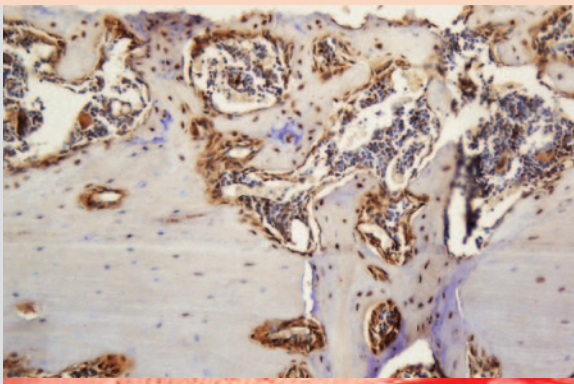
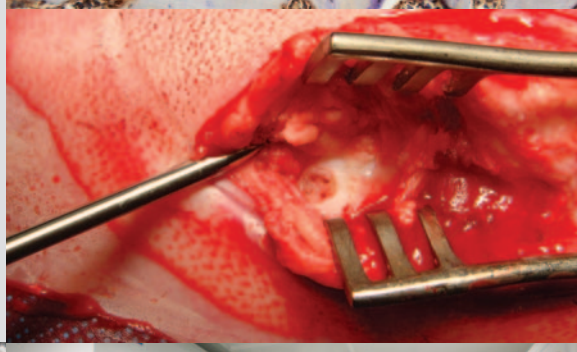
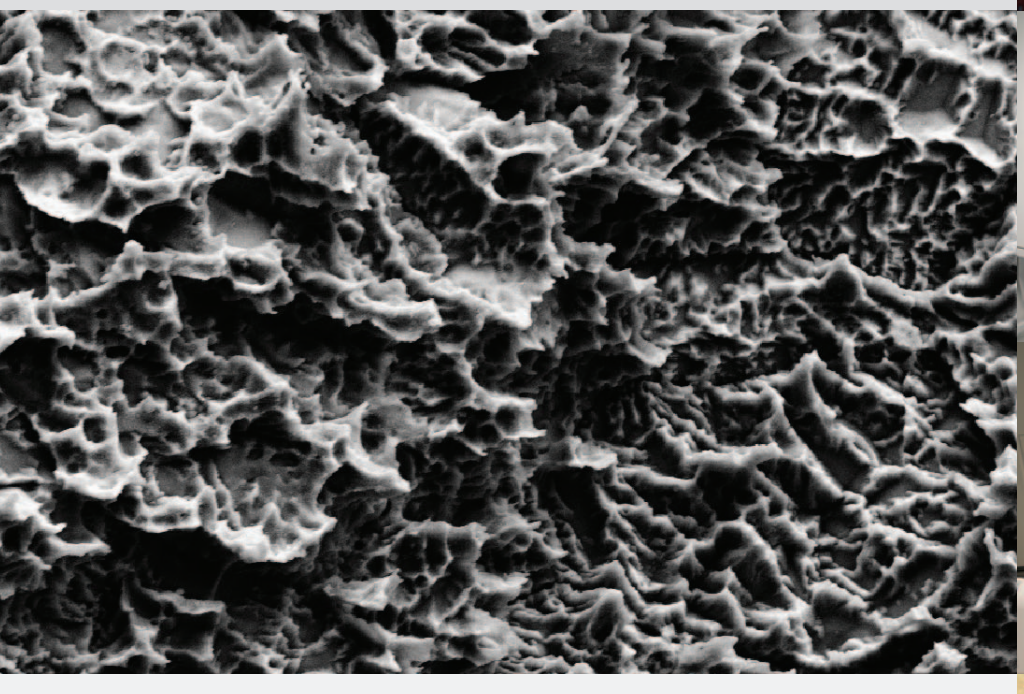
The Competence Center for Applied Biotechnology and Molecular Medicine







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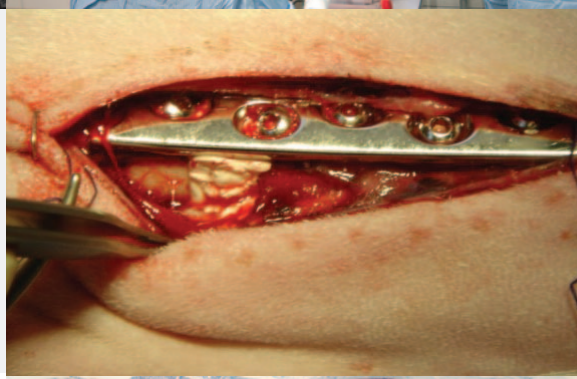

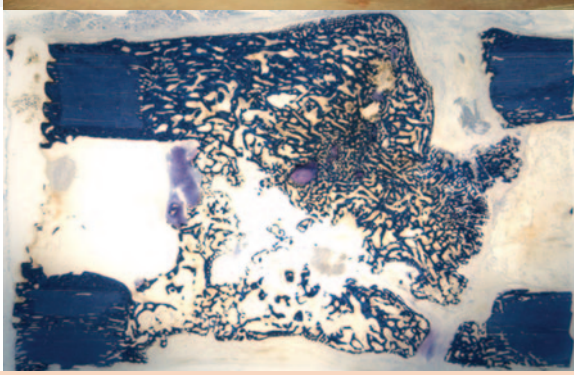

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From bench to bedside and back again

The Center for Applied Biotechnology and Molecular Medicine (CABMM)

The "Center for Applied Biotechnology and Molecular Medicine (CABMM)" is an official competence center 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 centers, the CABMM is not focusing on one particular medical field, but on translational and interdisciplinary aspects. Thus, range and diversity or 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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Regulatory affairs at the CABMM

The CABMM is unique in many ways within the research landscape for several reasons. One of them is the fact that research conducted at the CABMM fulfils regulatory requirements for having novel products or technologies accredited for their manufacturing, preclinical studies and/or clinical trial phases in human patients.

Nowadays, most medical products seek registration at the Food and Drug Administration (FDA), which guarantees entry into the largest markets in the world; especially novel strategies in regenerative medicine, where cells of various origins, together with natural or synthetic scaffolds and/or biomimetic agents, will be implanted in patients and have to undergo strict regulatory controls in many ways.

The FDA requires these products to be synthesised and/or produced under Good Manufacturing Practice Conditions (GMP), which include not only accredited and highly specialised infrastructure, but also current protocols, storage conditions and trained personnel to handle all related issues.

The CABMM is fortunate to offer this possibility in the facilities of the first Swiss Center for Regenerative Medicine under the leadership of Prof Dr med, Dr rer nat Simon Philipp Hoerstrup, a well known researcher in cardiovascular research and regenerative medicine (www.remedi.uzh.ch/aboutus/team/hoerstrup-1.html). This centre is the first officially accredited GMP institution at a University in Switzerland and offers great possibilities to prepare and store primary cells and cell-based therapies for preclinical animal studies or clinical studies in human patients for research collaborators.

Research in permanence

The University of Zurich's Professor Dr Brigitte von Rechenberg discusses research in osseointegration of permanent medical devices

Permanent implants have their fixed place in medicine today, such that they replace functions of organs permanently and allow patients to lead an almost normal life. There are different types of permanent implants replacing either soft tissue (breast implants, surgical mesh in hernias, gastric bands, cardiovascular stents, etc.) or hard tissue in the form of prostheses. The majority of the latter replace joint functions, such as in the hip, shoulder, knee or phalanges. Furthermore, cages are used for the fusion of vertebral bodies in the case of spine instabilities. In dentistry it has become a widely accepted surgical procedure to reconstruct lost teeth functionally as well as aesthetically using implants. In fact, the development and integration of cutting-edge technologies for innovative treatment concepts in implantology currently turn out to form a central part of state-of-the-art treatment concept in orthopaedics and dentistry.

Biocompatibility and osseointegration

Although these implant applications are variable in their biological function and location, the basic questions to be solved for a good clinical outcome are very similar. Biocompatibility with the surrounding tissue is one of the main requisites and for implants anchored in bone osseointegration long-term, is one of the major challenges.

'Biomechanical resistance to wear and tear determines the suitability of a permanent implant together with biocompatibility issues. Pure titanium or titanium alloys are among the most biocompatible materials nowadays and are frequently used for non-cemented permanent implants in orthopaedics and dentistry.'

Osseointegration describes the functional connection of the synthetic implant and the living bone creating a direct interface between them. Good osseointegration means direct primary bone deposition on the implant without the formation of a fibrous interface membrane between implant and bone. Originally it was Brånemark who coined the "direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant" as osseointegration in dental implants. A fibrous interface membrane between implant and bone indicates biomechanical instability and/or in combination with chronic inflammatory cells (lymphocytes, plasma cells) also bio-incompatibility. Nowadays, it is a desirable modern concept to shorten the time of osseointegration towards minimising the healing period before an implant finally is exposed to biomechanical forces. In this context a reliable and stable anchorage of implants (primary stability) under functional loading conditions is considered as the vital point for a positive treatment outcome. Different measures to enhance osseointegration are scrutinised.

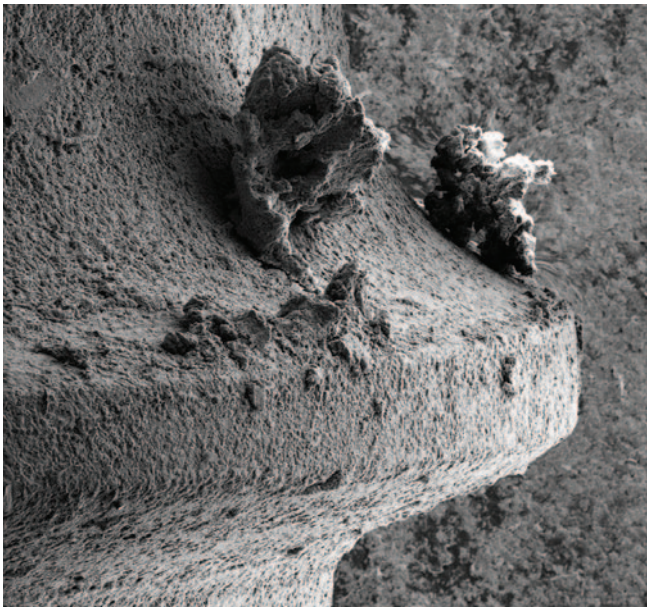

Fig. 1 Bone cells are attached to a titanium surface that was functionalised with a biomimetic to enhance osseointegration. The implant surface is presented after two months in vivo *and after removal torque test (TEM picture)*

Implant material properties

Different materials are used for permanent orthopaedic implants. Apart from the biomechanical properties of the materials, cost efficacy and production issues have to be considered for implant design. Biomechanical resistance to wear and tear determines the suitability of a permanent implant together with biocompatibility issues. Pure titanium or titanium alloys are among the most biocompatible materials nowadays and are frequently used for non-cemented permanent implants in orthopaedics and dentistry. Their high tensile strength, corrosion resistance and acceptance from the adjacent bone makes them very attractive materials for permanent implants. Bone cells attach directly on the surface and deposit bone matrix on the surface of the implant (Fig. 1). Therefore, this material is also often used not only as a primary implant (dentistry), but often as a coating of a less biocompatible implant like cobalt chrome, zirkonia or even newer polymers such as Peek (polyether ether ketone).

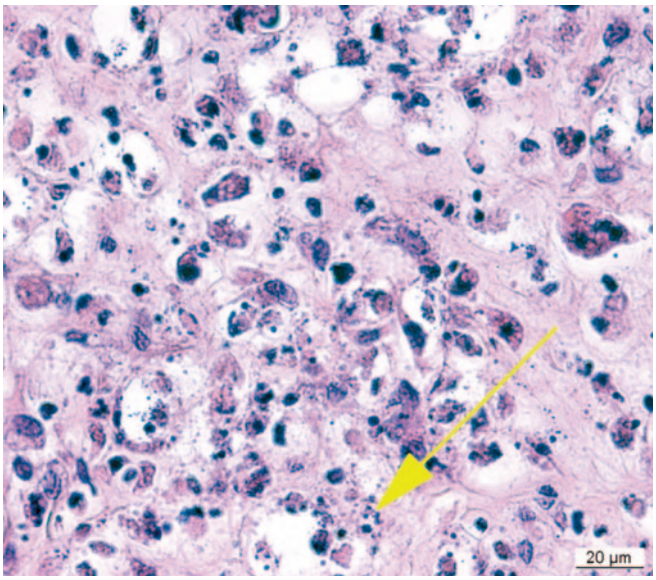

Fig. 2 Numerous wear particles are detected in macrophages in the interface membrane after primary instability of a titanium implant

'Aseptic loosening is a complex process that has many factors leading to final failure. Although wear particles are considered as the main reason, this is too simplistic and demonstrates clearly the short coming of translating *in vitro* results directly to the etiopathogenesis of clinical problems.'

Implant corrosion

For long-term metal implants, corrosion behaviour is very important. Under the term 'corrosion' the chemical reaction of the metal to its environment is summarised. Ion release of metals on their surface is normal and is called 'surface corrosion'. It can be accelerated by moisture, electrochemical changes in the environment (pH) and needs to be controlled for medical implants. A passive surface or oxide layer on the metal surface mostly induced by exposure to air can be protective and is especially good in titanium implants.

However, this passivation layer can be destroyed through different mechanisms, such as galvanic corrosion (electrochemical action between two different types of materials), pitting corrosion (accumulation of small pits at the metal surface), crevice corrosion (friction between two metals) or stress corrosion (mechanical overload and breakage on grain boundaries within the metal). Often combinations between electrochemical and mechanical issues lead to severe changes of the metal surface through tribocorrosion. The effect of tribocorrosion is highest in passive metals with a thin oxide layer. Once friction and electrochemical changes occur, it leads to the formation of wear particles (nanometres to micrometres) that are literally removed from the implant surface as a result of the movement between two opposing surfaces. These wear particles can be generated from metal as well as polymer surfaces and are considered

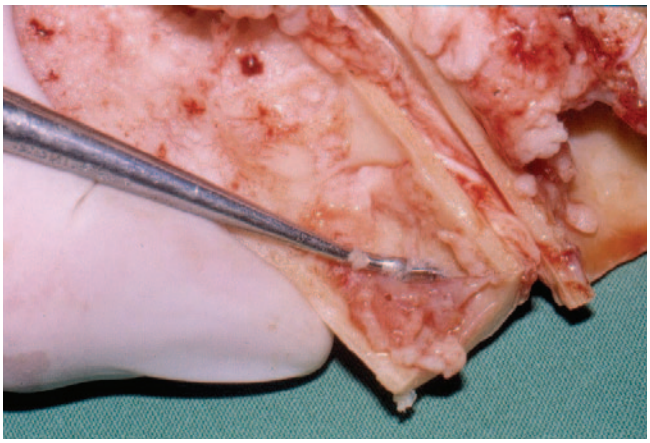

Fig. 3 The formation of a thick interface membrane between implant and bone is present at eight months after insertion of a primary unstable femoral component consisting of cobalt chrome

instrumental in the process of implant loosening. Actually, mostly they are incriminated as 'the reason' for the process of aseptic loosening of permanent implants.

Aseptic loosening of implants

Aseptic loosening is a complex process that has many factors leading to final failure. Although wear particles are considered as the main reason, this is too simplistic and demonstrates clearly the short coming of translating *in vitro* results directly to the etiopathogenesis of clinical problems. Translational medicine (from bench to bedside) includes many issues, in this case of permanent implants it is a mixture of biomechanics, surgical technique, basic mechanisms of wound healing including inflammatory responses, material properties, implant design and, last but not least, the patient's individual response to the implant (allergies, immune status and patient compliance). These factors taken together lead to a complex cascade where on each level failures can occur and if not stopped or prevented will lead to a vicious cycle and final implant failure.

The cascade starts with the quality of the host bone and whether the inflammatory status is under control. The surgical technique and fine tuning of implant bed preparation is the next step (training and quality of surgeon), but in any case – even with the best surgeon – will result in a massive lesion, for instance if hip, knee or shoulder prostheses are inserted. Inflammation will be the body's response and tissue debris has to be removed by cellular mechanisms, mainly macrophages. Inflammation in bone is always accompanied by local bone resorption. If the implant has good primary stability and is well anchored within the bone, the inflammation will subside rather quickly and bone resorption will be minimal. However, if primary (micro) instability of the implant is present, inflammatory signals of tissue are enhanced through the additional mechanical overload and bone resorption will be increased at the bone-implant interface. Local inflammation will not only lead to recruitment and activation of osteoclasts, the bone resorbing cells, but also to a change of local milieu.

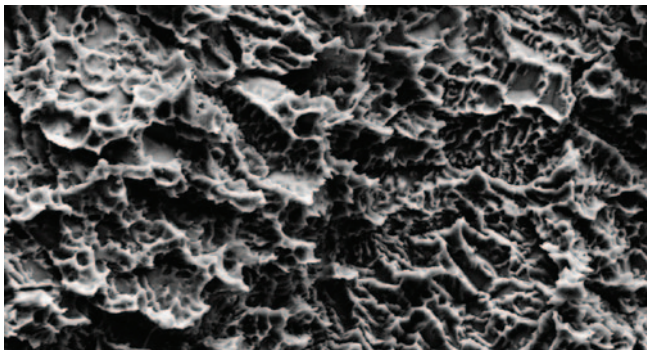

Fig. 4 The surface topography of a titanium implant

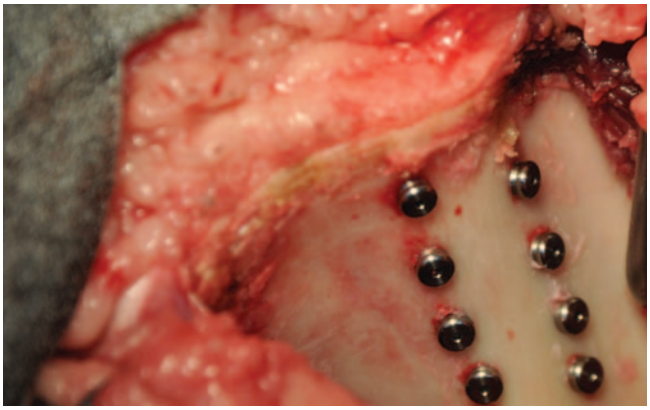

Fig. 5 Implants are placed in the ilial wing of the pelvis in sheep to test osseointegration

‘Physical, chemical or biological characteristics of the original material may be changed such that roughness, surface charge and energy, reactivity and finally biocompatibility are altered. Surface topography of metal implants is optimised through adapting surface macro and microporosity.’

The local pH will decrease and become more acidic, which in turn changes the surface corrosion behaviour of the (metallic) implants. Together with the mechanical instability, tribocorrosion is enhanced which generates a dramatic increase of wear particle production (Fig. 2). The tolerance of macrophages that normally ingest wear particles and remove them from the implant surface is exceeded and inflammation is perpetuated at a dangerously high level. Eventually this process leads to the formation of a rather thick interface membrane that further enhances instability (Fig. 3). At this stage the perfect ‘perpetuum mobile’ is established with no way out but removing the implant and replacing it with a new one in a subsequent revision surgery.

By then the local bone quality is compromised and chances for an uncomplicated outcome are somewhat reduced. It has become clear from this cascade that aseptic loosening is determined early in the course and thus, modern research efforts are concentrated on early osseointegration by providing the best implant designs and sophisticated surface finishing and/or coatings.

Implant design

Implant designs in orthopaedics and dentistry are highly complex and include many features. The size and geometry of implants have to mimic the anatomy and physiological function at their best. Modern computer technology in imaging and implant design make it possible to fabricate ideal implants out of suitable biomaterials for different and special clinical indications. Implant body design, thread pattern as well as pitch distances are mechanical implant features, which are related to implant macro design. Modular systems allow for the selection of different implant components increasing the variability and fitting of implants for individual patients. Biomechanical analyses of retrieved implants from patients with aseptic loosening reveal mechanisms contributing to the problem of implant failure. Although the high variability of modular systems are attractive, material problems, such as cold welding or metal on metal friction, seem to be involved in severe cases of aseptic loosening that are accompanied by tumour-like changes of the inflamed tissue. In cases of prostheses replacing a joint, the two opposing surfaces (e.g. head and cup in hip prosthesis) need to be perfectly aligned to prevent uneven gliding of the two materials and focal overload and material degradation.

Implant surface

Much research nowadays is devoted to surface modification of implants. Physical, chemical or biological characteristics of the original material may be changed such that roughness, surface charge and energy, reactivity and finally biocompatibility are altered. Surface topography of metal implants is optimised through adapting surface macro and microporosity (Fig. 4). Various technologies are available, among them sandblasting, anodisation, acid etching and laser treatment as the most important. Additional surface biofunctionalisation and nanostructuring can be achieved through plasma polymerisation, covalent binding of poly (ethylene glycol) (PEG), heparinisation, peptide functionalisation and calcium phosphate deposition, etc. All these technologies aim at improving the direct attachment of osteoblasts and encouraging the deposition of bone matrix macromolecules at the implant surface.

Testing osseointegration in animal experiments

Testing osseointegration of implants can only be done in animal experiments. Suitable animal models are mandatory and need critical review. According to international standards, species suitable for testing implants in bone include dogs, mini pigs, sheep and rabbits. Although dogs and rabbits are some of the most frequently used models, they offer certain drawbacks and constraints like e.g. significant differences in bone composition, bone metabolism, healing rate and anatomy. While rabbits produce bone very easily, positive results may not always be reproduced in larger species, such as in dogs or sheep. In addition, the use of dogs in experimental surgery poses ethical questions that cause problems in our modern society where the animal-human bond is illustrated with dogs as the most beloved pets and companions. A well suited and highly standardised animal model in sheep was established in

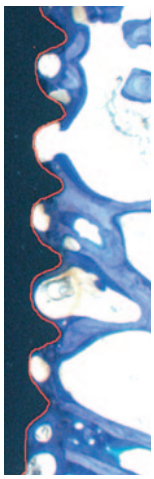

Fig. 6 A histology section shows two different metal implants in situ *(PMMA, 30-40µm, surface staining with toluidine blue). Note the differences between bone formation at the implant surface and within the adjacent bone*

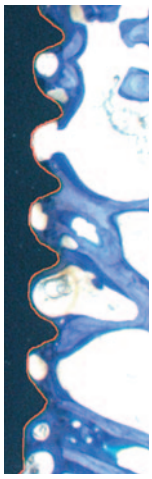

Fig. 7 Measuring the bone-implant-contact (BIC) in ground section is demonstrated. Specialised software (Qwips, Qwin, Leica, Heerbrugg, Switzerland) is used to quantify the total implant surface and the bone directly attached to the implant. The BIC is calculated in percentage of the total implant surface

our laboratory, where implants can be tested for biocompatibility and osseointegrative properties in the iliac wing of the pelvis (Fig. 5). It proved its reliability (>1,000 implants) in testing different implant designs and surface modifications. Especially the high number of implant test sites in one animal (n=18) as well as the possibility to place implants of a length of up to 10mm and diameter up to 6mm under aseptic conditions render this model ideal for translational implant research. Furthermore, known from orthopaedic research, where sheep are commonly applied for analysing fracture healing, new osteosynthesis techniques and also osseointegration of implants, the bone metabolism is similar to humans. In contrast to other frequently used anatomical implant locations in sheep like e.g. the tibia or mandible, implant placement in the pelvis allows a differentiation and comparison between cortical and trabecular bone structures. Last but not least, this animal model allows assessing osseointegration without interference of mechanical issues.

For the analysis of osseointegration and the contiguous bone-to-implant-contact a wide variety of different image-guided and biomechanical research methods are currently available. It is the combination of various test methods that facilitate drawing conclusions and compare implant performances. Bone samples embedded in polymethylmetacrylate (PMMA) allow cutting histological sections with the implant *in situ* (Fig. 6). The bone-implant-contact (BIC) can be measured (Fig. 7) as well as the percentage of new bone formation as a response in the adjacent bone. Pending the implant design biomechanical test such as removal torque, push-out or pull-out tests indicate the forces required to loosen the bone implant bone contact. The application of intravital fluorescent dyes facilitates the assessment of bone remodelling over time (Fig. 8) in response to the implant surfaces, which is especially important if biological modifications were applied. Modern three-dimensional imaging technologies, such as micro-computer tomography (µCT) or atomic force microscopy provide unprecedented opportunities in material science to specify and define the surface topography on a micro and nano level. Further state-of-the-art imaged guided technologies include scanning electron microscopy, microradiography and resonance frequency analysis.

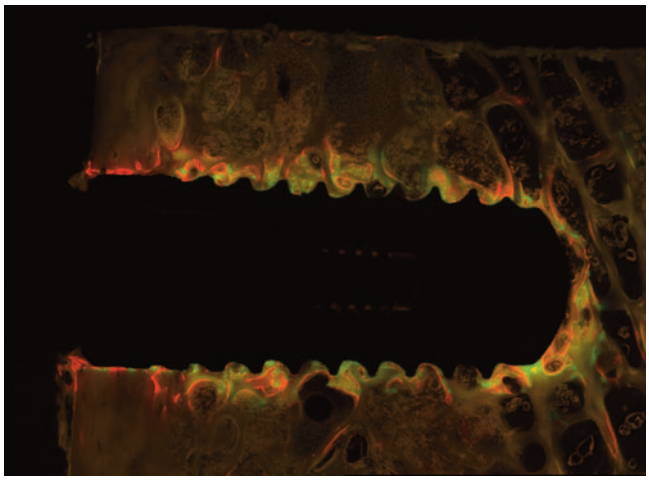

Fig. 8 Fluorescence dyes can be visualised in native sections using special filters

Conclusion and outlook

Permanent medical devices will increase tremendously in the future especially in a society more and more formed by the elderly. Furthermore, sports and subsequent overuse of joints and cartilage degeneration, accidents and, in the case of dental implants, a higher focus on aesthetics, will contribute to this development. Increasing age of the population will also increase the demand of longer lasting permanent implants. Therefore, research with osseointegration of medical implants will be important in translational medicine. Advanced immediate and early loading protocols of implant-borne restorations and novel treatment approaches are requested, where implant stability will always be the primary focus.

The CABMM's mission, vision and values

The CABMM is dedicated to fostering advances in clinically oriented research in the fields of regenerative medicine, experimental medicine and surgery, applied biotechnology, and molecular medicine. Embedded within both the Vetsuisse and Medical Faculties of Zurich University, it provides an interdisciplinary research platform on which basic scientists and clinicians are able to exchange scientific information and create collaborations for the purpose of developing novel therapeutic approaches for the treatment of dysfunctional and diseased tissues.

In addition, the CABMM takes responsibility for training and mentoring junior scientists and newly founded groups. Based on these concepts, it also aims to establish relevant research activities at the University of Zurich and to strengthen already existing bonds, especially between the Vetsuisse Faculty and the Medical Faculty for Clinical Research. Uniting clinically oriented research activities not only creates a solid basis for core competencies, but also optimises the use of available infrastructure. It is envisaged that through the unique collaborative network provided by the CABMM, new and important advances can be made in our ability to understand, treat and manage human disease.

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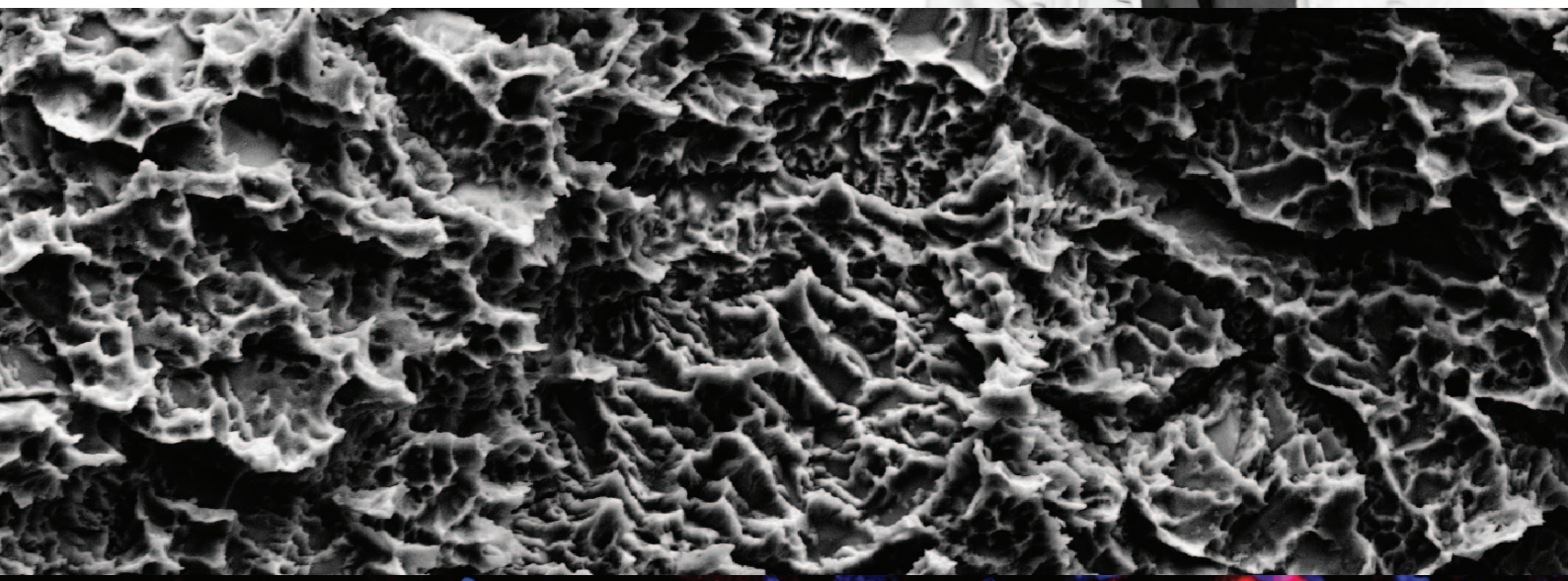

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