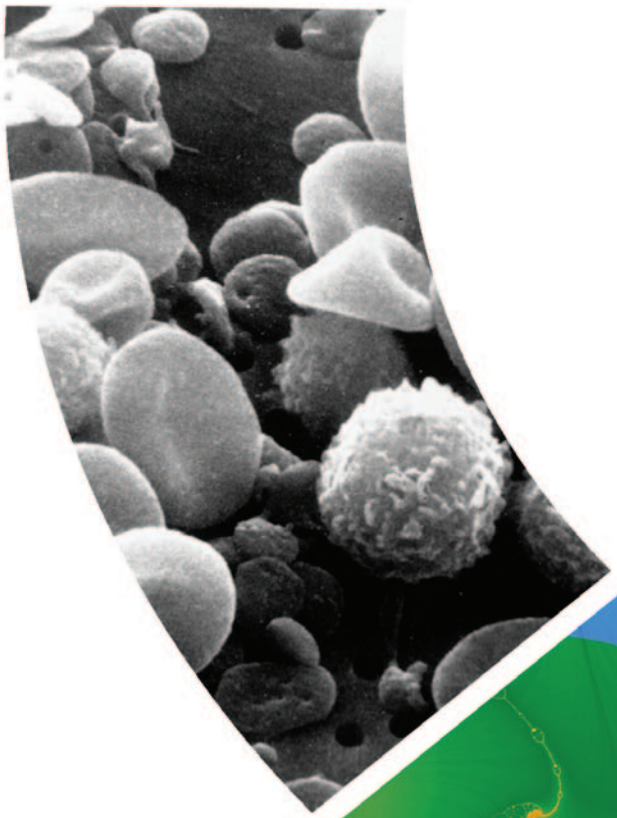
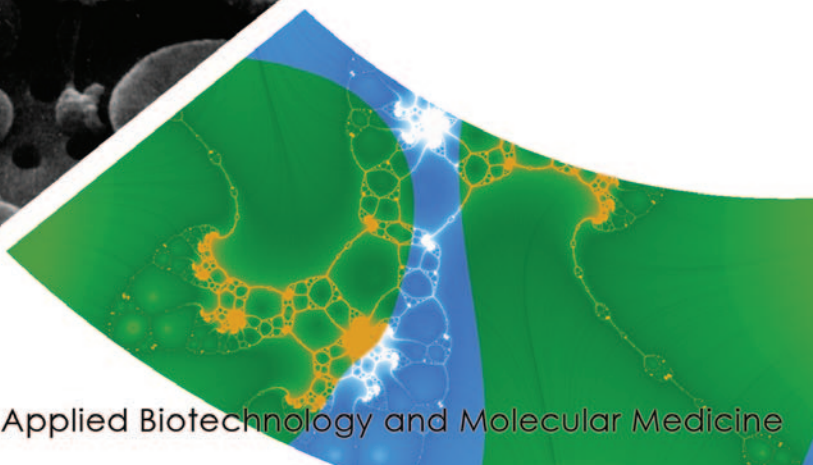
cabmm

center for applied biotechnology and molecular medicine





The Competence Center for Applied Biotechnology and Molecular Medicine

Brigitte von Rechenberg

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The Competence Centre for Applied Biotechnology and Molecular Medicine (CABMM) is a cutting-edge and innovative medical research organisation, working within both the VetSuisse and Medical Faculties of Zürich University, to provide novel therapies for humans and animals. It provides a platform for interdisciplinary research across the fields of regenerative medicine, experimental medicine and surgery, applied biotechnology and molecular medicine. Because of an interdisciplinary and collaborative approach, the CABMM is also able to pioneer translational medicine, using research findings to create medicines and treatments for diseased and dysfunctional tissues.

Over the past year, the organisation has presented some of their groundbreaking research in Pan European Networks' publications. This research covers a wealth of topics from the CABMM's broad range of research interests, but is united by the shared goal of improving human and animal health. Some of the best of the CABMM's articles are now collated in this booklet, highlighting a few key aspects of the varied and important work that the organisation is undertaking.

Here, the centre writes on the development of new methods of transcutaneous treatment for skin problems, cartilage lesions and deep wound infections; the creation of new therapies for the treatment of chlamydial infections in both humans and animals; and an investigation into the possible role that certain proteins found in genomic regions of cat chromosomes could have on obesity. Each of these articles presents new innovations or advancements in medical research which could have a profound impact upon the future of medicine, therapy and patient care.

As well as presenting research and results, the CABMM has written a thought provoking piece on the ethics of animal testing, proposing the introduction of better frameworks across Europe that could co-ordinate pan-European research and prevent unnecessary animal experimentation. Another article explains the importance of understanding and applying the principle of translational research. Implemented effectively, translational research offers to revolutionise how human and animal testing is carried out, involving careful planning from the preclinical tests through to human clinical trials, ensuring that the results of this testing go from 'bench to bedside and back'.

This booklet contains only a selection of the CABMM's publications, and many more are available on Pan European Networks' websites. The organisation has written on an even wider variety of topics than are included here; further articles explore the science and ethics of anaesthesia and the technology of Bonewelding®, which allows for the anchorage of implants in bone. The collected body of the centre's research speaks for itself, demonstrating the CABMM's role as an organisation at the pinnacle of experimentation, and at the forefront of developing new medicines for humans and animals alike.

Professor Dr Nicole Borel and her research group at the Center for Applied Biotechnology and Molecular Medicine illustrate new therapeutic strategies for treatment of chlamydial infections in humans and animals

More than meets the eye

Trachoma is the leading cause of infectious blindness in the world. This devastating disease is caused by the ocular serovars (A, B, Ba, and C) of the Gram-negative, obligate, intracellular bacterium *Chlamydia trachomatis,* which is hyperendemic in sub-Saharan Africa, the Middle East, as well as parts of Asia and central and South America. Globally, 84 million people, the majority of whom are children, suffer from active ocular *C. trachomatis* infection, and nearly eight million people are visually impaired as a result (Fig. 1).

Trachoma, a disease of poverty and poor hygiene, is categorised as a 'neglected tropical disease', and a number of global health organisations are working together to eliminate blinding trachoma by 2020; this includes the GET 2020 Alliance and the SAFE strategy of the World Health Organization.

The primary frontline antibiotics used to treat ocular chlamydial infection and prevent trachoma are oral azithromycin and topical tetracycline. However, there are drawbacks to antibiotic treatment of chlamydial infections, including unwanted and sometimes dangerous side effects, expenses (which may be prohibitive in poorer countries where trachoma is endemic), and the on-going and problematic emergence of antibiotic-resistant bacteria. Furthermore, incomplete or failed elimination of chlamydiae by antibiotic treatment may lead to deregulation of chlamydial development.

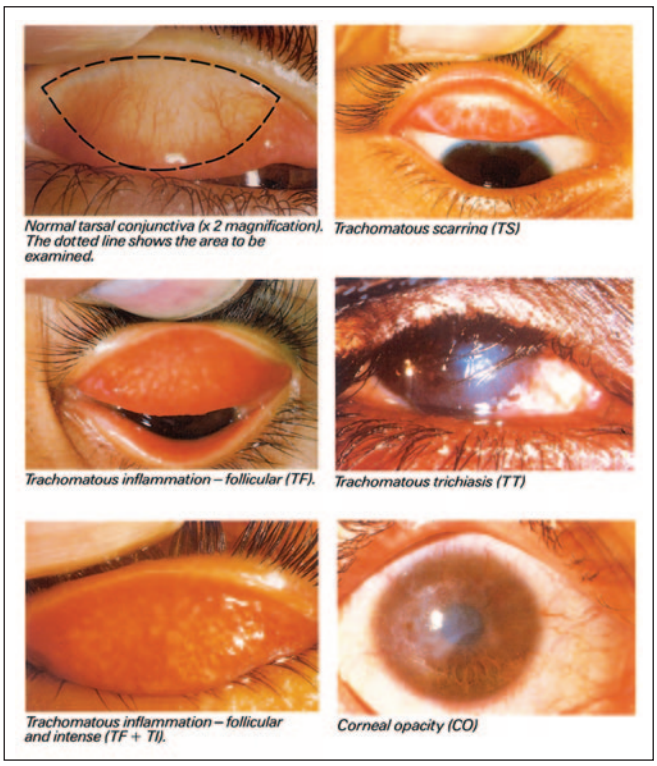

Fig. 1 Trachoma grading, adapted from the World Health Organization trachoma simplified grading card

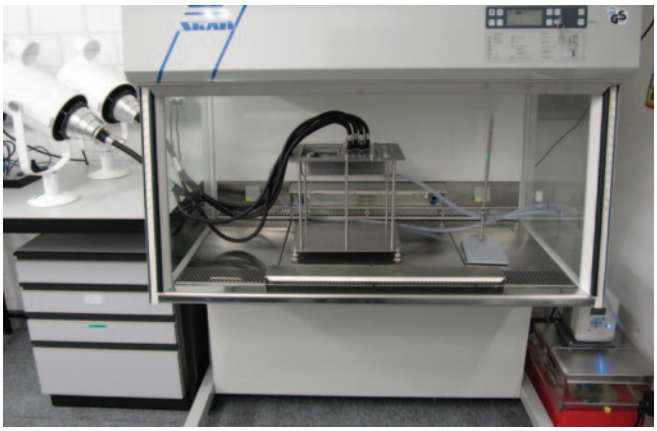

Fig. 2 wIRA setting for *in vitro* experiments

Recent *in vitro* and *in vivo* studies indicate that a break in the normal chlamydial developmental cycle can result in chlamydial 'persistence' and long term infection that is refractory to antibiotic treatment. Such 'persistent' infections can cause a cascade of on-going inflammatory-induced sequelae, resulting in scarring of the conjunctiva and trichiasis (Fig. 1) which cannot be reversed by antibiotic treatment and can only be corrected by ocular surgery. Thus, novel but non-pharmacologic therapeutic strategies for trachoma are of high interest.

New therapeutic strategies

Water-filtered infrared A (wIRA) is short wavelength infrared radiation with a spectrum ranging from 780 to 1440nm. Light from a halogen bulb, passing through a water containing cuvette, emits wIRA and visible light (VIS) (Fig. 2). wIRA alone, or in combination with VIS (wIRA/VIS), has been used in various clinical settings, and its efficacy has been proven in acute and chronic wound-healing processes.

Due to its high tissue penetration and low thermal load on the skin surface, wIRA does not cause the skin irritation and overheating associated with unfiltered IRA irradiation. This makes wIRA an effective means to increase tissue temperature, oxygenation and perfusion, all important factors positively influencing wound healing. Consequently, wIRA has been shown to reduce the frequency of secondary wound infections.

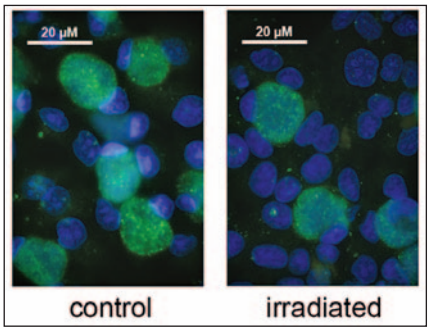

Fig. 3 Irradiation of chlamydial elementary bodies reduces their infectivity on host cells

Following abdominal surgery, a lower rate of wound infections was observed after post-operative wIRA/VIS irradiation compared to treatment with VIS alone. However, few preliminary data on the treatment of infectious conditions with wIRA irradiation have been reported so far. The direct effect of wIRA on pathogens *in vitro,* and in particular on obligate, intracellular agents such as chlamydiae, has not been shown before.

Acute chlamydial infection

Recent *in vitro* investigations by our group have revealed the exposure of chlamydiae prior to host cell infection. The exposure of Chlamydia-infected cells to wIRA/VIS irradiation reduces both the number of chlamydial inclusions that develop within host cells and the subsequent production of infectious chlamydiae, without any negative impacts on host cell viability. The efficacy of wIRA/VIS irradiation in reducing the infectious chlamydial forms (the elementary bodies (EB)) was demonstrated in animal-infecting as well as human-infecting chlamydial species.

Fig. 3 shows *C. trachomatis* EBs either irradiated with wIRA/ VIS (20 minutes, 3700W/m^2) or not irradiated (control), prior to infection of HeLa monolayers (human-derived cervical epithelial cell line). Cultures were incubated for 43 hours, fixed, and immune-labelled with anti-chlamydial LPS (green, chlamydial inclusions) and DAPI (blue, host cell nuclei). Frequency of inclusions per nucleus was calculated, and irradiation resulted in an approximately 50% reduction in the number of host cells infected (not shown; $p \leq 0.05$, $n = 3$, *t* test). Representative microscopic pictures at 1,000 times magnification are shown.

Furthermore, multiple-dose irradiation, as applied in clinical settings of wound healing and reduction of wound infection, resulted in an even more profound reduction of chlamydial burden *in vitro*. Importantly, we showed that wIRA/VIS does not induce cytotoxicity in two different permanent cell lines, one of human origin and one of non-human primate origin, even at high doses of wIRA/VIS or long term exposure.

Additionally, in a collaborative project with a group of the Darmstadt Technical University in Germany, wIRA treatment has been demonstrated to be undamaging to the pig eye. Incidentally, because wIRA/VIS reduces infectivity of directly irradiated extracellular chlamydial infectious EBs, potential applications for decontamination of healthcare and/or agriculture settings may be viable.

The benefits of wIRA treatment

Potential advantages of using wIRA to treat trachoma patients as an alternative or combination therapy in the future are manifold. The wIRA technology is already commercially available and has been shown to speed wound healing, reduce inflammation, and decrease secondary wound infections in clinical trials.

The wIRA device is applicable in the field, easy to use and does not require skilled medical personnel. The efficacy of a single dose of wIRA has been shown *in vitro* to reduce the chlamydial infectious burden by 50% without detriment to the host cells. Additionally, the anti-chlamydial effect of wIRA is most effective late in the chlamydial developmental cycle when anti-chlamydial drugs are less effective.

Inappropriate or inconsistent antibiotic therapy is a primary factor leading to the emergence of drug-resistant bacteria, which is particularly problematic in developing countries where antibiotics can be acquired only periodically and where use of expired or counterfeit medications is common. Because the use of wIRA may facilitate reduced dependence on antibiotic treatment, it has the potential to help reduce the incidence of bacterial antibiotic resistance. Because wIRA exposure reduces production of infectious chlamydiae, it may be expected to reduce/prevent *C. trachomatis* transmission as well as trachoma disease progression.

Current investigations

When it comes to treating trachoma patients, wIRA shows promise as a valuable therapeutic strategy. On-going work in our lab aims to demonstrate proof of concept for ocular wIRA treatment in an *in vitro* conjunctival cell culture model and an *ex vivo* animal model of ocular infection, respectively.

The transition to a more clinically relevant human conjunctival epithelial cell culture and ocular *C. trachomatis* strain wIRA exposure model, from our current successful and established non-conjunctival permanent epithelial cell and non-ocular *C. trachomatis* strain model, is now one of the main aims in the Borel research group at CABMM. It is expected that wIRA will reduce inclusion formation and production of infectious *C. trachomatis* EBs in conjunctival epithelial cells by 50% or more. Multiple or longer wIRA doses will likely further reduce inclusion formation and production of infectious EBs without deleterious effects on the host cells.

Our second aim will be to establish an *ex vivo* animal eye model (sheep) for infection with the animal chlamydial pathogen *C. pecorum* followed by exposure to wIRA. Natural infection with *C. pecorum* in sheep causes conjunctivitis. Preliminary *in vitro* data have already shown the effective reduction of wIRA/VIS in *C. pecorum*-infected cells. We expect that the *ex vivo* sheep eye infection model will show that wIRA exposure reduces chlamydial inclusion formation, shedding of infectious *C. pecorum,* and/or ocular inflammation.

The next step will be to establish an *in vivo* sheep model to evaluate the therapeutic effect of wIRA on conjunctivitis induced by *C. pecorum*. There are no existing animal models for *C. trachomatis* infections with the exception of a non-human primate model. The sheep model will be more technically and economically feasible than a non-human primate model. Shedding of infectious chlamydiae and subsequent inflammation will be scored and statistically evaluated so that both the anti-chlamydial and anti-inflammatory effects of wIRA *in vivo* can be determined.

In trachoma patients, the wIRA device could theoretically be used in conjunction with antibiotic therapy or as an alternative to antibiotic therapy, both to treat eye infections and to prevent disease transmission. The wIRA device has the potential to be utilised by technical personnel and/or physicians working to treat trachoma in a variety of settings.

Related projects

Chlamydiae not only induce trachoma but can also cause a wide range of acute and chronic diseases in animals and humans worldwide. They are responsible for economically important diseases such as mastitis, endometritis, conjunctivitis and pneumonia, and those which cause abortion in livestock. More recently, their role in asymptomatic gastrointestinal infections with recurrent chlamydial faecal shedding has been re-emphasised in veterinary medicine.

A recent investigation in our group demonstrated that more than 90% of the Swiss fattening pigs harbour *C. suis*, the pig-infecting chlamydial species, in their intestines. These chlamydiae, frequently resulting in entirely asymptomatic infection in swine, might be considered to be a part of the non-disease associated gut microbiome. However, on the other hand, *C. suis* is the sole chlamydial species demonstrated capable of acquiring stable antibiotic resistance (tetracycline resistance gene), presumably by lateral gene transfer. The gut may therefore represent an ideal niche and potential reservoir for dissemination of antibiotic-resistant chlamydiae.

The ability of chlamydial organisms to establish chronic, frequently subclinical infections has been hypothesised to correlate with chlamydial 'persistence' or 'the chlamydial stress response'. Fig. 4 shows that infectious EBs and replicative reticulate bodies (RB) comprise the characteristic biphasic chlamydial developmental cycle. Various stressors, including the host cytokine interferon-gamma and beta lactam antibiotics, such as penicillin, can cause the chlamydiae to enter persistence. Persistent chlamydiae are defined as viable but non-infectious and can resume production of infectious EBs upon removal of the stressor. *In vitro* and *in vivo* animal studies have shown persistent chlamydiae to be resistant to killing with azithromycin, a frontline anti-chlamydial drug.

This *in vitro* phenomenon is speculated to be associated with chronic clinical conditions in humans such as chronic bronchitis, asthma, atherosclerosis, reactive arthritis and genital infections in women leading to infertility. Notably, genital serovars of *C. trachomatis* are responsible for the most common bacterial cause of sexually transmitted diseases, and understanding the factors affecting the pathogenicity of chlamydial infection is therefore of broad human clinical and veterinary relevance.

Outlook

A current project in our lab investigates the mechanism leading to the 'persistence' chlamydial state in a co-infection model with chlamydiae and viruses naturally occurring in the porcine gut. Additionally, we have recently determined that damage/danger-associated molecules, typically released from mammalian cells/tissues in the course of pathogen infection and/or non-infectious trauma, inhibit both human-infecting and porcine-infecting chlamydial development *in vitro*. This suggests that these molecules might play a role in chlamydial development *in vivo*, both in humans and in animals of agricultural and economic importance, particularly in the context of poly-microbial infections.

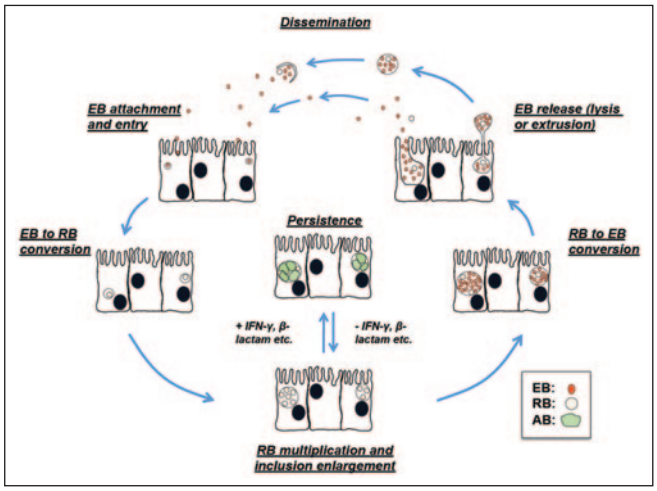

Fig. 4 Chlamydial developmental cycle, courtesy of R V Schoborg, *Microbes and Infection*, 2011

The inhibitory effect of wIRA/VIS on chlamydial infection has been proven *in vitro*. However, more preliminary experiments are necessary before the wIRA device can be implemented for the treatment of trachoma patients still suffering from the devastating leading cause of infectious blindness worldwide. Research in our lab is on-going in an effort to determine the mechanism(s) responsible for the anti-chlamydial effect of wIRA/VIS. Our current studies indicate that thermal as well as non-thermal effects contribute to the inhibitory impact of wIRA/VIS exposure on chlamydiae.

The long term goal of this project and the described related projects is to provide further insight into the host/pathogen interactions, pro-inflammatory mechanisms, and immune evasion strategies of this fascinating pathogen.

HORIZON 2020

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Everyday ethical considerations

The ethics of animal testing should be a consideration that is woven into the everyday business of researchers

Let's put it straight from the very beginning: no living, ordinary person could be enthusiastic about using animals for research and making them suffer in the laboratories of the world to advance medicine. But the serious researchers among us know that we cannot find new insights and new therapies for humans and animals without using them, and this is for several reasons. Among them are basic insights to studying disease mechanisms and thereby understanding where the chain of events is broken in cases of pathology. Cell and organ cultures can tell us a lot and are widely used, however, they cannot explain everything. For this, living organisms are too complex to just be quickly mimicked in cell culture wells or bioreactors. Therefore, animal experiments cannot be avoided, since on the other side of the coin weighs the suffering of both human and animal patients desperately awaiting cures for their diseases.

Although we divide research into basic and applied research, in reality these borders are not so clear and distinguishable – it's a transient business. How can we find good strategies for therapeutic regimens if we don't know where to intervene? To give you a sample: millions of animals give their lives to study cartilage resurfacing with very limited success and why? Certainly hyaline cartilage is a tricky thing to repair to start with. However, one of the main reasons is that nobody has yet found out what the normal regeneration and repair mechanisms of hyaline cartilage are, and how this is connected to the underlying subchondral bone.

Granted, this is difficult to find out, but if we can't understand this fundamental physiology of our joints, there is little hope that we will ever be able to regenerate new cartilage after a defect occurred on the surface. This is true for humans and animals. Dogs and horses have a lot of joint problems and wait for new therapy strategies as humans do. For horses this is one of the most common reasons to be euthanised or slaughtered. This shows impressively that animal experiments are not just for humans but also for animals – to save their lives.

Ethics and philosophy

The ethical and philosophical aspects of why animal experiments can be justified in science are widely discussed and are not the focus of this essay. There is also the issue of the famous 'three Rs' (3Rs), which stand for Reduce, Replace and Refine, leading to more conscious use of animals in research, and this issue is mainly left to the philosophers. One of the main problems, however, is very often neglected, but is instrumental for animals in research and directly connected to the individual animal's wellbeing or suffering. It's about the quality of how animal experiments are conducted and who conducts them. Some aspects are covered by legislations within the different countries. They are mainly related to infrastructure and environment of animal facilities. It also includes personnel with their training and very rudimentary documentations.

Most of the FELASA accredited laboratories nowadays include veterinarians for staff members. However, these are often not veterinary specialists for either small rodent facilities or large animal surgery, respectively anaesthesia. Nowadays, this should be unacceptable in all laboratories. It should be required by law that veterinary specialists with board certifications of the European or American Colleges of Laboratory Animals (ECLAM/ACLAM) are leaders of such facilities. It should also be the law that specialists of the European or American Veterinary Colleges of surgery (ECVS/ACVS) and of anaesthesia and analgesia (ECVAA/ACVAA) must be involved in every single surgery for experiments with larger animals like sheep, goats, pigs, calves, heifers, dogs and cats or primates.

Medical doctors, biologists or other basic scientists should not be allowed to be alone at the table in future when surgeries on these animals are conducted. Surgeries should only be permitted in

collaboration with veterinary specialists which, in the case of larger animals, should have both ECVS/ACVS and ECVAA/ACVAA diplomas. The latter are just as important for conducting correct anaesthesia and analgesia regimens. Too many serious errors happen and have been witnessed around surgeries and aftercare by the author, when specialists are not involved.

Quality

If ethical concerns are taken seriously, it starts right there: with the quality of the experiments performed at the table with each individual animal, and not only at the drawing board or with administrative legislation. Involving true specialists would reduce incidents that include administration of wrong human dosages to sheep for instance with muscle relaxants, heparin, or other incompatible drugs leading to experiment-unrelated death of hundreds of experimental animals in the world, some including serious suffering of animals (bleeding or suffocating to death).

Quality in animal experiments also includes their standard documentation and considerations of regulatory affairs if therapeutic approaches are studied and are part of the project. This goes hand in hand and should be part of initial planning in animal experiments to avoid repeating them later. This is imminent for reducing animal numbers right from the start (3Rs). Regulatory affairs relate to accreditation of preclinical experiments by either European agencies (TUV) or the FDA (Food and Drug Administration) in the United States, a prerequisite for allowance of clinical trials, Phase I-III and later registration of any medication, medical devices or combination products. Depending on the study and the technology tested, preclinical experiments should comply with Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP).

Once clinical studies are attempted these have to be conducted according to Good Clinical Practice (GCP). Different to the United States, where projects can be conducted according to GLP given the correct audits, in Europe the facility has to be officially acknowledged by the authorities (e.g. Swissmedic in Switzerland) prior to any GLP experiment being conducted. In most countries GLP or GMP approved facilities are private companies without the academic background of universities and other academic institutions (e.g. Fraunhofer institutes in Germany), where the expertise for most projects would be considerably broader.

This major gap between academia and later industrial needs is responsible for many animal experiments that have to be repeated, or are not accepted by the regulatory bodies due to the animal model used and missing or incomplete documentation. Therefore, it is advisable to include companies that should be involved in production and upscaling of test items, and contact regulatory bodies right from the start. To include these considerations in scientific translational and also basic projects means applying ethical considerations for animal experiments in everyday business of researchers.

Roadmaps of Horizon 2020 consider regulatory affairs in connection with animal experiments an important aspect for translation from bench to bedside.

The official Competence Center for Applied Biotechnology and Molecular Medicine (CABMM) is a unique professional network at the University of Zürich, Switzerland, for translational medicine where medical problems are investigated literally from "bench to bedside" (see http://www.cabmm.uzh.ch/index.html). The expert members of the CABMM deal with either: A) experimental medicine or surgery; B) molecular medicine; C) regenerative medicine; or D) applied biotechnology. Basic researchers focus on molecular regulation mechanisms, and material scientists place their emphasis on developing new (intelligent) scaffolds/matrices used for tissue engineering, one of the modern backbones of modern regenerative medicine. *In vitro* generated tissues are studied in preclinical experimental animal studies, where biocompatibility, integration and functionality tested before clinical trial phases in humans can be initiated.

The main strategic goal of the CABMM is the promotion of translational research based on excellent interaction between basic research and clinics, academic institutions and industrial partners. Through consolidation and optimisation of an already excellent infrastructure, the methodical knowhow is continuously improved and the expertise of all members and their national and international research partners allows the development of products and appropriate technology transfer. Through the uniqueness of the CABMM, the University of Zürich is the only European university with official accreditation in Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP). These are required to get products registered at the FDA for clinical application.

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The CABMM's Professor Dr Brigitte von Rechenberg and her fellow researchers outline their studies into transcutaneous drug delivery using a novel transdermal application technology

Transcutaneous drug delivery

Chronic deep wounds and chronic joint disease are always a problem for patients due to the constant pain and restrictions in daily living, for both people and animals. Chronic wounds may prove difficult to heal for many reasons. Among them one of the major inhibitions is scarce distribution of drugs to wound beds and edges due to excess fibrosis and scar formation. Missing vascularity, often alongside chronic infection within the scar tissue, is just one of the major underlying problems.

Hyaline cartilage damage within the joint, often resulting from synovial membrane inflammation or small lesions, will worsen over time and result in irreversible changes. Matrix destruction is the end-result for both chronic inflammation and small lesions. In both cases, matrix-degrading enzymes such as metalloproteinases, aggrecanases, hyaluronidases and cathepsins are triggered by inflammatory mediators (nitric oxide (NO), prostaglandin (PGE2)) and cytokines (interleukins (IL1, IL6) and tumour necrosis factor) and will finally degrade the collagen network and proteoglycans, thus changing the hydro-osmotic pressure of the hyaline cartilage. It is then only a question of time until fibrillation of the cartilage surface occurs, followed by fissures and cleft formation down to the subchondral bone plate. Erosion of cartilage down to the subchondral bone is the devastating result for patients, at which point stronger measures of therapy such as joint replacements through full or partial prosthesis may become necessary.

Fig. 1 MedDrop device

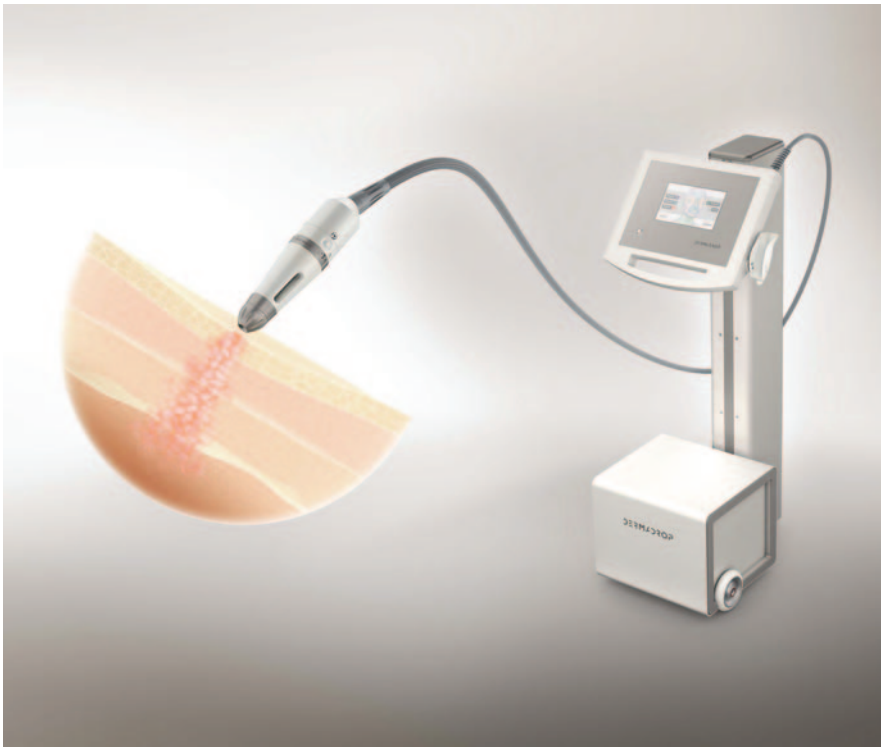
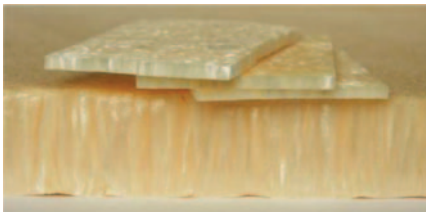


Fig. 2 Valeoskin scaffold



Although research in this field of osteoarthritis is extensive and has elucidated many important mechanisms about how the homeostasis of the hyaline cartilage is imbalanced, it is not yet possible to stop the degenerative processes, nor can full regeneration of the cartilage surface be achieved. Chondroprotective supplements or anti-inflammatory drugs can partially slow down the mechanism and take some of the pain away. Some approaches have engaged in cell-based therapies, but even these cannot fully restore joint integrity. Like in chronic wounds, one of the major problems in joint disease is also the local distribution of medication and keeping a therapeutic drug level over a longer period of time in the affected tissues.

Although non-steroidal anti-inflammatory drugs (NSAID) or cortison derivates may decrease the degree of chronic inflammation and pain, they cannot put a halt to the degradation process. In contrast, NSAIDs may dose-dependently accelerate the activity of metalloproteinases and cortison derivates may completely inhibit the synthesis of new macromolecules. Furthermore, chronic systemic application of NSAIDs and particularly cortison derivates may cause complications, such as gastric ulcers, liver and kidney failures.

For both deep wound infections and joint diseases, alternative and innovative drug

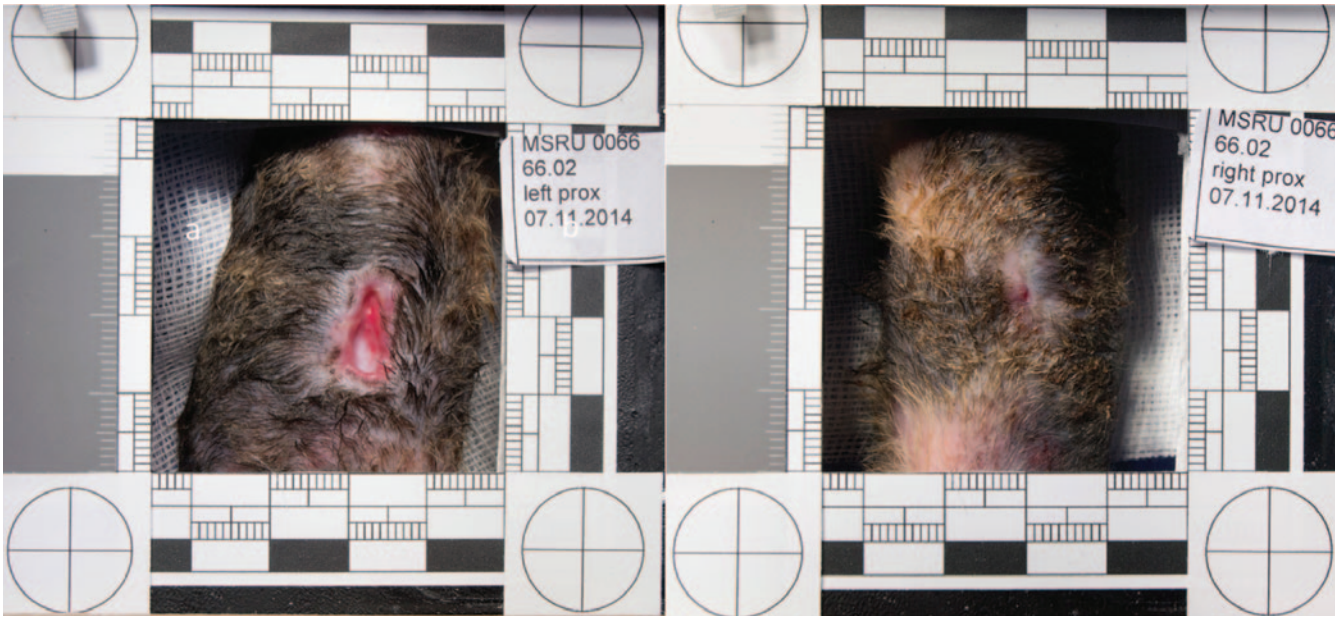


Fig. 3 Wound healing in cats at 24 days after experimental skin lesions: wound closure with hydrogel (Fig. 3a) is significantly slower compared to those treated with Valeoskin® (Fig. 3b)

application techniques resulting in effective tissue concentrations with fewer systemic side effects are needed.

Transdermal application (TDA) technology

The TDA technology we used was developed for human and veterinary applications ('MedDrop' and 'VetDrop'). The principle of this technology is based on a non-invasive, pain-free delivery of active ingredients through or onto the skin. Applications are meant for therapies of skin and also joint problems. The system has been applied successfully in human patients with a variety of skin diseases and in horses with lameness. In fact, a declared goal is the medical device registration for the human medical market and, therefore, preclinical studies in animals were and are still conducted at our institution.

The technology was developed by MedDrop Technology AG (Zürich, Switzerland) and is based on an oxygen flow through a Venturi valve as well as a computer-operated application system which produces a nanodispersion of a drug mixture in the oxygen flow (Fig. 1). Compressed oxygen is delivered through special tubing to the applicator system. The applicator is a nanodispersion device that has a drug reservoir (customised capsules). The oxygen propels the carrier vehicle and the active ingredients under pressure through the diffuser system, which is held to the skin at a distance of approximately 2cm. The combination of the oxygen and the carrier molecule transports the active ingredients through the skin into the deeper tissues.

TDA for skin problems

As for the skin, the TDA technology is used in deep wounds with a chitosan-based scaffold (Valeoskin®) in combination with porcine gelatin to give a vertical structure for the wound and ingrowing cells from the regenerating wound bed (Fig. 2). This scaffold is degraded into amino acids, oligopeptides and glucosamine monomers, which can be utilised for regeneration processes, thus improving cell proliferation and vascularisation within the wound bed. Together with the TDA technology, other molecules (such as NSAIDs) can further be delivered into the deeper wound areas, and thus reach areas unreachable with conventional application methods.

Ongoing experimental preclinical studies in rats and cats at the CABMM have shown that the combination of the Valeoskin scaffold and the TDA technology is far superior to conventional wound treatment, such that closure of standardised wounds is almost 50% faster compared to controls that were only treated with hydrogel alone (Fig. 3). Along the same lines, it could be shown in clinical studies with deep and chronically infected wounds in humans due to diabetic ulcers that long term wounds over several years could be closed within a few months using the TDA technology in combination with the Valeoskin scaffolds (clinical study ongoing).

TDA for cartilage lesions

For cartilage lesions, preclinical studies in sheep were conducted at the CABMM to study the regeneration potential of the TDA technology using the femoral condyles of the stifle joint as an animal model. Experimental lesions of 6mm diameter were created in the weight-bearing area of both condyles and treated with a microfracturing technique in combination with the TDA technology for post-operative treatment (Fig. 4). Several groups were formed with treatments immediately after surgery and then every two to three days for a total of 18 applications. The application area was of 10cm^2 over the femoral condyles. Sheep

either received the vehicle alone, vehicle plus chito-oligosaccharide, vehicle plus carprofen (*ca.* 6.7%), vehicle plus chito-oligosaccharides plus carprofen, or the carrier oxygen alone, and, finally as controls, carprofen at a dosage of 4mg/kg/BW given intravenously.

Blood concentrations of carprofen concentrations were measured at different time intervals from all animals, the controls and the ones where carprofen was also part of the TDA mixture. After the first treatment, concentrations were measured over 18 hours, and then six hours after each treatment up to 40 days. For the intravenous control group, concentrations were measured at several intervals for 12 hours for five days, and then at eight and 12 days after the intravenous applications of carprofen. In addition, synovial fluid measurements were conducted at weekly intervals over six weeks.

Results

For the TDA groups, carprofen concentration could already be measured at 30 minutes after treatment with the peak concentration at 18 hours. Plasma levels increased after each application and could be well maintained over the entire 18 applications. Although lower than in blood samples, carprofen concentrations in synovial fluid correlated well with the plasma concentrations and could be measured also with the peak at six weeks. The combination with oligosaccharides increased the permeability and the concentration in the synovial fluid. Not surprisingly, the control groups had the highest peak plasma concentration of carprofen at just ten minutes after intravenous application, and also about a 300-fold higher overall concentration compared to the TDA group.

Fig. 4 TDA application in living sheep at the medial aspect of the stifle joint

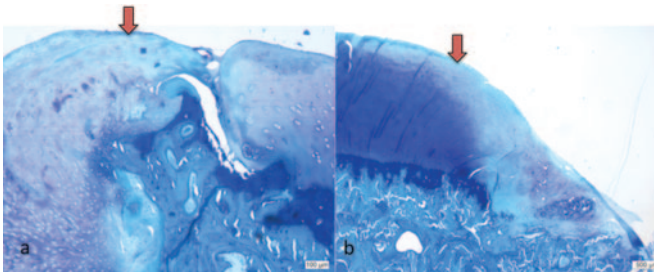


Fig. 5 Histology picture of cartilage lesion at three months after surgery and six weeks after the last TDA application with either vehicle alone (Fig. 5a) or vehicle + carprofen as a mixture (Fig. 5b): note the adjacent cartilage (block arrow), which shows considerable loss of matrix staining when vehicle alone is applied compared to the mixture of vehicle + carprofen

It has to be noted, however, that the carprofen concentration was higher in the classic intravenous injection than in the mixture of the TDA to begin with. Interesting was the fact that the TDA technology provided a longer lasting, steady and increasing concentration of carprofen after each application, whereas the high peaks after intravenous application decreased rather quickly and had no long-lasting effect. Although lower in concentration, with the TDA technology the local concentration was sufficient to have an improved effect on the degradation of the adjacent hyaline cartilage without having the same complication effects as seen in systemic applications.

Normally, the edges of a cartilage lesion degrade quickly and are almost acting as a 'nucleus' for degradation to spread further into the cartilage matrix – this being a major reason why cartilage degradation cannot be stopped once it has started. In our preclinical sheep experiments, it could be shown that the TDA technology using the vehicle plus chito-oligosaccharides plus carprofen combination improved the histological score of the adjacent cartilage above all other groups (Fig. 5). These results suggested that the lower intra-articular concentration of carprofen may have a more beneficial effect on maintaining a proper balance between regenerative and degenerative processes within the joint.

Further study

Encouraged by these results, another pilot study in sheep was conducted with an animal model for chronic hyaline cartilage degradation after the creation of a subchondral bone defect in the proximal metaphyseal area of the tibia. A rectangular defect of 1.5cm width, 1.5cm height and 1.8cm depth was created at the medial aspect of the tibia plateau, just 4mm below the articular cartilage surface. The bone defect was filled with autologous bone grafts for all six animals.

Earlier studies performed with this animal model of 136 experimental sheep at the CABMM demonstrated severe cartilage matrix degeneration in 100% of the animals within the first two to three months, starting with severe signs of matrix damage at just two weeks after surgery. In the pilot study comparing three sheep to three untreated animals, the TDA technology was applied immediately after surgery and for daily treatments thereafter until sacrifice of the animals at two weeks. This time, the TDA mixture for the active ingredients consisted of vehicle plus chito-oligosaccharides plus Diclofenac instead of carprofen (another NSAID).

Our earlier findings with the effect of TDA technology to prevent damage to the adjacent cartilage could be confirmed: the three animals treated with vehicle plus chito-oligosaccharides plus Diclofenac showed better cartilage surface preservation compared to the untreated animals, where fibrillation and cleft formation was readily visible. Overall proteoglycan loss was clearly less severe, indicated by improved matrix staining using toluidine blue. Interestingly, small clefts were also seen which, in contrast to the control groups, were still 'glued' together and where the edges were still filled with viable cells as if in an attempt the heal the lesion (Fig. 6). Clefts in cartilage surfaces are normally wide open and do not grow back together. In addition, more viable chondrocytes were seen in the TDA-treated cartilage samples compared to the untreated controls, which only received autografts to fill the bone defect.

Our hypothesis at this point is that the TDA treatments may have several positive effects on cartilage preservation: the relatively low concentration of the NSAIDs may just be enough to inhibit cartilage degradation locally while not interfering with the regenerative processes and required increase of macromolecule synthesis to maintain the homeostasis of the matrix. In addition, oxygen as the carrier system may beneficially influence chondrocyte viability and proliferation at the same time. Further studies are ongoing where optimal concentrations of the active ingredients and long term effects are tested in the same animal model.

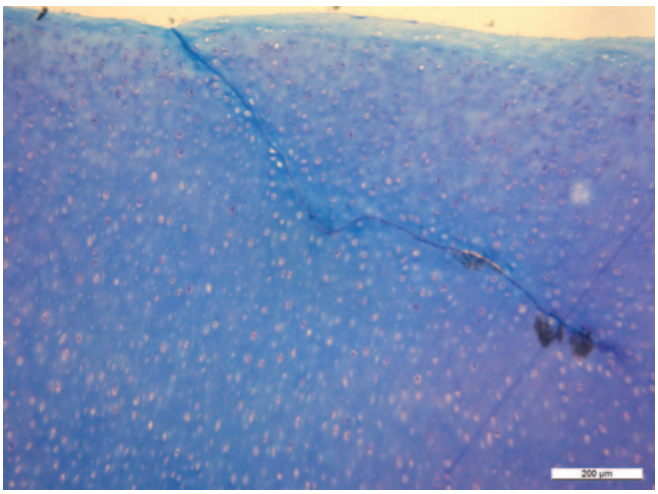


Fig. 6 Histology picture of hyaline cartilage after creating a subchondral metaphyseal defect in the proximal tibia: note that the cleft in the cartilage, which is a common sign for cartilage degeneration, is still sealed together. In addition, no loss of cellular viability or loss of metachromatic staining of the matrix is noted close to the cleft after TDA application

Outlook

The TDA technology is a promising tool for the transcutaneous delivery of active ingredients to deeper layers of tissue which in disease may not be amenable to classic ways of medication. Future studies at the CABMM will investigate the possibility of modulating regenerative and inflammatory processes with this type of regimen in wound and cartilage regeneration.

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TRANSLATIONAL RESEARCH

The Competence Center for Applied Biotechnology and Molecular Medicine defines the truth of translational research

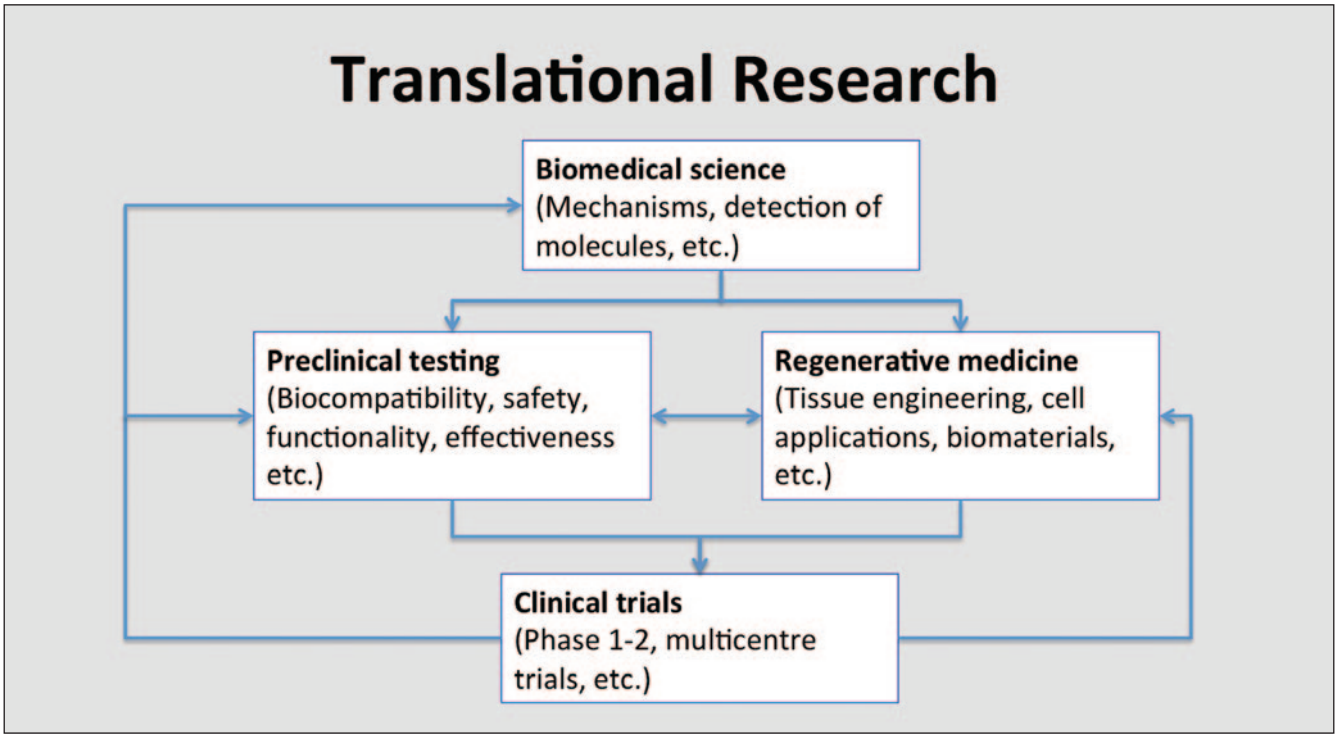
The ambition of medical research often undergoes fashion seasons in the same way as the textile industry – one summer it's the mini-skirt and high heels and the next it is long, ruffled skirts and sandals. 'Translational medicine' is the latest trend in medical research. Modern research groups and networks all over the world claim that they are involved in and contributing to this area – knowing, of course, that this is well received, in particular by funding agencies. However, looking a bit closer, there often isn't actually much translation behind the proposed projects, and the research is performed as ever before – on the bench alone – and it will never make it to the bedside of a patient.

Misnomer

The expression 'translational medicine' is a misnomer to start with. Medicine always comprises the translation aspect from basic scientific knowledge into clinical applications for the benefit of patients. The phrase should be 'translational medical research', and then the word 'translation' has to be better defined. Translational medical research aims to improve the health of individuals and the community by 'translating' findings into diagnostic tools, pharmaceuticals, procedures, policies and education, which basically indicates that knowledge gained from any area finally ends up with the patients for their benefit.

As an example: inventing a new pacemaker for heart patients is at best medical engineering but not yet translational research. This expression is only justified when the newly invented device undergoes all preclinical tests and finally receives permission to be used in a clinical human trial. The same is true for tissue-engineered constructs, such as in cell culture and bioreactors, engineered skin or vessel transplants. The translational aspect starts only after the proof of concept work has been done experimentally, e.g. at the point when the construct is implanted into an experimental animal in preclinical tests, with a very strong emphasis on, or in concrete preparation to perform, later human clinical use.

Ideally, the basic scientists in translational research (the researchers performing the animal experiments and the clinicians who at the end implant devices and tissue-engineered constructs or apply new medication in human patients) are involved from the very beginning of the innovation and plan the research throughout the experimental course as an interdisciplinary team. In an even more ideal translation, the clinician also provides material, such as biopsies, blood samples,



etc., from diseased patients for the basic scientists to investigate the mechanisms of the underlying disease or to study the benefit of the new treatment modality, thus driving the new innovation from the other end (from bedside to bench).

So translation is not a one-way street – it goes from 'bench to bedside and back'. If a researcher claims translation, then the direction of research has to be clearly directed towards a treatment modality that can later be used to combat disease. Finding a molecular regulatory mechanism of a disease or new molecule that inhibits an enzyme alone has nothing to do with translational research yet. This is still very valuable basic research, but for its translation it needs more than that.

Realistic undertakings

In medical reality, translation also involves regulatory affairs on many levels. For example, any tissue-engineered construct that was tested in preclinical studies in experimental animals, and is finally regulatory approved for clinical trials, has to involve the production of tissues under GMP (good manufacturing practice), preclinical testing under GLP (good laboratory practice) and finally clinical testing under GCP (good clinical practice) conditions. All three levels involve rigorous procedural protocols that are accredited by the medical authorities in each country and, due to market reasons, often at the end by the FDA (Food and Drug Administration, USA). Apart from the protocols, procedures also require accredited infrastructures that form the basis of these procedures, which are the safety basis for later use in human patients. These are expensive and extensive undertakings, and thus many, if not the majority of, good research findings get lost during this translational process.

The Competence Center for Applied Biotechnology and Molecular Medicine (CABMM) at the University of Zürich, Switzerland, was founded in 2008 to cope with this challenge. It is an interdisciplinary centre with the aim to perform, co-ordinate and promote clinically-oriented experimental research in the fields of biotechnology, regenerative medicine and molecular medicine. The promotion of scientific exchange and collaboration between basic scientists and clinically-oriented research groups, conducting joint research projects, and the transfer of gained project knowledge to sustain further development by filing patent applications and/or collaborations with industry are the core of the CABMM. In conjunction with the university hospitals, the University of Zürich is the only university, at least in Europe, that offers the full translation pipeline, including regulatory aspects with GMP (Swiss Center for Regenerative Medicine), GLP (Musculoskeletal Research Unit) and GCP (university hospitals).

In this context, the newly initiated Wyss Translational Center Zürich (in conjunction with the Swiss Federal Institute of Technology) further adds to the unique translational research competence by strictly focusing on the efficient translation of medical innovations and novel human-centric technologies in the fields of regenerative medicine and robotic technologies.

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WITH OBESITY REMAINING A CRITICAL GLOBAL CHALLENGE, RESEARCHERS FROM THE UNIVERSITY OF ZÜRICH DETAIL THEIR EFFORTS TO BETTER UNDERSTAND THE DYNAMICS AROUND THE ISSUE

The path to understanding

Working on the biochemical pathways leading to obesity, senior researcher PD Dr Brigitta Wichert, Institute of Animal nutrition (Director: Professor Dr Annette Liesegang), Vetsuisse Faculty Zürich, discusses problems observed due to genetic differences which were observed by chance.

Who is prone to obesity?

Overweight has become one of the major health risks worldwide. According to the World Health Organization (WHO) obesity was once considered as a problem only in high income countries, but is now dramatically on the rise in low and middle income countries as well, particularly in urban settings. Overweight and obesity in humans are major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. They are known to have a complex aetiology, influenced by a wide range of different factors including excessive food intake, inappropriate nutrient composition of food, lack of physical activity and genetic factors. Companion animals like cats live under similar living conditions as humans and therefore have the same risk factors. Comparable to humans, obese cats are in danger of developing Type 2 diabetes. Increasing body weight and the associated increasing body fat lead to decreasing insulin sensitivity and hyperglycaemia. Both are known as early warning signals for Type 2 diabetes. The induced insulin resistance can be reversible with weight loss in humans and cats. It is therefore important to prevent humans and cats from becoming overweight and to reduce existing excessive body weight.

How is obesity inherited?

We observed a segregating overweight phenotype in the cat breeding family located at the Institute of Animal Nutrition at the Vetsuisse Faculty, University of Zürich (Figs. 1 and 2).

The research of PD Dr Brigitta Wichert started to uncover a genetic background of the obesity phenomenon in the cat family of the institute. It all started with an observation that under identical feeding and housing regimes, we observed differences in phenotype. There were animals that gained weight very early during growth in contrast to those that retained their weight over years. A complex segregation analysis showed a genetic involvement and indicated the presence of an autosomal recessive major gene and a possible polygenic component.

Fig. 1 Phenotype of two male cats aged one year (fourth generation) of the named cat breeding family. Both cats were kept under the same conditions and on the same diet and feeding regimen. On the left the lean phenotype and on the right the obese one

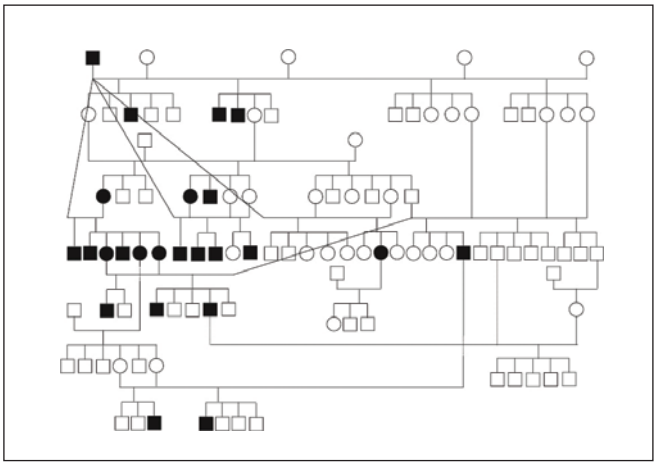

Fig. 2 Seventh-generation pedigree of the named cat breeding family with a segregating overweight phenotype. The cats were phenotyped with the help of the body condition scoring system, symbols: squares = males, circles = females; white = lean phenotype; black = obese phenotype

The animals were genotyped and a genome-wide association analysis was performed. The analysis identified genomic regions on chromosome 12 and nine associated with body condition score. The regions contain Proopiomelanocortin (POMC) and Melanocortin 4 receptor (MC4R) as positional candidate genes, and we are currently investigating the coding sequence of both genes to determine if variants or the combination of variants in those genes are associated with different body weight phenotypes. Mutations in the coding region of MC4R and POMC have been shown to be involved in development of obesity and obesity-associated phenotypes in rodents, as well as in humans.

Physiology of obesity

These findings can give new possibilities in providing an insight over the mechanisms lying behind the phenomenon of obesity.

The overweight phenotype in the cat family of the Institute of Animal Nutrition demonstrated that in addition to the genetic abnormalities, differences in physiological reactions exist; like a tendency to lower energy expenditure, a significant higher food intake (Fig. 3) and significant larger meal size. However, meal frequency and duration of a meal were not significantly different.

Although coding mutations have been shown to effect bodyweight, non-coding and epigenetic factors also play an important role in the development of obesity. This could, for instance, be the lack of an important signal for the termination of a meal. Due to our research programmes, which were already carried out at our institute, there is a large database which can be used as reference for other studies, such as the body weight development of kittens as well as growth of young animals before puberty, and body condition scoring of young animals' glucose and insulin concentrations at different timepoints of age and under different feeding regimes.

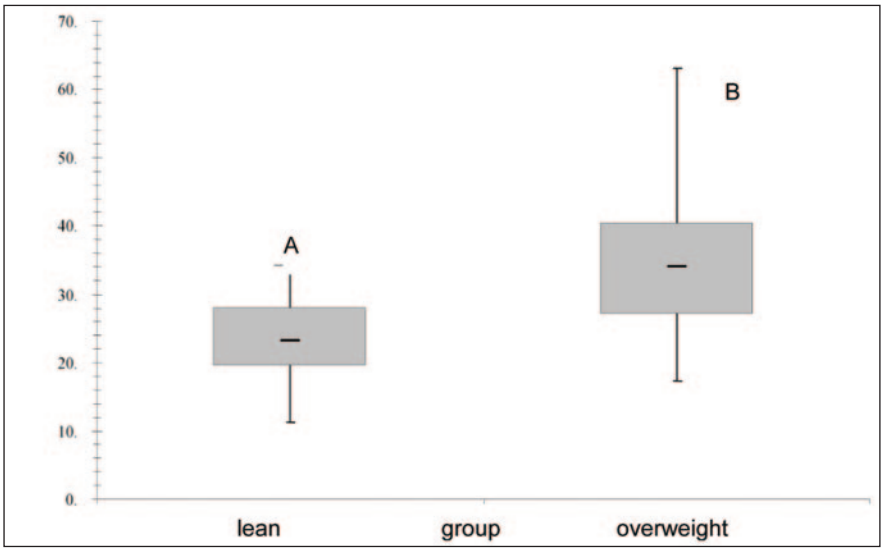

Fig. 3 Food intake (g DM/kg BW0.67) of two groups of male cats (one group belonging to the lean and one to the overweight phenotype)

Future opportunities

To further enhance the understanding of mechanisms involved in the development of obesity and obesity-associated diseases, the cat family of the Institute of Animal Nutrition is very promising due to the hereditary overweight phenotype. Besides the genotype, many other factors discussed to influence the development of obesity can be investigated in future. Among these especially is the influence of diet. The different nutrients like proteins, fat and carbohydrates may play an important role in the prevention or promotion of disease. This means higher contents of proteins might prevent diabetes in contrast to e.g. high carbohydrate diets, which might lead to a predisposition towards diabetes. Another interesting aspect would be the feeding of the mothers during gestation and lactation, the microbiome at different time points, under different feeding conditions and with different body condition scores, gene expression under differing conditions as well as other epigenetic factors. Last but not least, sterilised female cats may serve as a model for the increase of body weight during the postmenopausal period. In this context the mechanisms behind that phenomenon would also be of great interest.

Professor Dr med vet Brigitte von Rechenberg

Brigitta Wichert (second from right) and Annette Liesegang (third from right)

CABMM role

Within the Competence Center of Applied Biotechnology and Molecular Medicine (CABMM) at the University of Zürich, many possibilities are given, since the Institute of Animal Nutrition is able to use the whole infrastructure as well as playing a part within this infrastructure. The Institute of Animal Nutrition also delivers their expertise and laboratory skills to the other partners. This technically sophisticated surrounding of the CABMM is available to all institutions which need the specific expertise of the partners; and with this network a much higher impact is achieved.

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cabmm
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From bench to bedside and back again

The Center for Applied Biotechnology and Molecular Medicine (CABMM)

The 'Center for Applied Biotechnology and Molecular Medicine' is an official competence centre of the University of Zurich with the objective to create a stimulating environment for interdisciplinary and translational research in order to promote scientific exchange and collaborations between basic and clinical researchers.

The CABMM shows a unique structure, combining (i) a network of existing research groups interested in scientific exchange and collaboration on interdisciplinary and translational research projects and (ii) a platform for collaborative research, where basic scientists, clinicians and veterinarians work shoulder to shoulder for the purpose of developing novel therapeutic approaches for the treatment of dysfunctional and diseased tissue.

Thereby, unlike other research centres, the CABMM is not focusing on one particular medical field, but on translational and interdisciplinary aspects. Thus, range and diversity of research being conducted within the CABMM is broad, but all research follows one aim: to facilitate the development of new treatment regimes by building a bridge between basic and clinical researchers.

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