



Hand transplantation without side effects of immunosuppression



Hand transplantation

Ongoing studies at the **University of Bern**, Switzerland are seeking to develop novel on-demand drug delivery systems in order to reduce the risk of systemic immunosuppression following transplantation

FOR INDIVIDUALS WHO have suffered soft tissue or congenital defects, extirpation of tumours or severe burns or injury, the prospect of a hand transplant is often the last therapeutic resort. However, the procedure is widely regarded as extremely difficult to perform due to challenges with accurately replicating the sensations of touch, pressure and pain. In recent years, vascularised composite allotransplantation (VCA) – which involves multiple types of tissue – has emerged as a novel technique for both hand and face transplantations, and is now a widely used therapeutic option in reconstructive surgery.

Despite this significant recent advance, in many cases the risks associated with the chronic immunosuppression which is required to prevent transplant rejection are prohibitively high. In response to this obstacle, groundbreaking collaborative work between the Department of Clinical Research within the University of Bern and the Clinic of Plastic and Hand Surgery at the Bern University Hospital, Switzerland is seeking to improve the specificity and localisation of immunosuppressive (IS) drug delivery. The teams of Professors Robert Rieben and Esther Vögelin and Dr Thusitha Gajanayake hope that the studies will result in a significant shift in the transplant paradigm, reducing the risks associated with immunosuppression and enabling hand transplantation to become a more common procedure.

FK506-ENCAPSULATED HYDROGEL

Ultimately, Rieben, Vögelin and Gajanayake aim to create an on-demand drug delivery system which is able to release IS drugs in the appropriate concentration exactly when and where they are required. To this end, the team has been working to introduce the drug to a system governed according to the complex biochemical characteristics of an allograft,

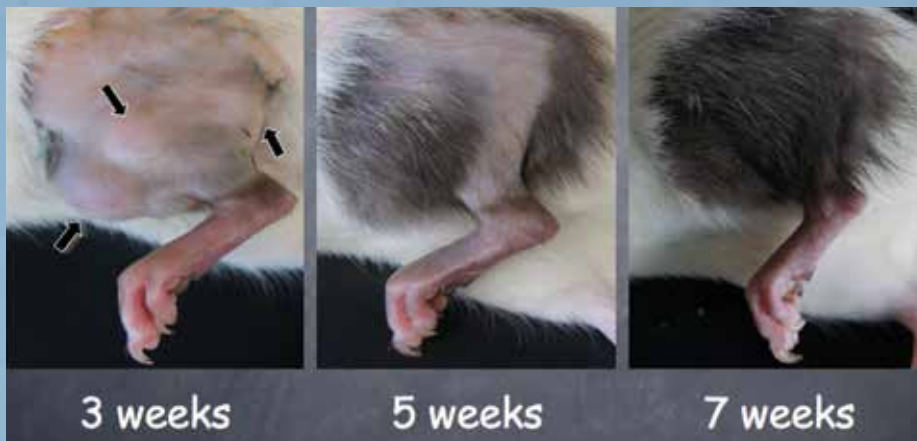
as Rieben elucidates further: "We loaded FK506 tacrolimus – a widely used IS drug – into an amphiphilic three-dimensional matrix, otherwise known as a hydrogel". Hydrogels can comprise any water-soluble polymer, either in macro- or nanoparticle form.

Recent developments, such as those presided over by Dr Praveen Vemula at inStem in Bangalore, India, have seen hydrogels becoming biocompatible. Vemula is an important partner of the Bern group and has been pioneering a system involving a self-assembled nanofibrous hydrogel, which has shown great promise in treating rheumatoid arthritis. The FK506-encapsulated hydrogel developed as a collaboration between Bern and Bangalore relies on the fact that under the influence of specific enzymes which are upregulated as a result of inflammation – the chief cause of transplant rejection – the hydrogel undergoes a process of cleavage, releasing its IS drug to the patient.

CLINICAL TRIALS

In order to trial this system, Rieben, Vögelin and Gajanayake have been carrying out tests involving the transplantation of a hind limb from a Brown Norway to a Lewis rat, injecting a single dose of the FK506-injected hydrogel into the model the day after the transplant. Over the course of these experiments, the graft went on to survive for 100 days – more than three times the median survival period for rats that were administered with the same dose of FK506 directly, without the hydrogel delivery system.

The hydrogel was injected under the skin and significant swelling was observed, which Gajanayake believes is attributable to increased water absorption. It was thus shown to remain in the locality of the graft for over 100 days, releasing the desired amount of FK506 each day. Graft survival under this method was also shown to be significantly higher than in recipient rats in which the hydrogel was injected into the contralateral limb, which lacks the inflammatory conditions provided by the graft



LONG-TERM SURVIVAL OF TGMS-FK06 TREATED GRAFTS

area. Additionally, the trials demonstrated that the hydrogel is highly responsive to environmental changes within the body, such as shifts in pH or temperature, causing it to undergo desirable changes both to its physiology and biochemistry.

The next step for Rieben, Vögelin and Gajanayake is to elucidate the exact mechanisms by which prolonged IS drug release via locally injected hydrogel is achieved, a goal which will see them experimenting with the administration of several doses of the FK506-injected hydrogel at intervals of three months. The researchers are confident that as their understanding of the properties of hydrogels develops, they can – in collaboration with the partners in Bangalore – continue to fine-tune the delivery system in order to further improve the prognosis for hand transplantation patients.

PATHOPHYSIOLOGY OF ISCHAEMIA/REPERFUSION INJURY

Alongside research on hydrogels, Rieben's lab has also made some interesting and significant progress in the field of injury involving ischaemia and reperfusion. In studies involving myocardial infarction in pig models, the researchers were able to employ endothelium-protecting drugs in order to almost completely prevent reperfusion injury, leading them to hypothesise that changes in endothelial cells during ischaemia can shift their anticoagulant and anti-inflammatory properties to procoagulant and pro-inflammatory ones.

"These changes have something to do with the glycocalyx – the layer of negatively charged sugar molecules on the endothelial surface," Rieben posits. By directly blocking the complement system which operates on the endothelium, the researchers effectively demonstrated that the prevention of reperfusion injury can be achieved. They have now moved on to explore a promising drug candidate for use in rat hind limb ischaemia/reperfusion models, within which the skeletal muscle's coagulation and bradykinin systems play a more crucial role. The researchers are confident that these studies will eventually lead to a breakthrough which could result in improvements in novel therapies for human ischaemia and reperfusion.

COLLABORATION

In addition to his collaboration with Vemula, who is working to develop a more effective biocompatible and biodegradable amphiphilic hydrogel system, Rieben has a number of other positive working relationships with fellow researchers. "Particularly for the Ischaemia/Reperfusion Research Group, collaboration with both clinical partners on the one hand and partners from the pharmaceutical industry on the other is very important," he enthuses. The majority of Rieben's clinical partners are surgeons or interventional cardiologists, with whom he greatly enjoys working because of their grounded approach and ability to root their thinking within the needs of individual patients. The Bern investigators also have a number of national and international partners, enabling an exchange of knowledge and expertise which benefits all parties, and without which meaningful biological and medical research would be almost impossible.

THE NEXT STEP

Keen to expand on the significant progress they have made to date, Rieben, Vögelin and Gajanayake are already planning a number of future studies. Perhaps most notably, their prospective research into the local effect of IS drugs on nerve regeneration in hand transplantation holds promise. Although FK506 is the current drug of choice in hand transplantations, due to its strong IS and neuroregenerative qualities it can demonstrate a number of side-effects, including nephrotoxicity, hyperglycaemia and central nervous system toxicity. For this reason, the researchers are seeking a more reliable system for local delivery in order to facilitate an increase in the clinical use of the drug.

"We will also work continuously on immunomodulation and immunoregulation in vascularised composite tissue allografts, with the ultimate goal of reducing the toxicity of IS drugs," Rieben outlines. They are hopeful that this work will not only benefit individuals who undergo hand transplantation, but will also have a wider knock-on effect within the transplantation field as a whole. By combining their own immunological knowledge with the surgical and biochemical expertise of their collaborators, the Bern team is confident that their findings will reach clinical application in the not too distant future.

INTELLIGENCE

HAND TRANSPLANTATION WITHOUT SIDE EFFECTS OF IMMUNOSUPPRESSION

OBJECTIVES

- To establish a clinically relevant immunosuppressive protocol in hand transplantation
- To optimise the effect of current immunosuppressive drugs by better understanding the pharmacodynamics and pharmacokinetics
- To develop a local delivery system for immunosuppressive drugs which would provide high local tissue levels of the active compound with considerably lowered systemic concentrations and thus weaker side-effects

KEY COLLABORATORS

Professor Esther Vögelin, Inselspital, Bern University Hospital, Bern, Switzerland • **Dr Thusitha Gajanayake**, Department of Clinical Research, University of Bern, Switzerland • **Dr Praveen Vemula**, inStem, Bangalore, India

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PROFESSOR ROBERT RIEBEN received his MA in 1987 and PhD in 1992 from the University of Bern. Postdoctoral positions followed in Bern and the Leiden University Medical Center, The Netherlands, before he became Senior Scientist at Bern University Hospital in 1997. He received his Habilitation in 2002, and was promoted to Associate Professor in 2007. Since 2005 he has been Senior Scientist and Research Coordinator in the Department of Clinical Research at the University of Bern.

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A hands-on approach

Professors Robert Rieben and Esther Vögelin and Dr Thusitha Gajanayake are dedicated to improving the prognosis for patients who have undergone hand transplantation procedures. Here, they talk about the importance of their work, and managing their substantial workload



Could you begin by providing a brief overview of the aims and objectives of your research?

Many experimental strategies have been used in an attempt to overcome toxicities associated with immunosuppressive (IS) drugs, but most are still far away from clinical application. Our approach is to establish a clinically relevant protocol for hand transplantation, optimising the effect of current IS drugs by better understanding their pharmacodynamics and pharmacokinetics.

IS drugs are rapidly absorbed by the blood stream, reaching a peak concentration systemically before being metabolised and cleared. Within this pharmacokinetic profile, there is a limited therapeutic window during which the desired therapeutic effect is seen. With standard, oral drug administration it is impossible to avoid higher plasma levels than are clinically desirable. Our goal is to develop a local delivery system for the IS drugs which would provide high local tissue levels of the active compound with considerably lowered systemic concentrations, reducing side-effects.

What is the current state of the art when it comes to hand transplantation?

Vascularised composite tissue allografts have long been of interest to transplant surgeons. A prime example of a vascularised composite allograft (VCA) is the hand, which comprises several tissues such as skin, fascia, muscle,

bone and cartilage. The first hand transplantation was performed in Ecuador in 1964, but due to the unavailability of an efficient IS protocol at that time, it was rejected after two weeks. The first successful hand transplantation came in 1998 in Lyon, France and since then over 70 hand transplantations have been carried out. Today, widespread clinical application of hand transplantation is prevented by the necessity of IS drugs which have potent health complications; immunosuppression use for a condition that is not life threatening is highly contentious.

There must be significant benefits in having a clinical partnership between the Inselspital, University Hospital Bern and the Department of Clinical Research at the University of Bern. Has this partnership proved fruitful for your investigations?

Our two departments are indeed collaborating very closely. Our research directly aims to find answers to the problems raised in clinical reconstructive surgery. In addition, the complex microsurgical animal models are routinely performed by talented surgeons from the Department of Plastic and Hand Surgery. We are continuously blending our immunological knowledge with that of the clinics to achieve a better understanding of hand transplantation as a whole.

On a personal level, do you encounter difficulties with effectively balancing your time between clinical work, research and administration?

This is indeed a big challenge. The economical pressure on our health system makes it increasingly difficult for clinicians

to dedicate time for research. Similarly, researchers at the University spend more and more time with administrative issues. Just one example: 10-15 years ago, a contract for investigator-driven lab research with a pharmaceutical company was a two-page document. We conducted the research and discussed the issues surrounding intellectual property and publication of data with our industry partners. Today, we get a 20-page document from the company, I have to get this checked by our technology transfer office and signed by the director of administration. Our university is really doing its best to keep the administrative hassle as low as possible but this is a general problem in contemporary society, common sense is disappearing and jurisdiction and quality control are taking over.

Finally, how do you rate the public's familiarity with hand transplantation and what should be done to improve awareness?

With the success of VCAs, this field has gained considerable public attention worldwide. However, since hand transplantation has never been performed in Switzerland, public awareness here is low.

To disseminate this topic, we plan to organise symposia and seminars and to publish our research in magazines and newspapers. Moreover, we encourage Master's students to join us to learn about VCAs.

Another issue which is of importance for our clinical partners is that hand transplantations are currently not paid for by our insurance companies. The issue of lifelong, systemic immunosuppression with considerable side-effects forms a major part of this discussion. Our research, which should lead to a significant reduction in the systemic impact of immunosuppression will therefore also have an impact on this ongoing political discourse.