± SD	9,9 ± 1,1	7,5 ± 0,7	8,7 ± 0,4	0,42 ± 0,03	418 ± 92	63 ± 9	6,2 ± 0,7	0,56 ± 0,21	33,5 ± 9,3	3,36 ± 1,17	2,79 ± 2,21	0,29 ± 0,25	0,05 ± 0,076	0,005 ± 0,008	1,42 ± 0,47	0,108 ± 0,037
		95% CI	9,2 – 10,7	7 – 8	8,3 – 9	0,4 – 0,44	354 – 481	57 – 69	5,7 – 6,7	0,42 – 0,71	27,1 – 39,9	2,55 – 4,17	1,26 – 4,32	0,11 – 0,46	0,000 – 0,102	0 – 0,01	1,09 – 1,74	0,082 – 0,133
	Male (n=8)	Mean ± SD	9,2 ± 1,9	7,4 ± 0,7	8,4 ± 0,5	0,41 ± 0,03	486 ± 166	64 ± 13	5,7 ± 0,9	0,68 ± 0,12	34,3 ± 13,3	3,32 ± 1,95	1,48 ± 0,7	0,13 ± 0,05	0,025 ± 0,046	0,003 ± 0,005	1,67 ± 0,63	0,127 ± 0,053
		95% CI	7,9 – 10,6	7 – 7,9	8,1 – 8,8	0,39 – 0,43	370 – 601	54 – 73	5 – 6,3	0,59 – 0,76	25,1 – 43,4	1,97 – 4,68	0,99 – 1,96	0,09 – 0,17	0,000 – 0,057	0,000 – 0,006	1,23 – 2,1	0,09 – 0,164
55	Female (n=8)	Mean ± SD	9,3 ± 0,7	7,9 ± 0,9	9,1 ± 0,9	0,43 ± 0,04	427 ± 81	62 ± 9	5,7 ± 0,9	0,6 ± 0,17	35,2 ± 10,3	3,27 ± 1,06	2,6 ± 1,1	0,24 ± 0,1	0 ± 0	0 ± 0	1,52 ± 0,59	0,121 ± 0,055
		95% CI	8,8 – 9,8	7,2 – 8,5	8,5 – 9,8	0,4 – 0,46	367 – 487	55 – 69	5 – 6,4	0,47 – 0,73	27,5 – 42,9	2,49 – 4,06	1,79 – 3,41	0,16 – 0,32			1,08 – 1,96	0,08 – 0,162
	Male (n=8)	Mean ± SD	8,5 ± 2,3	8,4 ± 0,6	9,7 ± 0,7	0,46 ± 0,03	455 ± 111	56 ± 12	4,6 ± 1	0,51 ± 0,23	42,2 ± 11,7	3,73 ± 1,79	1,58 ± 0,64	0,13 ± 0,04	0 ± 0	0 ± 0	1,42 ± 0,56	0,12 ± 0,05
		95% CI	6,9 – 10,1	7,9 – 8,8	9,2 – 10,2	0,43 – 0,48	378 – 533	48 – 64	3,9 – 5,3	0,35 – 0,67	34,1 – 50,3	2,49 – 4,97	1,13 – 2,02	0,1 – 0,16			1,03 – 1,8	0,085 – 0,155
68	Female (n=8)	Mean ± SD	11,5 ± 2,4	6,6 ± 1,4	8,6 ± 0,9	0,36 ± 0,08	340 ± 106	59 ± 9	6,9 ± 2	0,44 ± 0,23	37,5 ± 8,5	4,22 ± 1,06	2,6 ± 1,13	0,3 ± 0,15	0,113 ± 0,083	0,013 ± 0,01	0,8 ± 0,62	0,063 ± 0,055
		95% CI	9,8 – 13,1	5,6 – 7,6	8 – 9,2	0,3 – 0,41	266 – 413	53 – 65	5,5 – 8,2	0,28 – 0,6	31,7 – 43,4	3,49 – 4,95	1,81 – 3,39	0,2 – 0,41	0,055 – 0,17	0,005 – 0,02	0,26 – 1,34	0,015 – 0,112
	Male (n=8)	Mean ± SD	12,3 ± 1,3	7,5 ± 0,7	9,4 ± 0,4	0,43 ± 0,05	272 ± 77	55 ± 10	6,8 ± 1,6	0,28 ± 0,14	43,1 ± 9,7	5,29 ± 1,26	1,93 ± 0,85	0,24 ± 0,12	0,063 ± 0,052	0,006 ± 0,005	0,52 ± 0,2	0,041 ± 0,016
		95% CI	11,4 – 13,2	7 – 8	9,1 – 9,7	0,39 – 0,47	219 – 325	48 – 61	5,7 – 7,9	0,18 – 0,37	36,4 – 49,8	4,42 – 6,16	1,34 – 2,51	0,16 – 0,32	0,027 – 0,098	0,003 – 0,01	0,35 – 0,68	0,028 – 0,053
76	Female (n=8)	Mean ± SD	9,5 ± 1,2	7,5 ± 0,4	8,5 ± 0,2	0,41 ± 0,01	361 ± 87	58 ± 5	5,5 ± 0,7	1,13 ± 0,47	36,9 ± 5,8	3,54 ± 0,79	3,76 ± 1,85	0,36 ± 0,17	0,163 ± 0,16	0,015 ± 0,014	0,41 ± 0,15	0,031 ± 0,012
		95% CI	8,7 – 10,3	7,2 – 7,7	8,4 – 8,7	0,4 – 0,42	301 – 421	55 – 61	5,1 – 6	0,8 – 1,45	32,9 – 41	2,99 – 4,09	2,48 – 5,04	0,24 – 0,47	0,052 – 0,273	0,005 – 0,025	0,31 – 0,52	0,023 – 0,039
	Male (n=8)	Mean ± SD	11,3 ± 1,8	8 ± 0,6	9,7 ± 0,7	0,47 ± 0,04	301 ± 60	52 ± 6	5,9 ± 1,1	0,9 ± 0,23	43,5 ± 6,6	4,91 ± 1,1	3,1 ± 1,84	0,35 ± 0,2	0,088 ± 0,083	0,009 ± 0,008	0,45 ± 0,22	0,035 ± 0,016
		95% CI	10,1 – 12,5	7,6 – 8,4	9,2 – 10,2	0,44 – 0,5	259 – 342	49 – 56	5,2 – 6,7	0,74 – 1,06	38,9 – 48	4,15 – 5,67	1,82 – 4,38	0,21 – 0,49	0,03 – 0,145	0,003 – 0,015	0,29 – 0,6	0,024 – 0,046

Table 1
Hematology data - direct measurement.

Age (weeks)			Mean Cell Volume (fL)	Mean Cell Haemaglobin (fmol)	Mean Cell HG Conc (mmol/L)
6	Female (n=7)	Mean ± SD	51 ± 7	1,19 ± 0,29	23,3 ± 4,7
		95% CI	46 – 56	0,98 – 1,4	19,8 – 26,8
	Male (n=9)	Mean ± SD	52 ± 5	1,08 ± 0,16	21 ± 1,6
		95% CI	49 – 54	0,98 – 1,19	19,9 – 22
16	Female (n=8)	Mean ± SD	50 ± 4	1,02 ± 0,11	20,3 ± 0,4
		95% CI	47 – 53	0,94 – 1,09	20 – 20,6
	Male (n=8)	Mean ± SD	50 ± 2	1 ± 0,05	20,1 ± 0,3
		95% CI	48 – 51	0,97 – 1,04	19,9 – 20,4
20	Female (n=8)	Mean ± SD	52 ± 5	1,07 ± 0,12	20,4 ± 0,2
		95% CI	49 – 56	0,99 – 1,15	20,3 – 20,6
	Male (n=8)	Mean ± SD	51 ± 3	1,03 ± 0,06	20,3 ± 0,3
		95% CI	49 – 53	0,99 – 1,07	20 – 20,5
29	Female (n=8)	Mean ± SD	53 ± 6	1,1 ± 0,13	20,9 ± 0,2
		95% CI	49 – 57	1,01 – 1,19	20,7 – 21,1
	Male (n=8)	Mean ± SD	52 ± 4	1,09 ± 0,08	20,9 ± 0,2
		95% CI	49 – 55	1,03 – 1,14	20,8 – 21,1
42	Female (n=8)	Mean ± SD	56 ± 7	1,16 ± 0,14	20,8 ± 0,5
		95% CI	51 – 61	1,06 – 1,26	20,5 – 21,2
	Male (n=8)	Mean ± SD	55 ± 3	1,13 ± 0,07	20,8 ± 0,5
		95% CI	53 – 57	1,09 – 1,18	20,4 – 21,1
55	Female (n=8)	Mean ± SD	56 ± 7	1,17 ± 0,16	21,2 ± 0,1
		95% CI	50 – 61	1,05 – 1,29	21 – 21,3
	Male (n=8)	Mean ± SD	55 ± 3	1,16 ± 0,07	21,1 ± 0,2
		95% CI	53 – 57	1,11 – 1,21	21 – 21,3
68	Female (n=8)	Mean ± SD	55 ± 5	1,35 ± 0,24	25,1 ± 5,6
		95% CI	51 – 58	1,18 – 1,52	21,2 – 28,9
	Male (n=8)	Mean ± SD	57 ± 4	1,27 ± 0,15	22,2 ± 3,2
		95% CI	54 – 60	1,16 – 1,37	20 – 24,5
76	Female (n=8)	Mean ± SD	55 ± 3	1,15 ± 0,06	20,8 ± 0,2
		95% CI	53 – 57	1,1 – 1,19	20,6 – 20,9
	Male (n=8)	Mean ± SD	59 ± 4	1,22 ± 0,08	20,6 ± 0,3
		95% CI	56 – 62	1,16 – 1,27	20,4 – 20,8

Table 2
Hematology data – calculated.

Age (weeks)			Fibrinogen C (g/L)	Prothrombin time HS+ (s)	APTTsyn (s)
6	Female (n=7)	Mean ± SD	2,72 ± 0,27	13,85 ± 1,04	11,7 ± 1
		95% CI	2,5 – 2,94	13,02 – 14,68	10,9 – 12,5
	Male (n=9)	Mean ± SD	3,06 ± 0,49	14,03 ± 1	11,6 ± 1
		95% CI	2,75 – 3,38	13,38 – 14,69	10,9 – 12,2
16	Female (n=8)	Mean ± SD	2,63 ± 0,37	13,34 ± 0,8	11,4 ± 0,6
		95% CI	2,38 – 2,89	12,79 – 13,89	10,9 – 11,8
	Male (n=8)	Mean ± SD	4 ± 0,97	14,13 ± 0,69	10,7 ± 0,4
		95% CI	3,33 – 4,67	13,64 – 14,61	10,4 – 11
20	Female (n=8)	Mean ± SD	2,74 ± 0,37	13,73 ± 1,06	11,2 ± 0,6
		95% CI	2,48 – 3	12,99 – 14,46	10,8 – 11,7
	Male (n=8)	Mean ± SD	3,39 ± 0,67	14,15 ± 1,07	10,5 ± 0,1
		95% CI	2,93 – 3,85	13,41 – 14,89	10,5 – 10,6
29	Female (n=8)	Mean ± SD	2,7 ± 0,38	13,49 ± 0,72	11,4 ± 0,7
		95% CI	2,43 – 2,96	12,99 – 13,99	10,9 – 11,9
	Male (n=8)	Mean ± SD	3,27 ± 0,35	14,14 ± 0,52	10,5 ± 0
		95% CI	3,02 – 3,51	13,78 – 14,5	n=2
42	Female (n=8)	Mean ± SD	2,83 ± 0,25	12,96 ± 0,65	10,9 ± 0,5
		95% CI	2,65 – 3	12,52 – 13,41	10,5 – 11,3
	Male (n=8)	Mean ± SD	3,69 ± 0,96	13,51 ± 1,02	10,9 ± 0,5
		95% CI	3,03 – 4,36	12,8 – 14,22	10,5 – 11,2
55	Female (n=8)	Mean ± SD	2,87 ± 0,18	13,29 ± 1,06	12,1 ± 0,7
		95% CI	2,75 – 3	12,55 – 14,02	11,7 – 12,6
	Male (n=8)	Mean ± SD	4,42 ± 1,3	14,65 ± 0,73	11,5 ± 1
		95% CI	3,51 – 5,32	14,15 – 15,15	10,8 – 12,2
68	Female (n=8)	Mean ± SD	2,71 ± 0,39	13,23 ± 0,74	11,1 ± 1
		95% CI	2,44 – 2,98	12,71 – 13,74	10,4 – 11,8
	Male (n=8)	Mean ± SD	3,35 ± 0,67	14,06 ± 0,56	11,1 ± 0,9
		95% CI	2,89 – 3,82	13,68 – 14,45	10,5 – 11,8
76	Female (n=8)	Mean ± SD	2,78 ± 0,45	13,98 ± 0,59	11,1 ± 0,9
		95% CI	2,46 – 3,09	13,56 – 14,39	10,5 – 11,7
	Male (n=8)	Mean ± SD	3,61 ± 0,68	14,69 ± 0,31	10,7 ± 1,8
		95% CI	3,14 – 4,08	14,47 – 14,9	9,5 – 12

Table 3
Coagulation data.

Photo 2
Blood sampling in sling.

Age (weeks)			Alanine Aminotransferase (µk/L)	Aspartate Aminotransferase (µk/L)	Alkaline Phosphatase (µk/L)	Gamma Glutamyl transferase (µk/L)	Bilirubin (Total) (µm/L)	Cholesterol (mmol/L)	Triglycerides (mmol/L)	Carbamide Urea (mmol/L)	Creatinine (µm/L)	Glucose (mmol/L)	Calcium (mmol/L)	Magnesium (mmol/L)	Phosphorus (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Albumin (g/L)	Total Protein (g/L)
6	Female (n=7)	Mean ± SD	0,91 ± 1,27	0,83 ± 1,3	4,7 ± 2	0,97 ± 0,18	1,42 ± 0,67	4,7 ± 2,7	1,4 ± 0,46	1,85 ± 0,65	65,7 ± 17,3	5,5 ± 1,5	2,7 ± 0,18	1,03 ± 0,03	2,9 ± 0,6	139 ± 2	4,6 ± 0,5	98 ± 2	45,5 ± 2,4	55 ± 3
		95% CI	0 – 1,84	0 – 1,79	3,3 – 6,2	0,83 – 1,1	0,83 – 2,01	2,7 – 6,7	1,07 – 1,74	1,37 – 2,33	52,9 – 78,5	4,4 – 6,6	2,57 – 2,83	1,01 – 1,05	2,4 – 3,3	137 – 140	4,3 – 5	96 – 99	43,7 – 47,2	53 – 57
	Male (n=9)	Mean ± SD	0,47 ± 0,27	0,34 ± 0,11	4,3 ± 2,2	0,82 ± 0,08	1,49 ± 0,45	3,5 ± 1,1	0,7 ± 0,28	1,36 ± 0,37	68,6 ± 13,5	5,3 ± 0,6	2,75 ± 0,25	1,02 ± 0,08	2,8 ± 0,6	140 ± 4	5 ± 0,7	101 ± 1	44,5 ± 2,3	53 ± 3
		95% CI	0,29 – 0,65	0,27 – 0,41	2,8 – 5,8	0,76 – 0,87	1,15 – 1,82	2,8 – 4,2	0,51 – 0,88	1,11 – 1,6	59,8 – 77,3	5 – 5,7	2,59 – 2,91	0,97 – 1,07	2,3 – 3,2	137 – 142	4,5 – 5,5	100 – 102	43 – 46	51 – 55
16	Female (n=8)	Mean ± SD	1,12 ± 0,32	0,44 ± 0,13	4,1 ± 0,8	1,09 ± 0,09	1,1 ± 0	2,1 ± 0,1	0,57 ± 0,18	2,02 ± 0,61	89,4 ± 12,9	4,7 ± 0,8	2,71 ± 0,06	1,01 ± 0,09	2,5 ± 0,2	141 ± 3	6 ± 0,6	100 ± 2	44,3 ± 2	55 ± 2
		95% CI	0,9 – 1,34	0,35 – 0,53	3,6 – 4,7	1,03 – 1,15	n=2	2 – 2,1	0,44 – 0,69	1,59 – 2,44	80,4 – 98,3	4,2 – 5,2	2,67 – 2,74	0,95 – 1,08	2,4 – 2,6	139 – 144	5,6 – 6,4	99 – 102	43 – 45,7	54 – 57
	Male (n=8)	Mean ± SD	1,06 ± 0,21	0,53 ± 0,18	3,7 ± 0,9	1,19 ± 0,12	1,5 ± 0,76	1,7 ± 0,4	0,29 ± 0,09	2,17 ± 0,97	79,5 ± 13,9	4,9 ± 0,6	2,61 ± 0,06	1,01 ± 0,04	2,5 ± 0,1	141 ± 3	4,7 ± 0,9	99 ± 2	45,7 ± 1,8	55 ± 2
		95% CI	0,91 – 1,21	0,4 – 0,65	3,1 – 4,4	1,11 – 1,27	0,83 – 2,17	1,4 – 1,9	0,23 – 0,35	1,49 – 2,84	69,9 – 89,1	4,4 – 5,3	2,56 – 2,65	0,98 – 1,03	2,4 – 2,6	139 – 143	4,1 – 5,3	97 – 100	44,5 – 46,9	54 – 57
20	Female (n=8)	Mean ± SD	0,87 ± 0,18	0,34 ± 0,09	4,2 ± 0,8	1,09 ± 0,11	1,1 ± 0,52	2 ± 0,2	0,57 ± 0,18	2,04 ± 0,82	103,5 ± 13,3	4,3 ± 0,5	2,69 ± 0,11	0,97 ± 0,07	2,3 ± 0,3	142 ± 5	5 ± 0,6	100 ± 4	44 ± 2,3	54 ± 3
		95% CI	0,74 – 1	0,28 – 0,4	3,6 – 4,7	1,01 – 1,16	0,51 – 1,69	1,9 – 2,1	0,45 – 0,69	1,47 – 2,6	94,3 – 112,7	4 – 4,7	2,61 – 2,76	0,92 – 1,02	2 – 2,5	139 – 145	4,6 – 5,4	98 – 103	42,4 – 45,6	52 – 56
	Male (n=8)	Mean ± SD	1,01 ± 0,13	0,4 ± 0,06	3,8 ± 0,6	1,16 ± 0,14	1,15 ± 0,35	1,3 ± 0,2	0,27 ± 0,12	2,09 ± 0,54	89,8 ± 12,8	5,3 ± 1	2,65 ± 0,09	0,94 ± 0,05	2,3 ± 0,1	144 ± 2	4,9 ± 0,7	100 ± 2	46,2 ± 2,9	54 ± 3
		95% CI	0,92 – 1,1	0,35 – 0,44	3,3 – 4,2	1,06 – 1,25	0,66 – 1,64	1,2 – 1,5	0,18 – 0,35	1,72 – 2,46	80,9 – 98,6	4,6 – 6	2,59 – 2,71	0,91 – 0,98	2,3 – 2,4	142 – 146	4,4 – 5,3	98 – 101	44,2 – 48,2	52 – 56
29	Female (n=8)	Mean ± SD	1,14 ± 0,35	0,34 ± 0,04	2,2 ± 0,3	0,97 ± 0,13	1,37 ± 0,72	1,8 ± 0,2	0,56 ± 0,21	2,29 ± 0,73	99,6 ± 14,6	3,9 ± 0,2	2,7 ± 0,11	1,01 ± 0,07	2,1 ± 0,2	141 ± 6	5,6 ± 0,5	100 ± 4	46 ± 2,7	58 ± 4
		95% CI	0,89 – 1,38	0,31 – 0,37	2 – 2,4	0,88 – 1,06	0,55 – 2,19	1,6 – 1,9	0,42 – 0,71	1,78 – 2,8	89,5 – 109,8	3,8 – 4,1	2,62 – 2,77	0,97 – 1,06	2 – 2,3	137 – 144	5,3 – 5,9	97 – 103	44,1 – 47,8	55 – 61
	Male (n=8)	Mean ± SD	1,19 ± 0,17	0,43 ± 0,04	2,7 ± 0,5	1,15 ± 0,13	1,42 ± 0,48	1,2 ± 0,2	0,31 ± 0,13	2,44 ± 0,6	105,5 ± 14,1	4,1 ± 0,3	2,7 ± 0,08	1 ± 0,05	2 ± 0,1	140 ± 4	5,4 ± 0,7	99 ± 4	47,4 ± 1,8	57 ± 2
		95% CI	1,07 – 1,3	0,41 – 0,46	2,4 – 3	1,06 – 1,23	1 – 1,84	1 – 1,3	0,21 – 0,4	2,03 – 2,85	95,8 – 115,2	3,9 – 4,3	2,64 – 2,75	0,96 – 1,03	2 – 2,1	137 – 143	4,9 – 5,9	96 – 101	46,1 – 48,6	55 – 58
42	Female (n=8)	Mean ± SD	1,33 ± 0,36	0,42 ± 0,07	2,3 ± 0,6	0,92 ± 0,1	<LOD	1,7 ± 0,1	0,61 ± 0,21	1,9 ± 0,59	90,5 ± 11,5	4,2 ± 0,4	2,67 ± 0,07	0,96 ± 0,04	2,2 ± 0,2	144 ± 2	5,3 ± 1	101 ± 2	45,5 ± 3,7	60 ± 3
		95% CI	1,08 – 1,58	0,37 – 0,47	1,9 – 2,7	0,85 – 0,99		1,7 – 1,8	0,46 – 0,75	1,49 – 2,31	82,6 – 98,4	3,9 – 4,5	2,62 – 2,72	0,94 – 0,99	2,1 – 2,4	142 – 145	4,6 – 5,9	100 – 102	42,9 – 48,1	58 – 62
	Male (n=8)	Mean ± SD	1,11 ± 0,29	0,46 ± 0,13	2,5 ± 0,4	1,06 ± 0,16	1,62 ± 0,54	1,3 ± 0,3	0,3 ± 0,08	1,83 ± 0,3	100,8 ± 14,9	4,4 ± 0,5	2,6 ± 0,03	1,01 ± 0,08	2,2 ± 0,3	142 ± 2	4,5 ± 0,8	98 ± 2	46,5 ± 3	58 ± 3
		95% CI	0,91 – 1,3	0,37 – 0,55	2,2 – 2,8	0,94 – 1,17	1,14 – 2,1	1,1 – 1,5	0,24 – 0,36	1,62 – 2,03	90,5 – 111	4,1 – 4,8	2,58 – 2,63	0,96 – 1,07	2 – 2,4	141 – 143	3,9 – 5,1	97 – 100	44,4 – 48,6	56 – 60
55	Female (n=8)	Mean ± SD	1,03 ± 0,16	0,38 ± 0,18	1,7 ± 0,3	0,89 ± 0,11	1,2 ± 0,35	1,8 ± 0,3	0,57 ± 0,15	2,08 ± 0,6	92,3 ± 10,8	4,1 ± 0,3	2,7 ± 0,1	1 ± 0,06	2,1 ± 0,2	140 ± 6	5,7 ± 0,6	98 ± 4	49,8 ± 2,8	64 ± 3
		95% CI	0,91 – 1,14	0,25 – 0,51	1,5 – 2	0,82 – 0,97	0,94 – 1,46	1,6 – 2	0,46 – 0,67	1,66 – 2,49	84,7 – 99,8	3,9 – 4,3	2,63 – 2,77	0,96 – 1,04	2 – 2,2	136 – 144	5,3 – 6,1	95 – 100	47,9 – 51,7	62 – 66
	Male (n=8)	Mean ± SD	1,02 ± 0,34	0,37 ± 0,11	1,6 ± 0,3	1,1 ± 0,16	1,23 ± 0,29	1,1 ± 0,2	0,34 ± 0,07	2,68 ± 0,43	107,9 ± 14,7	4,3 ± 0,4	2,69 ± 0,11	1,03 ± 0,05	2 ± 0,3	140 ± 5	5,3 ± 0,4	96 ± 4	52 ± 1,6	64 ± 2
		95% CI	0,78 – 1,25	0,3 – 0,45	1,4 – 1,8	0,99 – 1,21	0,94 – 1,51	1 – 1,3	0,29 – 0,38	2,38 – 2,98	97,7 – 118	4 – 4,6	2,61 – 2,76	1 – 1,06	1,8 – 2,2	137 – 144	5 – 5,6	93 – 99	50,9 – 53,1	62 – 65
68	Female (n=8)	Mean ± SD	1,28 ± 0,21	0,44 ± 0,13	2,5 ± 1,4	1,17 ± 0,29	1,73 ± 0,75	1,8 ± 0,3	0,48 ± 0,15	2,33 ± 0,63	84,8 ± 14,3	3,6 ± 0,7	2,62 ± 0,07	1,01 ± 0,05	2 ± 0,2	143 ± 2	4,8 ± 0,6	99 ± 2	47,7 ± 2,8	66 ± 5
		95% CI	1,13 – 1,42	0,35 – 0,53	1,5 – 3,5	0,97 – 1,37	0,99 – 2,46	1,6 – 2	0,37 – 0,58	1,89 – 2,77	74,9 – 94,6	3,1 – 4,2	2,57 – 2,67	0,98 – 1,05	1,9 – 2,2	142 – 144	4,4 – 5,2	98 – 100	45,7 – 49,6	63 – 69
	Male (n=8)	Mean ± SD	1,13 ± 0,28	0,51 ± 0,09	2,2 ± 0,4	1,21 ± 0,23	1,65 ± 0,74	1,1 ± 0,1	0,4 ± 0,1	3,22 ± 0,78	107,5 ± 15,2	4,9 ± 1,6	2,69 ± 0,08	1,03 ± 0,05	2,2 ± 0,2	145 ± 3	4,7 ± 0,3	100 ± 1	51,7 ± 2,5	67 ± 2
		95% CI	0,94 – 1,32	0,45 – 0,57	1,9 – 2,4	1,05 – 1,37	1,05 – 2,25	1 – 1,2	0,34 – 0,47	2,68 – 3,76	97 – 118	3,8 – 6	2,64 – 2,75	1 – 1,06	2 – 2,3	143 – 147	4,4 – 4,9	99 – 101	49,9 – 53,4	65 – 68
76	Female (n=8)	Mean ± SD	1,23 ± 0,17	0,41 ± 0,1	1,8 ± 0,7	1,07 ± 0,2	<LOD	1,8 ± 0,4	0,49 ± 0,15	3,27 ± 0,62	99,1 ± 6,2	2,8 ± 0,8	2,61 ± 0,09	1,02 ± 0,06	2,1 ± 0,2	146 ± 4	6,2 ± 0,7	102 ± 2	46,8 ± 1,6	67 ± 3
		95% CI	1,11 – 1,35	0,34 – 0,48	1,3 – 2,3	0,94 – 1,21		1,6 – 2,1	0,38 – 0,59	2,84 – 3,69	94,8 – 103,4	2,3 – 3,3	2,55 – 2,67	0,97 – 1,06	2 – 2,2	143 – 148	5,7 – 6,8	101 – 103	45,6 – 47,9	66 – 69
	Male (n=8)	Mean ± SD	1 ± 0,31	0,37 ± 0,07	3,7 ± 1,9	1,09 ± 0,15	1,2 ± 0,14	1 ± 0,1	0,28 ± 0,07	3,97 ± 0,79	101,3 ± 8,1	4,2 ± 1	2,63 ± 0,16	0,98 ± 0,05	2,1 ± 0,2	145 ± 5	5,8 ± 1,2	101 ± 2	51,7 ± 3,1	68 ± 2
		95% CI	0,78 – 1,22	0,33 – 0,42	2,4 – 5	0,98 – 1,19	1,06 – 1,34	0,9 – 1,1	0,23 – 0,33	3,42 – 4,52	95,7 – 106,8	3,5 – 4,9	2,52 – 2,74	0,94 – 1,01	2 – 2,2	142 – 149	5 – 6,7	100 – 102	49,6 – 53,8	66 – 70

Table 4
Clinical chemistry data.

Conclusion

In general, the obtained values were within Scantox A/S in-house Göttingen Minipigs hematology, coagulation and clinical chemistry reference values (obtained in a period between 2013-2018), however levels for reticulocytes, fibrinogen and creatinine were in several cases observed to be outside of the range. For reticulocytes this was primarily observed in animals between 29 and 55 weeks of age. For fibrinogen this was observed in animals aged 16-20 weeks and again from week 55 and onwards. For creatinine levels this was observed from week 29 and onwards.

The observed differences in these values are likely due to differences in environment, housing, and diet, and overall, the data from the IgG humanized Minipigs are comparable to the standard Göttingen Minipigs. It confirms that the IgG Humanized Minipigs does not differ significantly from the standard Göttingen Minipigs, other than the intentional and intended change to their genome and hence tolerance to human antibodies.

Results

The data shared in this article is available for download at minipigs.dk/about-gottingen-minipigs/background-data.

Photo 3
Humanized IgG Göttingen Minipigs aged 20 weeks.

Extract from the paper:

Accelerated Wound Healing in Minipigs by On-Site Production and Delivery of CXCL12 by Transformed Lactic Acid Bacteria

By Emelie Öhnstedt[1,2], Hava Lofton Tomenius[1,2], Peter Frank[2], Stefan Roos[3], Evelina Vågesjö[1,2] and Mia Phillipson[1,4].

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Non-healing wounds are a growing medical problem and result in considerable suffering. The lack of pharmaceutical treatment options reflects the multistep wound healing process, and the complexity of both translation and assessment of treatment efficacy. We previously demonstrated accelerated healing of full-thickness wounds in mice following topical application of the probiotic bacteria Limosilactobacillus reuteri R2LC transformed to express CXCL12. In this study, safety and biological effects of a freeze-dried formulation of CXCL12-producing L. reuteri (ILP100) were investigated in induced full-thickness wounds in minipigs, and different wound healing evaluation methods (macroscopic, planimetry, 2D-photographs, 3D-scanning, ultrasound) were compared. We found that treatment with ILP100 was safe and accelerated healing, as granulation tissue filled wound cavities 1 day faster in treated compared to untreated/placebo-treated wounds. Furthermore, evaluation using planimetry resulted in 1.5 days faster healing than using 2D photographs of the same wounds, whereas the areas measured using 2D photographs were smaller compared to those obtained from 3D scans accounting for surface curvatures, whereas ultrasound imaging enabled detailed detection of thin epithelial layers. In conclusion, topical administration of the drug candidate ILP100 warrants further clinical development as it was proven to be safe and to accelerate healing using different evaluation methods in minipigs.

1. Introduction

The skin serves as an important barrier to the environment, and wounding of the skin rapidly initiates a healing process. Non-healing wounds are a growing medical problem associated with aging populations and the prevalence of metabolic diseases [1]. In addition to causing discomfort and pain, such wounds increase the risk of amputation due to infections and result in associated care costs that can account for over 3% of the healthcare budget in industrialized countries [3,4]. There are currently very limited options for active treatment, i.e., treatments that accelerate wound healing.

The development of treatments to accelerate wound healing is associated with many challenges, which explains the limited range of available options. For instance, topical administration of drug candidates is limited by the proteolytic microenvironment of the wounds, which greatly reduces bioavailability [6]. We recently developed a means to circumvent this issue by transforming a strain of the probiotic bacteria *Limosilactobacillus reuteri* R2LC (*L. reuteri* R2LC, previously known as *Lactobacillus reuteri* R2LC) to express murine or human CXCL12, which allows continuous expression of the protein at the wound site while inhibiting degradation of the chemokine [7]. Topical application of this genetically engineered *L. reuteri* R2LC was demonstrated to accelerate healing of full-thickness wounds in otherwise healthy or diabetic mice, and mice with peripheral hind limb ischemia, as well as to improve re-epithelialization using an ex vivo model of human skin disks [7]. This effect was proven to be macrophage-dependent, and both macrophage numbers and their transforming growth factor β (TGF-β) production increased by CXCL12-producing *L. reuteri* R2LC treatment, which ultimately resulted in increased proliferation of keratinocytes and accelerated wound healing [7].

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In contrast, wound closure in pigs and humans solely depends on the formation of granulation tissue and re-epithelialization, as wound contraction does not occur since the skin is firmly attached to the underlying connective tissue and lacks the required muscle layer [8-10]. In addition, non-healing wounds form a heterogenous group as they are the result of several underlying and complex conditions, making them impossible to fully replicate preclinically. The available non-healing wound models usually consist of wounds induced in an animal model of a primary condition associated with non-healing wounds, such as ischemia and diabetes. However, non-healing wounds have a more multifaceted pathophysiology and also depend on factors such as age and bacterial load [1, 5, 11].

The main objective of this paper was to increase the quality of wound healing evaluation by exploring and comparing classic as well as novel methods for the assessment of healing of induced, full-thickness wounds in minipigs. In parallel, the effect of the freezedried formulation of human CXCL12-producing *L. reuteri*

R2LC, ILP100 a new-in-class drug candidate, on wound healing was evaluated in two separate cohorts of minipigs.

2. Materials and methods

2.1. Study design

The primary objective of both studies was to assess safety and toxicity for regulatory compliance. The two studies also contained a number of complimentary technical and analytical exploratory endpoints which are reported herein.

2.2. Animals

Cohort A was performed in 18 male, and Cohort B in 15 female Göttingen SPF minipigs (Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark) at CitoxLabs (Ejby, Denmark). At cohort inclusion, the pigs were randomized to the different treatment groups, weighed 19–25 kg, and were between 7 to 11 months old. All experiments were approved by The Danish Veterinary and Food administration Council (Ethical permit number; 2015-15-0201-00713).

2.3. Limosilactobacillus reuteri R2LC Encoding Human CXCL12

A strain of probiotic bacteria *Limosilactobacillus reuteri* R2LC (*L. reuteri* R2LC) genetically engineered to encode human CXCL12 1 alpha has been designed as reported elsewhere [7], and developed in a freeze-dried formulation as the drug candidate ILP100. In brief, the sequences encoding the human chemokine CXCL12 1 alpha were inserted into an expression vector, after which the constructs were transformed into *L. reuteri* R2LC. The CXCL12 expression is induced by the addition of an inducing peptide, SppIP, resulting in the transformed *L. reuteri* R2LC expressing human CXCL12 following activation (for details [7]).

2.4. Wound Induction

Two to three circular full-thickness wounds (20 mm diameter, area 3.14 cm^2) were induced on each side of the spine on the back of each animal.

2.8. Wound Treatment

The wounds were treated and the dressings were changed on days 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 25, and 28 following wound induction (Figure 1). Cohort A comprised three subsets in which the wounds: (i) did not receive any treatment, (ii) were treated with wild type *L. reuteri* R2LC (500 µL, 2.5 x 10^9 CFU/wound), (iii) were treated with ILP100 (100 µL, 7 x 10^9 CFU/wound). In Cohort B, the wounds were treated either with (i) placebo (500 µL) or (ii) ILP100 (500 µL, 2.5 x 10^9 CFU/wound). Before treatment of the wounds, the freeze-dried formulations were reconstituted in buffer and activated with abundant amounts of SppIP (100 to 1000 ng/mL).

2.9. Wound Evaluation

The wounds were macroscopically evaluated and photographed in a standardized manner at day 1 (only photo), 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 28 in Cohort A, and at day 1 (only photo), 3, 7, 9, 11, 13, 15, 21, 25 and 28 in Cohort B (Figure 1). The macroscopic evaluation performed on-site included scoring of granulation, presence of hypergranulation, wound edge inflammation, surrounding skin inflammation, hemorrhaging, and exudation. The scoring ranged from 0–not present, 1–minimal, 2–slight, 3–moderate, and 4–marked.

2.9.1. Two-Dimensional Photographs of Wounds

Two-dimensional (2D) photographs were taken in a standardized manner with a flash using the same camera and at a fixed distance with a 5.5 cm x 5.5 cm frame placed around the wound. From the photographs, area measurements were performed using ImageJ2 software (National Institutes of Health, Bethesda, MD, USA) where the frame in the photos served as the scale.

2.9.2. Planimetric Assessments of Wounds

In Cohort A, the wounds were measured using planimetry days 2, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 28. For the planimetry assessment, a sterile transparent sheet was placed on top of the wound, on which both the area of the wound and the newly formed epithelia were outlined. In the wound, the area covered with granulation tissue was outlined and the remaining wound area was marked as unspecific tissue. The sheets were later analyzed using PictZar Pro (7.5.1) (Advanced Planimetric Services, Elmwood Park, NJ, USA).

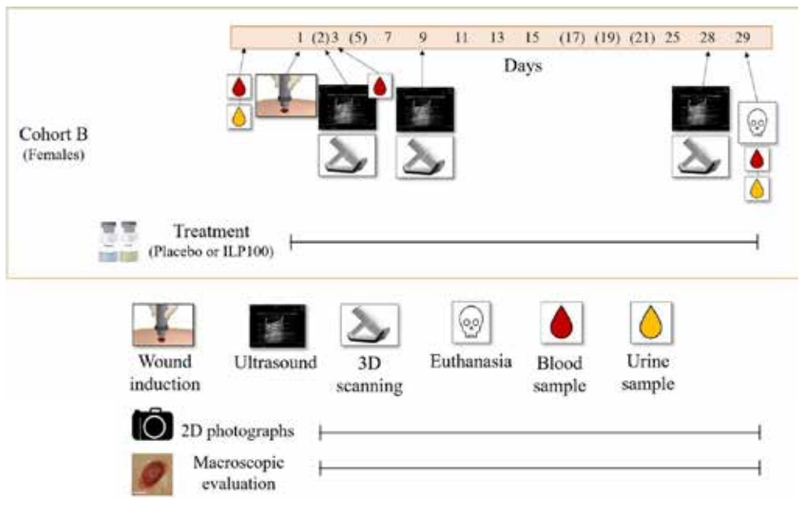

Figure 1 *(See full figure in the complete paper)* Schematic illustration of the protocol design. The study consists of two cohorts: one in males (Cohort A, blue box) and one in females (Cohort B, orange box). In the illustration, the numbers in the filled blue (Cohort A) and orange (Cohort B) boxes indicate the days where 2D photographs, macroscopic evaluation, treatment, and planimetry (only Cohort A) were performed. No 2D photographs, macroscopic evaluation, or planimetry were performed on the days in parenthesis. Time points for wound induction, collection of blood (for hematology, clinical chemistry, plasma levels of CXCL12 and SppIP, and for CFU counts of ILP100), collection of urine samples, and for additional imaging (only Cohort B) with ultrasound and 3D scanning are indicated with arrowed symbols.

2.9.3. Three-Dimensional Scanning of Wounds

In Cohort B, three-dimensional wound measurements were carried out using a stereoscopic optical system, a Cherry Imaging platform consisting of a hand-held 3D scanner, and TraceTM version 5 software (Cherry Imaging, Yokneam, Israel, 2019). The 3D scanner acquires thousands of images with a speed of 15 frames/second at a resolution of 100 µm, that are rendered into a 3D surface [15]. For each pig, three of the wounds were scanned on days 2, 9, and 28. The wound margins were manually marked on the 3D surfaces created in the TraceTM software, and the program then calculated the area, volume, and depth of the wound. Wound depth was designated as the average of 10% of the measured spots with the deepest values.

2.9.4. Ultrasound Imaging of Wounds

In Cohort B, the three wounds that were 3D scanned were imaged using ultrasound on days 2, 9, and 28 following wound induction using Arietta V60 with linear probes L64, 5–18 MHz. The probe was placed in the same direction on all occasions for all wounds, in order to produce scans that visualized one transversal section of the entire wound. Scanning directly on the wound was only possible using saline flushed into the wound cavity (Days 2 and 9). On Day 28, the wounds were completely healed and epithelialized, and ultrasound gel was used instead of saline.

3. Results

3.1. Evaluation of Methods Assessing Wound Granulation, Re-Epithelialization and Area

Wound healing was assessed in minipigs using consecutive measurements of areas and volumes of induced wounds, as well as of the formed scars. Areas of wounds and early scars were measured and analyzed by three approaches: 2D photographs with ImageJ2 software, planimetry with PictZar Pro software, and 3D scans with the TraceTM software (Figure 1). Wound diameters were also measured by 2D photographs, 3D scans, and ultrasounds. Wound and scar volumes were assessed by 3D scanning, and all wounds were evaluated macroscopically for assessment of granulation tissue at different time points following induction.

In Cohort A, wound areas were assessed using planimetry and 2D photographs according to standardized protocols. On days 2, 5, 9, 11, and 13 following wound induction, there were statistically significant differences between the measured mean wound areas (93–96 wounds per time point) using planimetry or 2D photographs. However, of these time points, only days 9 and 11 had area differences exceeding 0.1 cm^2, and the planimetrymeasured areas were 0.36 (± 0.05) cm^2 and 0.20 (± 0.04) cm^2 smaller for the respective days compared to the areas measured from 2D photographs (Figure 2A,B). Epithelialization was first noted on day 9 (Figure 2C) and might account for the observed area differences, as it is more difficult to detect thin epithelial layers from the 2D photographs acquired with a flash compared to those assessed by planimetry. In fact, the time to 50%, 75%, and 100% re-epithelialization differed depending on the method used, as the time to 100% re-epithelialization occurred on average 1.5 days later ($p \leq 0.0001$) when assessed by 2D photographs (planimetry: 12.2 ± 2.3 days, 2D photographs 13.6 ± 2.1 days, Figure 2D). Similarly, the 75% and 50% re-epithelialization

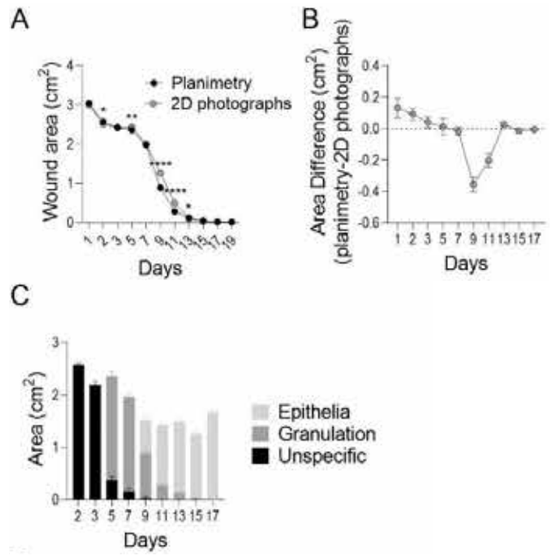

Figure 2 *(See full figure in the complete paper)* Comparisons of different wound assessment methods. Wound areas from Cohort A were measured by on-site planimetry (A) and from 2D photographs (B), n = 18, N = 93–96), and the areas were compared to reveal method-dependent differences. From the planimetric data, the areas of newly formed epithelia, granulation tissue, and unspecific tissue for all wounds in Cohort A (C) were retrieved (n = 18, N = 96). On days 2, 9, and 28, the wounds in Cohort B were imaged with ultrasound and representative images are shown (L), where the yellow lines delineate measured diameters and depth showing that the wounds are fully epithelialized and considered healed at d28.

were reached 1.0 day ($p \leq 0.0001$) and 0.2 day (p = 0.08) earlier, respectively, when assessed by planimetry compared to 2D photographs (Figure 2E,F).

To allow measurements of the wound area, depth, and volume, as well as the height and volumes of scars, a technology with stereoscopic scanning generating 3D surface models was utilized in Cohort B. When the areas obtained from 3D scanning was compared to those obtained from the 2D photographs of the same wounds on day 9 and day 28, it became evident that smaller wound areas were detected by 2D photographs when compared to those from 3D scans (day 9: 16 ± 8% smaller, $p \leq 0.0001$, day 28: 27 ± 12% smaller, $p < 0.0001$, Figure 2G,H).

The formation of granulation tissue in the wound cavity is a prerequisite for reepithelialization as it enables epithelial cell migration and wound closure (Figure 2I). The 3D scanning of wounds results in a negative volume corresponding to the wound cavity, while a positive volume depicts clot formation or hypergranulation, i.e., granulation tissue elevated beyond the level of the surrounding skin. The 3D scans were complemented with a macroscopic evaluation of the wounds before each treatment, and the wounds were scored (0–4) for the formation of granulation tissue, where higher scores indicate that a larger extent of the cavity is filled with granulation tissue. The

granulation scoring and 3D volume measurements at day 9 were then plotted against each other to test for correlation. On day 9, the majority of the wounds had a granulation score of 4 and a wound volume close to 0 (Figure 2J). If wounds with blood clots were excluded (red dots in graphs), the wound with the largest cavity was the one receiving a lower granulation score, as expected. Due to the fast formation of granulation tissue in the induced wounds, 3D scanning should be performed at earlier time points to evaluate if those results correlate with the macroscopic observations. Interestingly, all wounds that were scored as hypergranulating from the macroscopic evaluation also showed a positive volume when measured by the 3D scanning, and a significant trend (p = 0.0001) towards higher hypergranulation scores with increasing volume was demonstrated using Jonckheere's trend test (Figure 2K).

Thus, wound healing can be evaluated using several parameters, and one method is not enough for assessing all aspects of the healing process. As demonstrated above, different methods might give different results when measuring the same parameters. Therefore, these differences should be taken into account when making informed decisions on which method to use in designing controlled studies assessing wound healing in animals and humans.

3.2. Treatment with CXCL12-Producing L. reuteri R2LC Accelerates Wound Healing

The biological effect of ILP100-treatment was demonstrated in Cohort A by accelerated re-epithelialization and increased formation of granulation tissue as compared to untreated wounds (Planimetry). Accelerated re-epithelialization by ILP100 was observed as reduced wound area and increased percentage of the wounds being re-epithelialized when compared to untreated wounds and wounds treated with wild-type *L. reuteri* R2LC (Planimetry, Figure 3B,C). In addition, larger areas of newly formed epithelia were demonstrated following ILP100-treatment on days 9 and 11, and the treated wounds became fully re-epithelialized 3 days earlier than untreated wounds (Planimetry, Figure 3D,E), even though no differences were detected for the time leading to 50% or 75% re-epithelialization of the wound area (Planimetry, Figure 3F,G). The accelerated re-epithelialization demonstrated by planimetry was also supported by assessments from the 2D photographs, as the ILP100-treated wounds were fully epithelized almost 2 days faster than the untreated wounds (12.6 ± 0.4 days versus 14.4 ± 1.4 days, respectively, Figure A1). However, no differences were detected between treatments when absolute wound size, percent reepithelialized wound area, or time to 50% or 75% re-epithelialization were analyzed in the 2D photographs. The discrepancy observed for results obtained by planimetry and 2D photographs were in line with our evaluation that planimetry reported faster wound healing compared to 2D photographs (Figure 2D). In addition, the formation of granulation tissue was accelerated by the ILP100 treatment at day 5, and the time to complete wound coverage of granulation tissue occurred 1.5 days earlier in the ILP100-treated wounds when compared to untreated wounds (Planimetry, Figure 3H,I). This observation was strengthened by the macroscopic evaluation, as ILP100-treated wounds received higher average scores for the assessment of granulation, starting at day 5 (Figure 3J,K).

In Cohort B, macroscopic evaluation again demonstrated increased granulation of ILP100-treated wounds at day 7 and day 9 when compared to placebo (Figure 4A,B), whereas no granulation scoring was performed at day 5 as part of the protocol. Planimetry was not performed in Cohort B, but data from 2D photographs revealed an increased portion of re-epithelialization of wounds following ILP100-treatment at day 7 and day 9, resulting in the ILP100-treated wounds reaching 75% and 50% re-epithelialization area faster than the placebo-treated wounds (Figure A2B,D,E). No differences in wound area or time to complete re-epithelialization between treatments could, however, be detected in analyses from the 2D photographs (Figure A2A,C).

The wounds in Cohort B were also imaged by the 3D scanner on three occasions (day 2, 9, and 28, Figure 4C). In accordance with the observations from the 2D photographs, a reduced wound

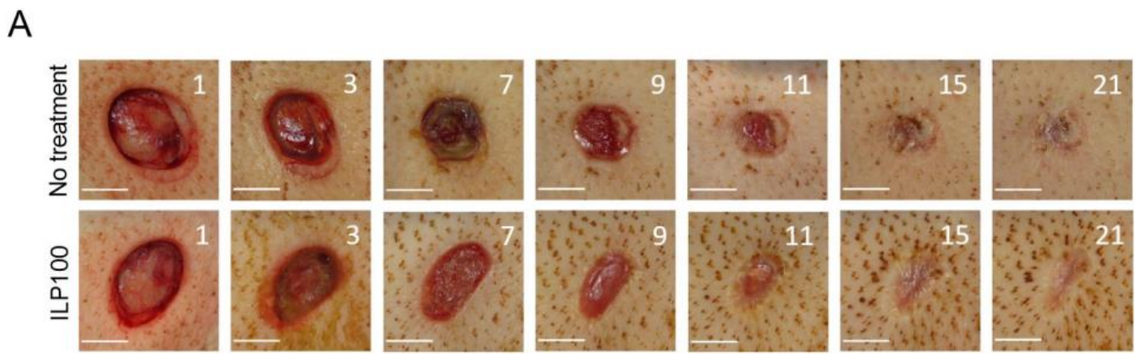

Figure 3 *(See full figure in the complete paper)*
Wound healing assessed by planimetry and macroscopic evaluation in cohort A. (A) shows representative photographs of healing over time where the inserted numbers depict days post-induction and the scale bar corresponds to 1 cm.

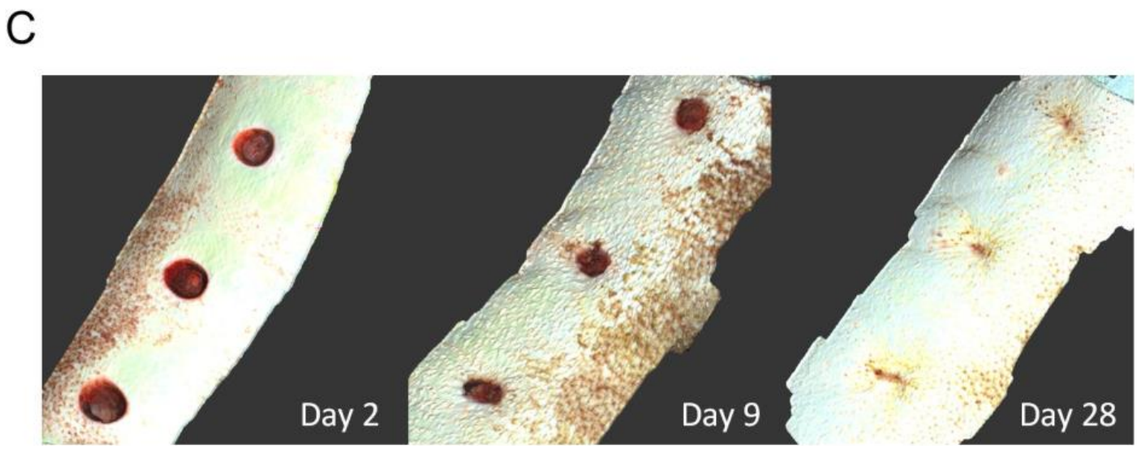

Figure 4 *(See full figure in the complete paper)*
The healing was also assessed using 3D scans, where panel (C) shows representative projections of 3D scans from day 3, day 9, and day 28.

area was observed on day 9 for the ILP100-treated wounds using the 3D scans (Figure 4D). The 3D scans also revealed a reduced depth (calculated as the mean of the deepest 10% of measurements of the wound) in the ILP100-treated wounds compared to placebo (Figure 4E), even though no statistical differences in wound volumes were observed (Figure 4F). Early scarring was evaluated using the 3D scans at day 28, where no statistical differences could be observed between treatments, even though a trend of reduced scar area (p = 0.0923) and reduced scar height (p = 0.0975, Figure 4G–I) were detected for the ILP100 treated wounds.

Taken together, topical ILP100 treatment to induced full-thickness wounds accelerated wound healing in both male and female minipigs.

4. Discussion

Despite being a significant societal burden in industrialized countries, available treatment options for non-healing wounds are today very limited. This study investigates the biological effects of the drug candidate ILP100 on induced wounds in minipigs by evaluating different and novel methods for the assessment of wound healing. Of the methods evaluated, we found that planimetry reported reduced wound areas (day 9 and 11) and faster healing when compared to 2D photographs, which in turn reported smaller wound areas than 3D scans. Wounds treated with ILP100 demonstrated accelerated healing by advanced re-epithelialization, as revealed by planimetry, 2D photographs, and 3D scans, in addition to higher granulation scores and increased area of granulation, as measured by planimetry.

For successful translation of preclinical projects, the clinical relevance of the models used is essential. The most widely used experimental animals are inbred mice due to their small size, as well as the wide palette of available genetically modified strains. However, rodent skin contains a muscle layer (panniculus carnosus) that enables the contraction of wounds, which does not exist in human skin and complicates translation. In contrast, pig skin not only lacks the contractile muscle layer but also resembles human skin with its sparse haircoat, firm attachment to underlying connective tissue, and epidermal turnover time [10, 16, 17]. When the translational success was evaluated in 25 wound healing studies, the agreement between the pre-clinical and clinical outcome was higher for pre-clinical evaluation in pigs (78%) than in smaller mammals (53%) or using in vitro studies (57%) [18]. In the current study, our previous observation of accelerated wound healing in mice treated with CXCL12-producing L. reuteri R2LC was confirmed to also occur in minipigs, even though with different kinetics.

Another factor for the limited success of clinical trials is that the only accepted primary endpoint to date is complete wound healing, reported as the time to heal, or the fraction of healed wounds at a relevant time point [14]. However, healing of wounds not only involves a reduction of the wound area through re-epithelialization, but also requires regeneration of tissue in the wound cavity, namely the formation of granulation tissue. In fact, the absence of healthy granulation tissue is a characteristic of non-healing wounds [2, 19]. For this reason, solely evaluating wound healing by repeated measurements of wound area does not readily account for the wound healing process. In addition, the depth of the wound has been shown to be a predictor of its healing rate [20], as well as being associated with the risk of amputation in diabetic foot ulcers [21, 22]. Therefore, assessment of the granulation tissue and depth measurements are part of many of the assessment tools that have been developed for non-healing wounds, such as Pressure Ulcer Scale for Healing (PUSH), SussmanWound Healing Tool (SWHT), and Bates-Jensen wound assessment tool (BWAT) [20, 23-26]. Of these tools, only BWAT considers the amount of granulation tissue while the others only assess the presence or absence of healthy granulation tissue. Other factors such as exudation, inflammation, and the presence of necrotic tissue or slough may also give an indication on how the healing is progressing [27-29]. Thus, extensive efforts have been made to identify appropriate new primary endpoints for wound healing studies [13, 14, 30]. Even though the primary endpoint of complete

healing remains, the FDA recently announced that it is open to discussing new primary endpoints, including (1) Percentage area reduction (PAR), (2) Reduced infection, (3) Reduced pain/reduced analgesia usage, (4) Increased physical function and ambulation, and (5) Quality of life [12].

Translation of preclinical wound therapies is also limited by the fact that there is no current gold standard for evaluating wound healing. Planimetry and wound area measurements from 2D photographs have been demonstrated to have good interand intra-investigator reliability [31-33].

Immune cells are known to contribute to the distinct phases of the wound healing cascade by different means [34-35]. While innate bactericidal functions are crucial in the early stages following wounding, the tissue restorative functions of predominantly macrophages drive the healing and remodelling processes during the later phases. The healing process is orchestrated by a cascade of growth factors, chemokines, and cytokines, and delivery of these to the wound site has been explored as treatment options for wound healing. Indeed, in the current study in minipigs, the initial reduction of wound size is not seen, even though treatment with ILP100 (freeze-dried formulation of human CXCL12-producing *L. reuteri* R2LC) still reduces the number of days to complete re-epithelialization. Here, the effect on shortened time to wound healing was demonstrated in two separate cohorts, as Cohort A reports 3 days' faster complete re-epithelialization using planimetry, while Cohort B demonstrates a reduced number of days to 50% and 75% re-epithelialization measured from 2D photographs. In fact, the human variant CXCL12 has previously been shown to have a biological effect on wound healing in Yorkshire pigs where healing was accelerated in full-thickness incision wounds treated with scaffolds soaked in human CXCL12 protein or plasmid DNA coding for CXCL12 [36].

In the current studies, both the presence of granulation tissue and the proportion of the wound cavity filled with granulation tissue were scored, with a similar scoring system to BWAT. In addition, the area of the wound covered with granulation tissue was measured by planimetry. We found that wounds treated with ILP100 demonstrated an accelerated formation of granulation tissue, shown by increased granulation scoring on day 5 and day 7 (Cohort A) and day 7 and day 9 (Cohort B), as well as increased area covered with granulation tissue on day 5 (Cohort A). Both studies revealed a reduced number of days to reach a granulation score of 4, indicating that the whole cavity is filled with granulation tissue more quickly.

One limitation of the current experimental design is that the two cohorts were studied 2 years apart, which might have resulted in a slight shift in the grading criteria of the granulation tissue. Further, Cohort A included only male pigs whereas Cohort B was conducted in only females. However, all animals in the respective cohort were included in the study within a week and housed in the same stables, limiting the environmental differences and allowing for intra-cohort comparisons. Despite some observed differences in wound healing between the two cohorts following no treatment or treatment with placebo or wild-type bacteria, similar results were observed for treatment efficacy of ILP100. Thus, ILP100-treated wounds in two separate cohorts demonstrated the accelerated formation of granulation tissue and re-epithelialization, signifying the translational potential of our previous observations in mice.

5. Conclusions

We found that topical treatment with the new drug candidate ILP100 to full-thickness wounds in minipigs accelerates healing and is well tolerated. The current study also reveals the need for standardized methods to assess wound healing since differences between methods can be substantial. In addition to educating evaluators to use the same criteria for wound assessment, careful consideration should be taken when choosing methods, including the need for high accuracy, mode of action of the drug candidate, user-friendliness, traceability, and costs, as well as the risk of infection or other disturbances to the wound healing process.

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Science

Method development for in vivo direct injection to the dorsal root ganglion (DRG)

ABOUT STUDY INSIGHTS: Göttingen Minipigs are increasingly selected for all aspects of pharmaceutical research and are fully recognized as a reliable and established animal model by all regulatory authorities worldwide. This section aims at providing an insight into the wide use of Göttingen Minipigs within biological research.

Insight provided by:
Sigal Meilin | Chief Scientific Officer at MD Biosciences, Israel.

What is the purpose of the study?
Over the last few years we have experienced a shift in therapy development for pain. Pain therapy is moving in the direction of systemic to local treatment or is becoming a more complex biological therapy, such as gene editing. Gene editing technologies require direct penetration of the gene to the cell body. The DRG is the cell body in this case of the peripheral sensory system.

Why is it important?
Intra-DRG injections in large adult animal pain models allows for the evaluation of gene-related therapies for chronic pain.

What makes this study particularly interesting?
The world of pain therapy is changing, and a number of companies are working to develop gene editing or other technologies that require direct access to the peripheral nerve cell bodies. The method for DRG exposure and injection is challenging because the spinal cord in swine extends past the lumbar spine making the DRGs difficult to access. In vivo dosing into the DRG opens the door for more research on gene silencing, editing, and cell manipulation.

Which challenges have you met during the study?
Intra-DRG dosing is a great challenge. Published methods for DRG dosing cannot be applied on a larger scale for pharmaco-biologic studies. We successfully developed a proprietary method to perform administration into the DRG reproducibly, consistently and safely for the minipig. This success enables our Sponsors to reliably evaluate the efficacy of their newest developments including gene editing therapeutics.

Image 1
L5 DRG immediately after injection cut to 4 slices. Theinjected dye can be clearly observed in green.

How did you ensure that the method developed worked?
The validation was performed by tracking specific dye traces and measurement of their infiltration into the DRG and the spread. The figure below demonstrated dissected L5 DRG post in vivo dosing.

What was the monitoring system post-procedure?
The animals were monitored post-procedure using the following parameters:
General veterinarian health and function check-up:

- Body weight
- Response to mechanical stimuli (von Frey) [1]
- Open field assay for locomotor activity [1, 2]

The tables on the next page summarizes the results.

In general, the results suggest that the animals were in general good health following the DRG injection. As expected intra-DRG administration resulted in a decrease in the withdrawal force, indicating increased sensitivity to mechanical stimulation. The open-field assay result shows no locomotor changes following the DRG injection. Overall the data suggest that the intra-DRG injection method is safe, reproducible, and enables the testing of new pain therapeutics.

How do you recommend going about species selection?
Intra-DRG dosing is a new approach for introducing pain therapies to the sensory system. Göttingen Minipigs is a good species because their nervous system is similar to humans. These similarities were well described in a review by Meijs et al.[3] Chronic neuropathic pain was also previously characterized in pigs, making them a good candidate for this method.

Any learnings you would like to share?
Therapy development for pain is changing, and new models and methods are required to meet the complexity of today's technologies.

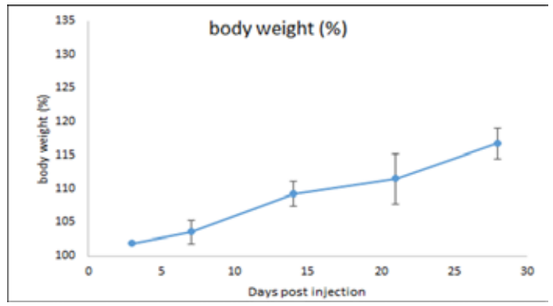

Figure 1
The animals gained weight throughout the entire study period which reflects general good health and a fast recovery.

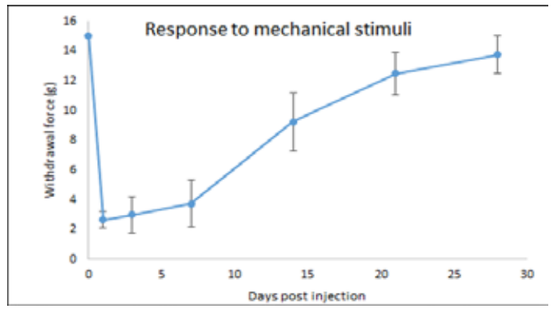

Figure 2
This graph shows the force applied to the hind leg to achieve a withdrawal response throughout the duration of the study.
The animals developed an expected transient sensitivity in the first 10 days post-injection. This was expressed as a low withdrawal force response.
The animals gained full recovery 2-3 weeks post-injection as expressed by the increase in withdrawal force.

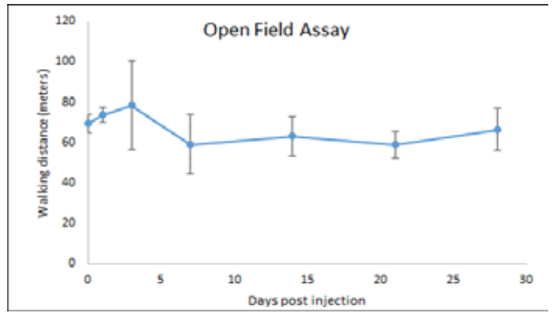

Figure 3
This graph shows the total meters that the animals walked in 5 minutes of exposure to the open field.
The injection did not affect the animal's locomotor activity throughout the study.

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The 15th Minipig Research Forum

Thank you for a great MRF 2023!

From 10-12 May 2023, nearly 100 scientists and laboratory technicians met in Amsterdam, the Netherlands, for the 15th annual meeting of the Minipig Research Forum (MRF). Once again, the MRF meeting presented a unique opportunity to get updates on research results and new data as well as to learn new techniques. Also, the opportunity to share experiences and network with fellow colleagues from all over the world using or working with Göttingen Minipigs was highly appreciated by the participants as being a very important part of the meeting.

The scientific program, which was organized by the Scientific Steering Committee, presented 21 inspiring speakers who addressed their specific areas of interest within four key session topics:

- Göttingen Minipigs used in advanced therapeutics
- Biomarkers in Göttingen Minipigs
- Animal welfare - next level
- Göttingen Minipigs in drug formulation development

This year, the meeting was kicked off by a keynote presentation held by Arne Hinrichs from the Ludwig-Maximillian University in Munich, Germany. The presentation introduced an entirely new strain of small sized pigs for biomedical research, Göttingen Micropigs, which has potential to (yet again) transform non-rodent species selection in preclinical studies.

Keynote presentation, "Introducing the Göttingen Micropig", by Arne Hinrichs.

Vivid networking during the poster viewing session.

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In addition to the four sessions, the scientific program also included two break-out sessions addressing the topics:

- Non-standard sampling in the minipig: Example of brain micro dialysis
- Rehoming of minipigs: Discussion of regulations, challenges, and solutions

The traditional and very popular poster presentation was back on the agenda, followed by the viewing session enabling close dialogue between the poster presenters and the participants. This part of the program also presents great opportunity to gather ideas for future collaborative projects using Göttingen Minipigs. A total of 13 informative posters were presented, and the winner of this year's Best Poster Award was Domenico Ventrella from the University of Bologna, who presented a very interesting poster on "The Göttingen Minipigs as an in vivo model to assess drug transfer via milk during lactation a contribution from the ConcePTION project".

Thank you to all our generous sponsors for supporting the 15th annual MRF and to all speakers and participants for your contributions to a truly successful conference! We look forward to welcoming all MRF members back in 2024.

Biomarker knowledge sharing group established

As an outcome of the MRF 2023, participants were given the opportunity to sign up for a knowledge-sharing group to work across companies and academia on the subject of qualified biomarker assays. Go to page 24 for more information on this great initiative and how to join the group.

Announcement of the Best Poster Award 2023: Congratulations to Domenico Ventrella.

MARK YOUR CALENDAR

- for the 16th Minipig Research Forum taking place 22-24 May 2024, once again in Amsterdam, the Netherlands.

Not a member yet?
Join the Minipig Research Forum community dedicated to advancing and refining minipig research. Go to minipigresearchforum.org or join the LinkedIn Group.

Feedback from participants at MRF 2023

"Biomarkers in minipigs. This topic showed that different CRO's and companies are already doing a lot of different assays (qualified and/or validated). This gives me more freedom to ask around if someone is already doing an assay."

"The mix of presentation topics, networking and meeting arrangements were all very nice. Well done!! "Kudo's" to the MRF Steering Committee for an excellent meeting experience."

Engaged audience after the keynote presentation by Arne Hinrichs introducing the Göttingen Micropig.

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Spotlights

Socialisation of Göttingen Minipigs at different stages

Proper socialisation of Göttingen Minipigs is crucial to ensure their well-being. This also creates a comfortable and cooperative environment for the animals and the caretakers, and ultimately results in healthier minipigs. Effective socialisation techniques vary across different stages, from piglets to adults. Animal Welfare Technician, Carina Anker, explains the different approaches applied at Ellegaard Göttingen Minipigs:

"In the farrowing sections we visit all the sows daily offering them a treat, so they have social contact and feel comfortable around the staff. This way they are also calm after farrowing, and lets the Animal Caretakers work in the pen and handle the piglets. This calmness can also be transferred to the piglets, who adopt the sow's acceptance of the caretakers' presence.

When the minipigs arrive at the early weaning section, they are socialised on a daily basis. The Animal Caretakers sit down in the pens and interact with the minipigs for at least 3 minutes in every pen. In the young stock section we always talk to the minipigs and often carry treats in our pockets. In the mating section, young females are also socialised by sitting in the pens and interacting with them. Breeding boars are housed separately, and it is therefore important that they are also socialised every day. This we do from outside the pen by scracthing them and offering them treats."

Publication

"First virological and pathological study of Göttingen Minipigs with Dippity Pig Syndrome (DPS)"

Dippity Pig Syndrome (DPS) is a well-known but rare complex of clinical signs affecting minipigs, which has not been thoroughly investigated yet. Affected animals show acute appearances of red, exudating, and painful lesions across the spine, also proven by an arching back (dipping). In general, the incidences of clinical signs appear suddenly. To understand the pathogenesis, this paper investigates histological and virological findings in affected and unaffected Göttingen Minipigs. Ultimately, different viruses were detected in the affected animals and in the affected skin, with one individual being of great interest due to having only PLHV-3. Other virus found in affected minipigs were also found in the unaffected, and the scientists behind the paper suggest, that DPS has a multifactorial cause.

Read the open access paper: DOI 10.1371/journal.pone.0281521

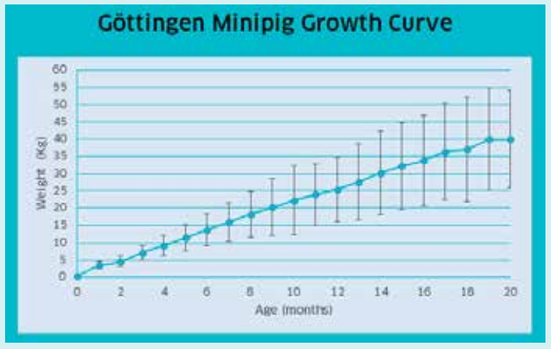
Publication

"Assessing extent of brain penetration in vivo ($K_{p,uu,brain}$) in Göttingen Minipig using a diverse set of reference drugs"

The study successfully determined brain penetration ratios ($K_{p,uu,brain}$) in Göttingen Minipigs through a pseudo steady-state approach involving intravenous dosing. Brain penetration data for various compounds were obtained and compared across species, and Göttingen Minipigs were found to be a suitable model for CNS drug safety testing and brain pharmacokinetics. The reference set of 17 compounds showed $K_{p,uu,brain}$ values between 0.02-2.4. Comparisons with rats indicated comparable values for many compounds, notably, differences were prominent for transporter substrates.

The findings support Göttingen Minipigs as a non-rodent CNS drug safety model and brain PK model for clinical translation, contributing to the understanding of drug disposition in minipigs.

Read the open access paper: DOI 10.1016/j.ejps.2023.106554



Health Monitoring Report: June 2023

Every 6 months the Health Monitoring Report (HMR), based on FELASA recommendations, is published for all three barriers at Ellegaard Göttingen Minipigs.

Laboratory Animal Veterinarian at Ellegaard Göttingen Minipigs, Maja Ramløse, who is responsible for reviewing the overall health monitoring plan, collecting, accumulating, and reporting the results, says: "We monitor the health of our colonies twice a year for a wide range of pathogens. In May/June we screen for selected agents, and in November/December we perform an extended analysis. For the latest report we are very pleased to confirm, that the June 2023 report shows no changes in the overall health status at our facility."

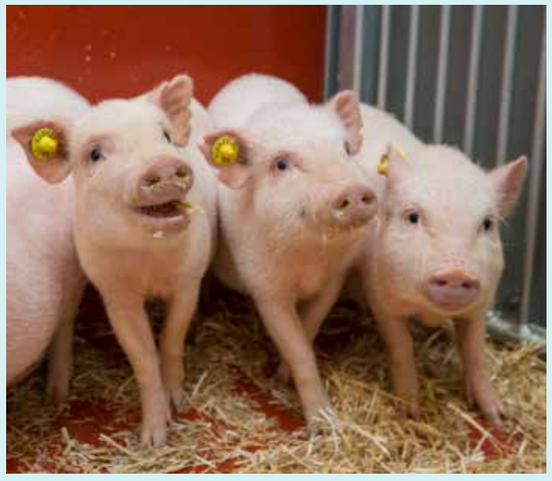
Download the full report from minipigs.dk/about-gottingen-minipigs/health-status.

Background data on Göttingen Minipigs

For decades Göttingen Minipigs have contributed to biomedical research and resulted in hundreds of scientific publications. As a support to scientists and their research, background data on Göttingen Minipigs is available online.

Go to minipigs.dk/about-gottingen-minipigs/background-data to download growth curve and data, hematology parameters, clinical chemistry background data, histopathology, hemodynamics and organ weights for Göttingen Minipigs.

New data: Now you can also download blood data for Humanized IgG Göttingen Minipigs.



Call for participants: New Pig Biomarker Knowledge Sharing Group

Are you interested in biomarkers in pig models and/or do you have assay qualification and validation skills?

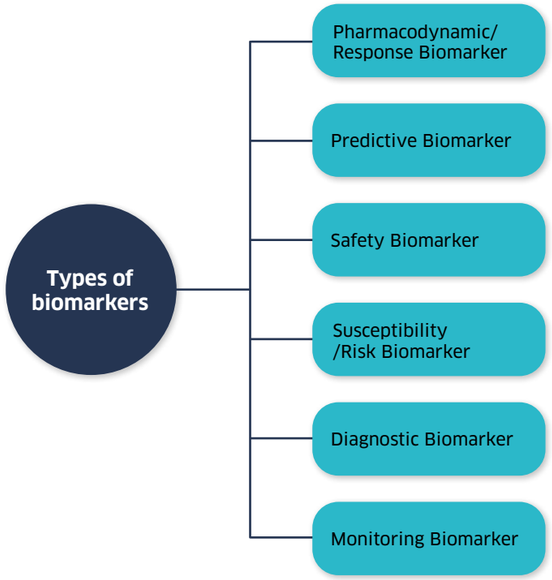
Background of initiative

To increase the use and the value of minipigs in pharmacology and toxicology more in depth knowledge on biomarkers is needed, to inform on target engagement, safety, mechanism of action and disease progress, among others.

Well-qualified assays for pigs are, however, not always available, and need to be set up and qualified from scratch.

Purpose

The aim of the group is to work across companies and academia to agree on criteria for assay qualification and validation, share data and establish a list of qualified biomarker assays for minipigs. If the list lacks relevant biomarkers of general interest in the group, we aim to work across companies to set these assays up. This will reduce time and resources for the individual laboratory, and ultimately improve the utility of minipigs in biomedical research.



How do I join?

Currently representatives from 26 companies covering pharmaceutical, contract research organizations, biotech companies, breeders and academia has expressed their interest in joining the group.

If you are interested in joining the Pig Biomarker Knowledge Sharing Group, please contact CSO at Ellegaard Göttingen Minipigs, Kirsten Rosenmay Jacobsen, at krj@minipigs.dk.

NEWS FROM

Ellegaard Göttingen Minipigs A/S

Ellegaard Göttingen Minipigs A/S is a leading international company supplying Göttingen Minipigs for biomedical research around the world. From our AAALAC accredited facility in Denmark we breed Göttingen Minipigs and enable the development of safer and more effective medicines, all based on our core values: Animal welfare, quality, respect, and collaboration.

Appointments and anniversaries

Lotte Schjølin joined as new Sales Coordinator on 15 August 2023.

Lotte has more than 30 years of experience within sales primarily from IT and the metal industry, and will fill a central role in our sales organisation.

Britt Anderberg joined as new Order Management & Logistics Coordinator on 15 August 2023.

Britt is the new member of our Order Management team and has worked with sales and logistics for over 20 years.

1 July 2023 Jørn Frydenberg celebrated his 15th anniversary with Ellegaard Göttingen Minipigs.

Jørn is one of our dedicated drivers, who ensures that our minipigs arrive safe and sound at our customers facilities.

Ellegaard Bioresearch on LinkedIn

Our American subsidiary, Ellegaard Bioresearch, is now on LinkedIn. Follow for information about local conference participation, news particularly related to North America, and news about Göttingen Minipigs in biomedical research.

You are also welcome to contact John Cameron, Regional Business Development Director, at jca@minipigs.com with questions or enquiries.

Where to meet us in 2023

CONFERENCE	DATE	LOCATION
EUROTOX	10-13 Sep	Ljubljana, Slovenia
SPS	18-21 Sep	Brussels, Belgium
LASACON	7-8 Nov	Bangalore, India
ACT	12-15 Nov	Orlando, Florida, USA

Göttingen Minipigs Academy

Course calendar 2024

In 2024 the physical courses will be supplemented with web academy courses, which can be attended online. Watch out for the 2024 course calendar, which will be published in September 2023.

To be notified of the course plan, subscribe to our scientific news at minipigs.dk/about-us/gottingen-minipigs-magazine or follow us on Linkedin.

About the academy

The Göttingen Minipigs Academy is for researchers, scientists, veterinarians, animal technicians, and others with an interest in the welfare, management, and use of Göttingen Minipigs in biomedical research.

The academy offers expert-led courses developed and conducted by experienced professionals within the field covering various aspects of working with Göttingen Minipigs, including their biology and behavior, husbandry, veterinary management and welfare, hands-on practical exercises, and animal models. From 2024 we will also offer course subjects such as ergonomics, disease models, specific study types such as wound healing, and animal training.

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New publications on Göttingen Minipigs

Ellegaard Göttingen Minipigs gives high priority to collaborative projects that aim to better characterize and validate Göttingen Minipigs as a translational animal model and which facilitate and refine the use of Göttingen Minipigs in research projects and safety testing. Do you have an idea for such a collaborative project? Please contact ellegaard@minipigs.dk.

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