# A customised endoprothesis for the stifle joints of rabbits for preclinical studies

The Competence Center of Applied Biotechnology and Molecular Medicine (CABMM) explores their findings in studying customisable implantation prostheses

The implantation of permanent endoprostheses has become a routine therapeutic measure in cases of failing joint structures. Full and partial prostheses are available for human patients depending on the joint structure to be replaced. Nowadays hip replacements are certainly the most frequently applied procedures, followed closely by knee replacements. But endoprostheses for shoulder, tibiatarsal and finger joints are also available, although less frequently used compared to hip and knee.

## The standard for implantation prostheses

Most of the endoprostheses are based on metal, although ceramics are becoming more popular. Both have their advantages and disadvantages. While metal-on-metal hips were once popular, problems with wear particles and granulomatous pseudo-tumour reactions of the soft tissue close to the articulation have almost removed them from the market over the last five years.

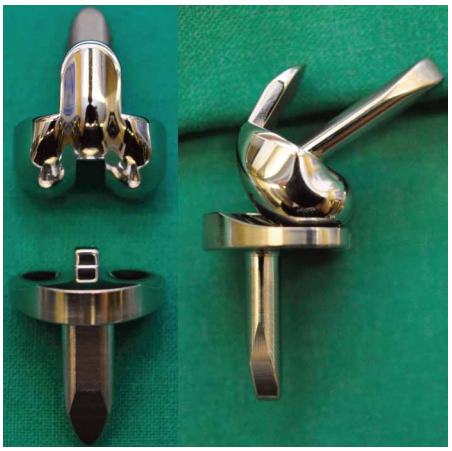

Fig. 1 Final rabbit knee prosthesis (CoCrMo), in anterioposterior (left) and mediolateral view (right)

Nevertheless, up until today, metals such as cobalt-chrome-molybdenum (CoCrMo) or titanium alloys are still often used in combination with plastic surfaces, as, for instance, polyethylene acetabular cups mounted on a metal basis.

Although results, on average, are excellent with modern surgical technology, wear and tear affects all permanent implants, shortening their in-life phase considerably by eliciting inflammatory reactions around the implant and finally aseptic loosening. Modular systems may also contribute to this phenomenon through tribocorrosion at the junction of the modular components. Therefore, modern research is still looking into the improvement of permanent implants by studying new coatings of prosthesis components that would make the implants more resistant against wear and tear, including tribocorrosion.

These coatings need to be tested for biocompatibility, excellent adhesion on the implant components, as well as their resistance against biomechanical load, resp. friction at the articulation. *In vitro* tests are good preliminary test systems for studying biocompatibility on a cellular level, corrosion and resistance against wear and tear under standardised conditions. However, at the end of the day, they cannot replace the reality test *in vivo*, where all these parameters are combined, including osseointegration of the permanent implant under physiological loading of the joint and normal ambulation. Therefore, standardised preclinical models for testing permanent implants are warranted.

## Studying the impact of prosthetic implants in rabbits

In the medical literature, no real standardised experimental animal model is described.

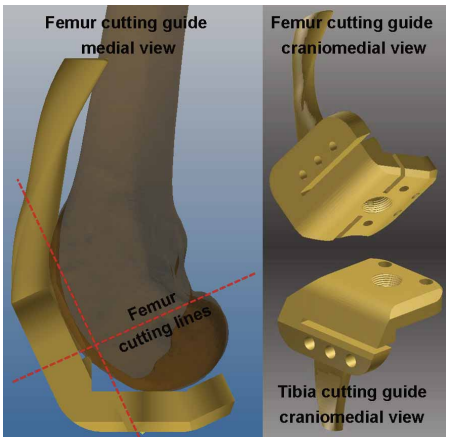


Fig. 2 Cutting guides for femur (left picture: placed guide on femoral condyle, right top: craniomedial view) and tibia (right bottom)

Preclinical experiments mostly focused on hip implants, but full and partial prostheses of stifle joints were also used in dogs, sheep, goats, minipigs and also rabbits. However, none of these were reported to be implants that were specifically customised to the anatomy and function of a joint of the species being tested. However, having an implant available that was adapted to the biomechanical load and anatomy of the selected species seemed of paramount importance when intricate questions such as wear and tear of a permanent implant, including new coatings, were evaluated. Therefore, part of our EU-project (FP7-NMP-2012-LARGE-6: LifeLongJoints, Grant agreement No: 310477) was to establish a standardised experimental animal model, where permanent implants could be tested and evaluated under appropriate anatomical and functional conditions.

The rabbit was selected as the species, where ethical and economical reasons formed the basis of this decision. The stifle joint was chosen as

model due to its easy access and standardisation potential as a more or less uniaxial joint. The fact that rabbits have a different angle of the stifle joint, due to its crouching position compared to humans, was considered. Since the purpose of the model was to study wear and tear of the permanent implant, this aspect could be neglected. As long as the physiologic load of the joint and normal ambulation of the experimental animal was guaranteed, the different angle was not expected to have an influence on the outcome.

A commercially available finger prosthesis for humans served as the basis of the customised implant for rabbits. 3D scans, CTs and motion analysis of the normal stifle joints of average-sized female New Zealand White rabbits (3.1-4.5kg BW, *ca.* eight months of age) were conducted, and results transferred to a computer-aided design system (CAD).

## Standardising prosthetic implantation

Prototypes developed from polymers were produced and tested in cadavers with the appropriate size. The final metal prostheses were produced either out of titanium or CoCrMo with traditional machining technology (Jossi Orthopedics AG, Islikon, Switzerland) (Fig. 1). The prosthesis was designed as a self-locking prototype, such that luxations of the two components could be avoided. For standardisation of the surgical technique, prototypes for cutting guides to remove the femoral condyles and tibia plateau were also designed using CAD (Fig. 2). Both prostheses, titanium and CoCrMo, had to be fixed with commercially available bone cement in the tibial and femoral shafts. Positioning of the prosthesis shaft was always checked by fluoroscopy before final cementing of the implant.

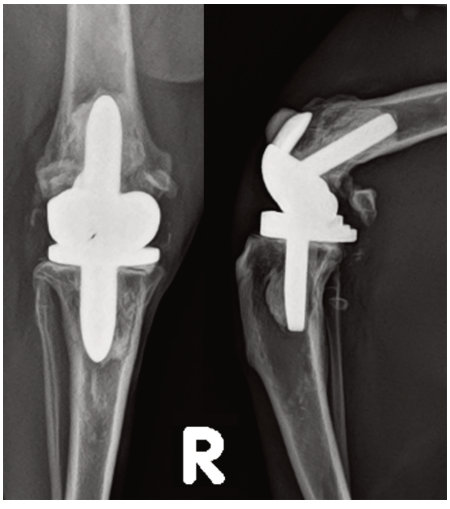


Fig. 3 Radiographs 8 weeks after surgery, in anterioposterior (left) and mediolateral view (right)

A standard operative procedure (SOP) was established that guaranteed repeatability of the procedure for the surgeons involved (KK, MC). SOPs were also created for evaluation of the in-life phase with scores for daily clinical observation, and anteroposterior and mediolateral radiographs postoperatively and every four weeks thereafter (Fig. 3). Intravital fluorescence dyes were used to document bone remodelling during osseointegration (Fig. 4). Furthermore, the macroscopic and microscopic evaluation procedures post-sacrifice were also fixed in SOPs for the implant *in situ*, the efferent lymph nodes in the popliteal and inguinal area of the operated limbs, and the synovial membrane. Microscopic evaluation was made according to ISO 10993-6 guidelines. All procedures were conducted under the Good Laboratory Conditions (GLP) standard. Animal experiments were performed according to the Swiss law of animal welfare (TSchG 455) and authorised through the local cantonal veterinary authorities.

## Assessing the suitability of customisable prostheses

To prove the suitability of the stifle prosthesis, a total of 34 rabbits were operated on, of which 28 rabbits were observed over 12 weeks after implantation. The surgical technique proved very reliable in all cases, with an average of between 40-60 minutes after gaining some routine. The rabbits took up normal eating and behaviour as soon as 70 minutes after surgery. They also started to move around on the second and third day after surgery. Lameness was noted, on average, for 37 days, although weight was placed on the operated limb already within the first week. Complications related to the prosthesis were rare, as such that one of 34 rabbits (1/34 rabbits) had to undergo a patella luxation correction and recovered without further complications; in one of 34 rabbits the prosthesis became loose on the second day after surgery due to cement failure; and one of 34 rabbits had a luxation of the two prosthesis components, which could not be corrected. At sacrifice it showed that the axis of the prosthesis was incorrect, as such that the angle of the tibia plateau was oblique. Four more rabbits had to be excluded for non-study-related reasons. One fractured its femur in the cage door and three were excluded due to severe coccidiosis (coccidiosis). The surgical technique and implant design proved successful in all other 28 rabbits.

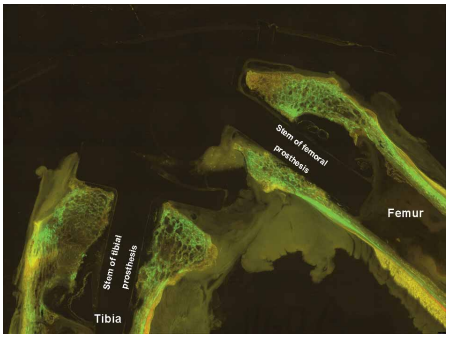


Fig. 4 Fluorescence picture, green: calcein green deposition at four weeks post surgery, red: xylenol orange deposition eight weeks post surgery, yellow: oxytetracycline 48h prior to sacrifice

Radiographic evaluation showed good alignment and positioning of the prosthesis. A sacrifice restriction of the extension angle of the stifle joint was recorded and varied between 80-130° (normal extension 180°). Restriction was associated with fibrosis of the surrounding soft tissue, which was again correlated to an inflammatory reaction to a new coating of the original metal components in these animals. Apart from the in-life observation, the developed score system for assessing tissue reaction in the synovial membranes or lymph nodes proved highly reliable and repeatable and, therefore, fulfilled the scope of high-degree standardisation.

Overall, the study showed that the customised stifle joint prosthesis for rabbits proved suitable for its original purpose: to establish a standardised animal model for testing biocompatibility for new materials and coatings of permanent implants.


## Co-authors

Stephen Ferguson, Prof. Dr.
Affiliation: ETHZ, CABMM

Maurizio Calcagni, PD Dr.med.
Affiliation: University Hospital Zurich, CABMM

Karina Klein, Dr.med.vet., PhD
Affiliation: MSRU

Isabel Heckel. med. vet.
Affiliation: MSRU

Professor Dr Brigitte von Rechenberg
Musculoskeletal Research Unit
Vetsuisse Faculty ZH
Competence Center of Applied Biotechnology and Molecular Medicine (CABMM)
University of Zurich