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Understanding artificial bones

Teams from the mechanical engineering and biology departments at TU Darmstadt are observing the growth of cells on bone implants. Therefore, a special testing chamber has been developed that should be able to make animal experiments unnecessary.

Photo: Katrin Binner

Hip bone implants formed from different materials and coatings: The research contributes to a better understanding of the bone integration to the implant surface (here: stem) to ensure the initial stability and permanent connection of the implant.

By Uta Neubauer

Hip and knee joints made of titanium, vertebral bodies made of plastic and other bone implants have already relieved many patients of their pain. However, many wearers of such endoprotheses suffer complications, for example when the artificial bones fail to grow in correctly. "What precisely happens in the body after implantation is still unclear", says the materials scientist Anne Martin from the Institute for Materials Technology in the mechanical engineering department at TU Darmstadt. Together with her former colleague Markus König and researchers from the biology department led by Bianca Bertulat, she has developed a model system that is intended to simulate the first days after a bone implant has been inserted.

The period after the operation is considered to be of particular importance for the healing process as this is the time when endogenous cells colonise the implant and ideally facilitate its integration into the body. "The surface of the implants plays a key role", Martin emphasises. By applying a solution that resembles our body fluids the researchers are keen to find out how attractive a surface is for the cells. "Good wetting means the surface and the fluid are compatible." Wettability depends, among other things, on the structure of the surface and is one of the basic requirements for the deposition of cells. Simply put: Only with a certain roughness cells feel well and develop in the right direction. Therefore, titanium implants are sand-blasted as a standard procedure.

"The surfaces could be designed in a way that they release substances that encourage the adhesion of the cells"

Titanium is the most common material for bone implants. Yet, it is not ideal, since the metal is significantly more rigid than our bones. "If the implant does all the work, the endogenous body tissue in the vicinity is no longer used and degrades", Martin says. That is possibly a cause for the loosening of endoprotheses. A meanwhile common alternative to titanium are synthetic materials such as PEEK (polyetheretherketone) that have similar mechanical properties as real bones. PEEK implants coated with titanium already exist.

Yet, which implant is best for patients? A clear answer to this question does not yet exist. That was the motivation for the Darmstadt materials technologists and biologists to examine the various materials used for bone replacement more closely. The focus of their joint project is on the interaction between living cells and the surface of implants. For this purpose, the interdisciplinary team has developed a compact testing chamber called SuBiTU (surface biology testing unit) and already registered it for a patent. The chamber is big enough to accommodate circular samples of materials with the diameter of a 2 Euro coin. The samples are exposed to living cells and a nutrient solution. The clever thing about the system: the lid of the chamber has a small glass window through which the cells can be observed with a microscope.

"Our aim with SuBiTU is to map processes that are similar to the body's own", emphasises Tom Engler, head of the surface technology competence group at the Institute for Materials Technology. Thus, a perfusion system that continuously supplies the cells with a nutrient likewise body fluids can be connected to the chamber. The influence of blood sugar levels and other parameters could also be examined in this way. Moreover, the biologists have developed a technol-

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Center for Structural Materials
Prof. Dr.-Ing. Matthias Oechsner
Phone: ++49(0)6151/16–24900
oechsner@mpa-ifw.tu-darmstadt.de
www.mpa-ifw.tu-darmstadt.de

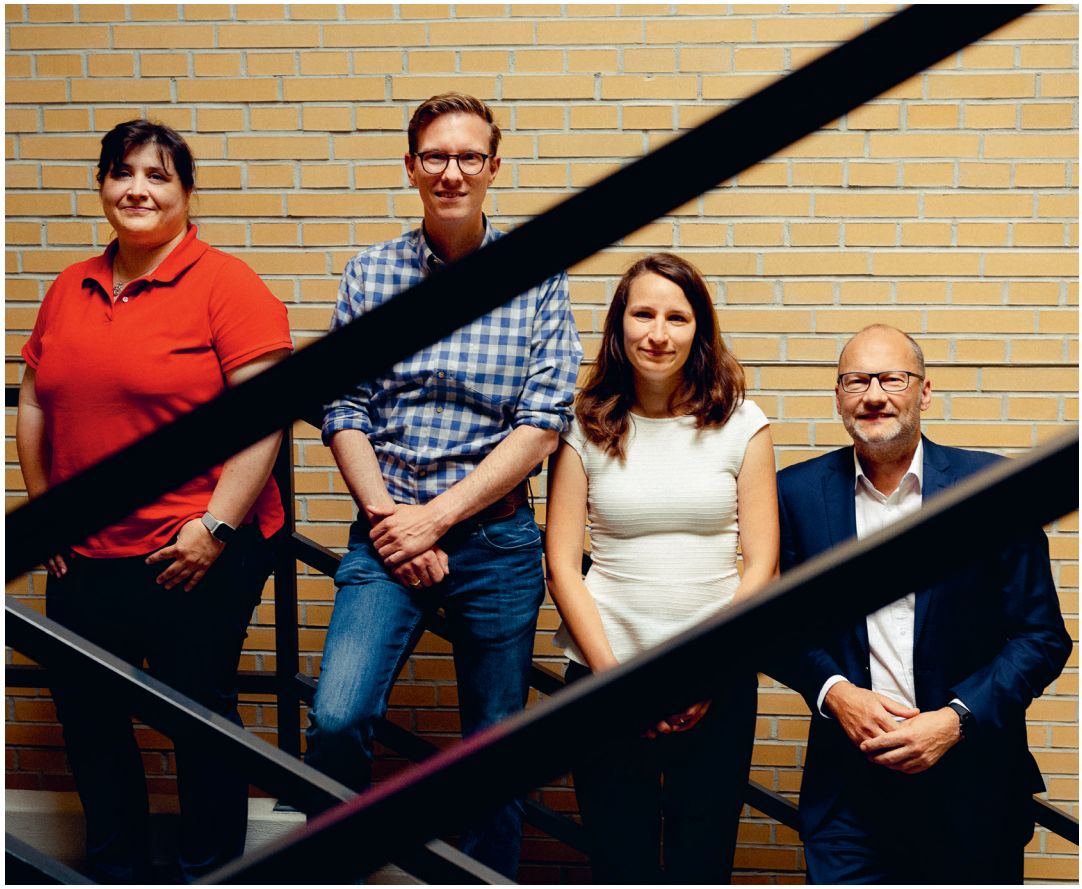

Abbildung: Katrin Binner

Interdisziplinary Work leads to success: Bianca Bertulat, Tom Engler, Anne Martin and Professor Matthias Oechsner (from left to right).

ogy that enables the cells in the chamber to grow not only two-dimensionally as in the Petri dish but three-dimensionally as in the human body. Therefore, they apply a drop of collagen to the implant samples. It forms a network on which the cells can orient themselves. "We thus offer the cells not only a meadow but also a house", Martin says and adds: "Our aim with the realistic tests is to reduce the number of animal experiments." Until now, there was no possibility of investigating the live interactions between cells and implant surfaces under body-like conditions for a period of several days. All available systems for the microscopy of living cells are simply too small for the examination of implant materials.

For their experiments, the researchers selected specific tissue cells that develop into bone cells under suitable conditions. The hope is that the formation of bone cells occurs in the chamber, initiated by the artificial bone material. To enable this development to be observed, markers were fed into the progenitor cells. Under the fluorescent microscope, they serve as recognition signs for the various cell types.

Some experiments have already been conducted in the new testing chamber. The detailed analysis, however, is still ongoing "At first sight, the cells look good", Martin thinks. Of particular interest to them is how the structure of the surface of the sample affects cell growth. As PEEK, cannot be sand-blasted, unlike the harder titanium, the Darmstadt materials technologists have developed an embossing method for the creation of rough PEEK samples. They press a stamp made of sand-blasted steel into the heated plastic. Should it turn out that cells on embossed PEEK surfaces grow significantly better than on smooth ones, rough PEEK implants could, for example, in the future be created using 3D printing.

Other modifications are also thinkable. "The surfaces could be designed in such a way that they release substances, which encourage the adhesion of the cells or have an anti-inflammatory effect", Engler says. A wafer-thin coating of silver, for example, has an anti-bacterial effect whereas certain proteins promote cell growth. Medicines could similarly be anchored to the surface. There are plenty of ideas – and with the new testing chamber, their suitability can finally be tested under body-like conditions.

The author is a science writer and holds a doctorate in Chemistry.

Support for interdisciplinary research

The forum for interdisciplinary research (FiF) at TU Darmstadt has been supporting TU scientists cooperating across specialist boundaries for more than ten years. The development of the SuBiTU testing chamber, in which researchers from the Institute of Materials Technology led by Professor Matthias Oechsner (department of mechanical engineering) and from the cell biology and epigenetics group led by Professor Cristina Cardoso (department of biology) are participating, was supported by FiF with start-up funding from May 2017 to April 2019. A follow-up project is planned.

Inspired by nature

Medical professionals and environment analysts would like to have microchips that measure substances directly on site. Scientists at TU Darmstadt have developed and patented a system based on nanopores with broad potential.

By Hildegard Kaulen

Anyone wishing to use laboratory readings for diagnosing a disorder or its progress monitoring or wanting to test the contamination of waste water with pesticides or medicines must almost always send the samples to a laboratory and wait for the results. That takes time. A "lab-on-a-chip system" determines the readings directly at the doctor's or the sampling site. As big as a credit card and with the functionality of a laboratory, these systems work faster, less costly and more efficiently than a classic laboratory while still having to be just as precise, robust and reliable. The research groups led by Wolfgang Ensinger, professor for materials technology and Helmut Schlaak, professor for electrical engineering and information technology at TU Darmstadt, have developed as part of the LOEWE project iNAPO a prototype for a "lab-on-a-chip system" that measures substances with sensors made of synthetic nanopores. The chemist Ivana Duznovic and the electrical engineer Mario El Khoury are participating in the project.

"Our aim is to develop a new generation of sensors that closely aligned to their biological models offer high sensitivity and performance capability".

The scientists were inspired by nature in their work. Integrated into the cell membranes, biological nanopores ensure that substances can be transported from the outside to the inside or vice versa. In so doing, they function as either sluices or selective transport systems that are specialised in certain substances. There has hitherto been no technical system that can compete with the sensitivity and specificity of biological nanopores. Their performance capability is unsurpassed. Biological nanopores are themselves, admittedly, unsuited to technical application as they are too fragile. Ensinger and Duznovic decided therefore to use synthetic nanopores, which they equipped with a chemical or biological sensor. To this end, the surfaces of the nanopores were correspondingly functionalised. "Our aim is to develop a new generation of sensors that closely aligned to their biological models offer high sensitivity and performance capability", Ensinger says. "When we then integrate these bio-inspired sensors into a microfluid system with portable analysis electronics, it becomes a "lab-on-a-chip system", Schlaak adds.

Admittedly, several steps are necessary for this. Firstly, the synthetic nanopores have to be produced. This occurs by way of bombarding polymer foils with heavy ions. This task is carried out by the GSI Helmholtz Centre for Heavy Ion Research in Darmstadt. After the foils have been bombarded, the nanopores are enlarged and brought into a conical shape. They then have a narrow and a wide opening and look like a funnel. The nanopores are enlarged by treating the foil on one side with an alkaline solution. "Via the caustic process, free carboxy groups arise via which the nanopores can then be functionalised using coupling chemistry", Ivana Duznovic explains. "We can basically attach anything we want to the carboxy groups. However, only such substances are suitable that are biologically or chemically relevant and with the help of which we can verify biomolecules relevant for diagnosis or environmental analysis purposes with high specificity and sensitivity. The sensor would otherwise make no sense".

The nanopores were functionalised for among other things the verification of histamine. Histamine plays a key role in allergic reactions and could also be important in connection with Alzheimer's dementia. The verification that Ensinger and Duznovic have created is based on a displacement reaction. A substance coupled to a carboxy group binds a metal ion that can also bind to histamine. If the

Information

LOEWE Research Cluster iNAPO
Prof. Dr. Wolfgang Ensinger
Phone: ++49 (0)6151/16–21992
ensinger@ma.tu-darmstadt.de
www.inapo.tu-darmstadt.de

sample contains histamine, the metal ion moves to the histamine, which can be recognised by a drop in current through the nanopores. The rule here is: the stronger the current falls, the more histamine there is in the sample. As the substance coupled to the carboxy group can be recharged with the metal ion, the nanopores can be regenerated and used for further verification.

The next step is the development of the "lab-on-a-chip system". "We already have a functioning microchip, but there are still some problems to be solved as the verification is supposed to function not only in watery solutions but also in a blood sample" Schlaak says. As the team would like to re-use the chip for cost reasons and as this is basically possible due to the regenerability of the nanopores, there should be no falsification of subsequent results from the initial usage. "We cannot have any memory effect", El Khoury puts the problem in a nutshell.

A "bottleneck" for commercialisation is also the search for a suitable candidate for corresponding verification, for medicine for example. "We need a marker that has already been correspondingly validated", Ensinger says. "It must be certain that the verification is actually sensible and helpful, for the diagnosis, screening or progress monitoring of a disorder, for example", the materials technologist continues. "We are currently on the lookout for interesting candidates and are already collaborating with Mainz University Hospital. However, we are open to other ideas."

A "lab-on-a-chip system" based on bio-inspired nanopores can only be created in close cooperation with other disciplines. Ensinger and Schlaak are therefore full of praise for the interdisciplinary procedure of the LOEWE project iNAPO. Chemists, biologists, materials technologists, physicists and electrical engineers were involved in the development. "We are very well prepared for such projects at TU Darmsatdt", Ensinger says. As the next step, the team would like to equip the surfaces of the nanoprobes for the verification of proteins to thus also identify complex biomarkers.

The author is a science writer and holds a doctorate in Biology.

LOEWE project ion-conducting nanopores (iNAPO)
14 working groups, 103 publications up to the beginning of the completion funding, two patents, one junior professorship, sixteen PhD students and the prize in the competition "Germany – Country of Ideas", at which iNAPO was honoured for its innovative research and development. The "balance sheet" of the project concluding at the end of the year and led by materials technologist Professor Wolfgang Ensinger and biologist Professor Bodo Laube at TU Darmstadt is impressive. This is why the projects are to be continued by way of the application of collaborative research centres. The declared aim of the project is to unite the functional properties of synthetic and biological nanopores and thus develop a new generation of molecular nanosensors. Great progress has been achieved along this path. The broad structure of the consortium and collaboration with foreign cooperation partners have also contributed to the success.

Professor Wolfgang Ensinger and Ivana Duznovic examine their highly sensitive product: A chip with sensors made of synthetic nanopore.

Abbildung: Katrin Binner

Improved diagnostics

Ulrike Nuber and her team have shown that a variety of structural brain changes can occur in Coffin-Siris syndrome. For doctors and affected individuals an important step.

By Hildegard Kaulen

The worst is the uncertainty. When parents realise that their child is not developing in line with his or her age and lags behind children of the same age, they want to know one thing above all: what does our child lack? Is there a name and a diagnosis for these developmental conspicuities, and how can we best help our child? Ulrike Nuber is aware of the desire for a precise diagnosis of a developmental disorder as the professor for stem cell and development biology at TU Darmstadt is also a doctor and worked in the field of genetic counselling for a while. Her wish has always been to understand how illnesses arise, what implications they have and how they can best be treated – notably brain diseases in children.

Nuber, who was professor at the Stem Cell Center at Lund University in Sweden prior to her move to TU Darmstadt, is interested in the Coffin-Siris syndrome besides other paediatric brain diseases. This is a complex developmental disorder with various symptoms. The syndrome is caused by spontaneous genetic changes in a certain group of genes in the germ line of one parent or during early embryonic development. As such, the Coffin-Siris syndrome occurs at random and can affect anyone. Although the disorder is considered to be rare, many doctors believe that there are far more persons affected whose symptoms are not attributed to the syndrome. This might also be due to the fact that it is unclear as to which structural changes can occur in the brain and thus ought to be visible on an MRT scan.

Among the genetic changes that cause Coffin-Siris syndrome are mutations in the so-called SMARCB1 gene. Mutations in this gene also predispose for aggressive brain tumours in children and it is unclear in detail as to how such disparate clinical outcomes can arise. Nuber and her team member Dr Alina Filatova therefore wanted to find out what happens when the activity of this gene is reduced in brain stem cells. Is brain development disturbed or do brain tumours arise? Their experiments showed that mice do not develop tumours but exhibit marked changes in brain development. It is already visible with the naked eye that the animals' brain is much too small and has conspicuous midline defects. In many mice, for example, the commissures connecting the two halves of the brain are underdeveloped or missing completely because the nerve fibres do not cross over to the opposite side. As such, many animals have no corpus callosum, the largest nerve tract in the brain of humans and mice. Moreover, many animals show pathological changes in the cerebellum and in the middle of the forebrain. In addition, the structure responsible for the production of brain fluid is too large.

The syndrome that bears the name of its discoverers, the American paediatrician Grange Coffin and the radiologist Evelyn Siris, stands for

Photo: Sandra Junker

Research group: Meike Stotz-Reimers, Professorin Ulrike Nuber and Michaela Becker-Röck (from left to right)).

Information

Stem Cell and Developmental Biology
Prof. Dr. Ulrike Nuber
Phone: ++49 (0)6151/16–24600
nuber@bio.tu-darmstadt.de
https://bit.ly/30sfdxW

Photo: Sandra Junker

The brain in the mouse model for the Coffin-Siris syndrome (on the right) is very much smaller than the brain of a control animal (on the left).

a general delay in development, a reduction in intelligence and reduced speech capability. The persons affected also have physical conspicuities. They are small, have dense eyebrows, a broad nasal bridge, low-set ears, a broad mouth and characteristic changes of the finger and toe end joints. Many persons affected also suffer from epilepsy and have eye or heart problems or other organic symptoms.

"When we saw the brain abnormalities in the mice, the next obvious question was if these structural changes can be seen on MRT scans of affected persons and why so little is known about it", Nuber says. "Are there no such changes or have they simply been overlooked because doctors do not know which structural details to search for when analysing MRT scans?".

"Perhaps our new findings will lead to the syndrome being considered more often in case of respective structural changes".

Thereupon, Nuber asked the human geneticist Professor Dagmar Wieczorek and the paediatric radiologist Dr Jörg Schaper from Düsseldorf University Hospital to take another look at the MRT scans of affected persons. Wieczorek numbers among the leading experts in the field of the Coffin-Siris syndrome worldwide and contacted a human geneticist at Hamburg University Hospital as well as colleagues in Poland and Holland. Detailed analyses then revealed that individuals with Coffin-Siris syndrome demonstrate a similar spectrum of structural changes. The extent to which this is the case was previously unknown.

"The mouse model was important for letting us see and recognise this spectrum in the affected persons", Nuber says. "Our findings have now provided doctors with an exact picture of what is possible. That is key additional knowledge for diagnostics". Nuber expects the syndrome to be diagnosed in the future also via such structural brain changes. "Perhaps our findings will lead to the syndrome being considered more often in case of respective structural changes and then genetically confirmed." Nuber, who sees herself as a genuine team player, was able to publish the results together with her colleagues in the respected open access journal "Nature Communications". Admittedly, the findings from mouse models cannot always be translated so well to humans as in this case. This is why the group headed by Ulrike Nuber is also working on stem cell-based human disease models.

Which function does the protein product of the SMARCB1 gene have, the activity of which was reduced in the brain stem cells? The protein is part of a complex that makes the DNA accessible so that genes can be scanned. Genes that are occupied by so-called nucleosomes are inaccessible and cannot be read. The complex, which the SMARCB1 protein is part of, puts the nucleosomes away and clears the path to the genes. If the complex is defective or missing, the genetic programme cannot be correctly read and implemented, ultimately leading to the symptoms of the Coffin-Siris syndrome or to a predisposition for brain tumours. To date, little is known as to which genes can no longer be correctly read due to such a change.

Nuber sees the mice also as an important animal model for testing potential therapeutic approaches. One approach could be to correct the nerve cell defects. "Perhaps we can succeed in restoring the contacts between the nerve cells connecting the two hemispheres or in counteracting the consequences of too few nerve cells." The stem cell researcher and doctor is grateful for the basic funding and personnel resources provided by TU Darmstadt. "Many projects are initially explorative and cannot be funded via external resources at that stage or take longer than the three years covered by standard external funding", Nuber says. "Our mouse model for the Coffin-Siris syndrome is such a project. I am therefore delighted that TU Darmstadt makes projects of this kind possible. Research needs such free space."

The author is a science writer and holds a doctorate in Biology.

Recent publication:
www.nature.com/articles/s41467-019-10849-y

Ceramic 2.0

Materials scientists at TU Darmstadt aim to reinvent the material ceramic. To this end, they are delving into its atomic structure. The German Research Foundation (DFG) is supporting this within the framework of a prestigious Reinhart Koselleck project.

By Christian Meier

When thinking about ceramics, the first thing that comes to mind is crockery or sharp knives. However, much more can be done with this hard and brittle material beyond the already impressive list of sensors, capacitors, etc., is the opinion of Professor Jürgen Rödel from the research group for non-metallic inorganic materials at TU Darmstadt. He is searching for new applications for the materials comprising many tiny crystals (polycrystals). Rödel's approach seems paradoxical initially. The materials researcher aims to improve ceramics by disrupting their atomic structure. "Our aim is to do this in a controlled way, however", he says. His team is focusing on a type of crystal defect, the creation of which appears trivial for metals, but for hard ceramics has hitherto appeared virtually unthinkable.

"For this purpose, we use methods that neither chemists nor physicists use", Rödel explains. These include the mechanical deformation of ceramics under controlled pressure and temperature. Moreover, as the Darmstadt team masters the methods for characterising materials, they see themselves as ideal experts for the task. Some types of crystal defects have been well researched. One of these is the absence of an atom in an otherwise regularly formed crystal lattice. Such a "point defect" resembles an empty seat in an otherwise full cinema. Point defects increase the conductivity of semiconductors in electronics. Two-dimensional defects have also been well researched. These are interfaces that separate two grains in a polycrystal. The intermediate case of a one-dimensional defect has, however, remained untouched territory as chemistry alone was not enough, Rödel says. In the case of such a dislocations, the defect takes the form of a straight line through the crystal. Seen in the light of the "cinema" metaphor, this would be an empty row of seats.

Dislocations in ceramics are electrically charged, which makes them technically interesting. They serve as channels for the electrical charge, thus increasing the conductivity. As they simultaneously retard the propagation of heat, they are well-suited as thermoelectric elements. These materials convert waste heat into electricity. Rödel mentions a further useful effect increasing the efficiency of fuel cells: "At the end of the dislocations, namely on the surface of the crystal, oxygen can be incorporated or removed". Furthermore, dislocations remain stable up to 500 degrees Celsius whereas point defects already move at around 100 degrees.

The prerequisite for technical usage is to incorporate dislocations into a ceramic in a planned way. This has hardly been achieved hitherto. Rödel's team aims to change that. "We are attempting to create as high a density of dislocations in ceramics as possible", Rödel explains. One of the challenges is to find the optimum temperature, electrical potential and other parameters for the mechanical deformation. "The result is still open", Rödel confesses. But we are making progress: The first partners in his network are already working on dislocation-based photovoltaic systems in England and on high-resolution electron microscopy in Japan.

The author is a science writer and holds a doctorate in Physics.

Reinhart Koselleck project

The "Research of Dislocations in Ceramics" is supported by DFG as a Reinhart Koselleck project with 1.25 million euros for five years. The programme targets areas for particularly innovative and in a positive sense risky research and thus attracts prestigious scientists and researchers. Professor Jürgen Rödel was the first person at TU Darmstadt to gain such DFG support.

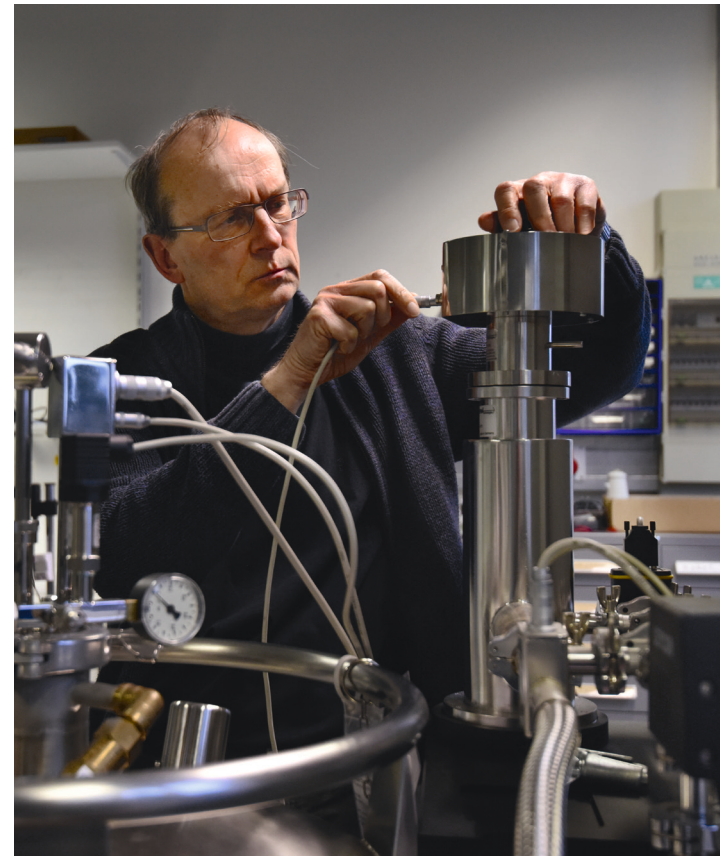

Photo: Claus Völker

Laboratory research: Professor Jürgen Rödel.

Information

Nonmetallic-Inorganic Materials
Prof. Dr. Jürgen Rödel
Phone: ++49 (0)6151/16–21698
roedel@ceramics.tu-darmstadt.de
www.mawi.tu-darmstadt.de/naw