What is the main focus of your research into hydrogel-based materials?

We focus on gaining a fundamental understanding of the processes which take place during gel formation, which in turn allows us to establish methods that control their development. In particular, we study the effects of additional inorganic materials that are either formed within the gel at the time of gelation, or may be formed within the hydrogel material at a later stage. Using this biomimetic (ie. inspired by nature) approach, we want to develop hydrogel-based composites (materials made of two or more constituent elements with significantly different physical or chemical properties). Natural composites often have an excellent range of physicochemical properties, combining strength, toughness, biocompatibility, etc., which is extremely difficult to achieve in synthetic materials.

Can you describe your past and present activities in this area of study?

We started to work on this particular topic in 2007 by testing whether it is possible to use bioinspired approaches to make composite materials based on polymeric hydrogels combined with an inorganic phase of calcium carbonate or calcium phosphate. For the hydrogel part, we have chosen to use alginate biopolymers. This is because alginates (a group of biopolymers of marine or bacterial origin) have been researched at the Norwegian University of Science and Technology (NTNU) for many years and the team could benefit from a wealth of accumulated knowledge. More importantly, however, alginate has some very valuable advantages: it is already used in medical and food applications (approved by regulatory organisations), and it readily forms hydrogels in mild conditions in the presence of calcium ions.

We quickly found that it is possible to make composites by forming inorganic crystals within the biopolymer, and specific interactions between the biopolymer and forming mineral crystals offer new ways in which the mineral phase can be controlled within the hydrogel.

You aim to better understand how to design and fabricate new types of hydrogels. How will you achieve this goal?

Our current activities focus on exploring mineralisation with calcium phosphate further, as this is the mineral most relevant for application in tissue engineering (since this is the mineral phase found in bone). In addition, we would like to learn how to construct more complex 3D structures from mineralised hydrogels and investigate how these structures influence cell growth within and on these materials.

Have you made significant progress towards improving the performance of hydrogel-based materials? What more needs to be achieved before the project’s end?

Significant progress has been made regarding fabrication and characterisation of developed materials, including improvement of mechanical properties, control over mineral phase composition, its properties and distribution. We focus on a more system-like approach, and...
we would like to combine newly developed materials and new fabrication strategies to make more complex structures and constructs. Our approach is to combine different types of mineralised and non-mineralised hydrogels, arranged in a controlled manner on different length scales. In this way, we hope to develop constructs with more than one function, where part of the material can supply mineral crystals, which cells encapsulated in a different part of the material could remodel and use to form new bone.

As far as the application of your biocomposites as bone defect repair scaffolds is concerned, do you believe there is significant promise?

There is great promise for these materials, and alginate already has a successful track record as a biomaterial, being used clinically in several medical devices such as wound dressings and dental impression materials. Calcium phosphate is the inorganic compound found in bones, and is therefore well tolerated by the body and can be tailored to have different responses *in vivo*. Indeed, this material is used clinically as a bone filler material in non-load bearing applications.

We believe that it is a good strategy to use materials that already have a good track record and are used clinically as a basis to develop new and improved devices and materials. This aids the approval process, as well as the likelihood of success. That said, the clinical approval process is understandably rigorous and takes many years to complete; we still have many outstanding experiments to carry out before we even begin that stage, so it will be some time before a product based on our materials is on the market.

Refining complex architectures

Researchers from the Norwegian University of Science and Technology have been exploring the use of hydrogel-based materials in tissue engineering and how to refine them so as to mimic sophisticated natural biomineralisation processes.

TISSUE ENGINEERING RELIES on innovation and the constant refinement of new and existing technologies. An important issue facing the field is the lack of synthetic biomaterials that perform as well or better than autologous grafts – ie. native undamaged tissue taken from the patient to repair lost or damaged tissue – in instructing tissue regeneration. Bioinspired or bioderived materials offer great potential in this regard and there is a huge research effort to find biomaterials that outperform current clinical tissue regeneration approaches.

Control over the cellular systems and materials responsible for healing or replacing a damaged tissue is vital in tissue engineering. In particular, factors such as biocompatibility (ie. the material invokes an appropriate host response), bio-instructive function (ie. the material stimulates natural regenerative mechanisms in the body), mechanical function and biological function are of paramount importance. These requirements are extremely difficult to achieve; for example, natural structural tissues, such as bone, cartilage and tendons have remarkable mechanical properties which cannot be mimicked by the synthetic materials of today. In the case of bone, its exceptional strength and fracture resistance is due to extraordinarily complex architectures of organic and mineral (inorganic) components that are precisely controlled over a large length scale from nano- to centimetres by a process known as biomineralisation.

Professor Pawel Sikorski and Dr David Bassett of the Norwegian University of Science and Technology (NTNU), as well as other experts from a range of disciplines and institutions across Europe, comprise a team that is investigating how to replicate natural biomineralisation processes in synthetic hydrogel-based materials. Sikorski explains the rationale for the research, and clarifies its potential application in practical terms: "Mineralisation of hydrogels is a good model system for this type of investigation, especially since developed materials could have direct applications in areas such as tissue engineering and regenerative medicine".

As a starting point for the research, a model system based on alginate gel beads (small spheres, around 0.5 mm in diameter) was chosen to study mineralisation and consider how the process could be improved and refined. The programme focuses on alginate gel beads rather than large blocks of hydrogels to allow for diffusion and encourage nutrient exchange, which are important factors to consider since these materials are intended to support cell growth. The initial goal was to synthesise mineralised gels at pH and temperature conditions compatible with human cells, and in particular stem cells which could be used together as a viable tissue engineering scaffold for bone regeneration. Furthermore, the project aims to carefully control and study the nanoscale deposition of inorganic material into a biopolymer gel network in an attempt to mimic natural biomineralisation processes.

REPLICATING NATURAL PROCESSES

Calcium phosphate (CaP) and calcium carbonate (CaC) minerals were used to mineralise the alginate gel beads, and the research team found that composite materials with nanoscale mineral crystals distributed within the gel network could be made in both cases. Beads mineralised using CaP were found to be characterised by more inhomogeneous distribution of the mineral phase. This observation inspired the development of a new mineralisation strategy intended to stimulate a more homogenous distribution. Here, an enzyme alkaline phosphatase (ALP) was encapsulated within the alginate network.

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HYDROGEL-BASED MATERIALS FOR TISSUE ENGINEERING

OBJECTIVES

• To understand how to design and fabricate new types of hydrogels, and to combine such hydrogels into materials with hierarchical structures resembling, to a large extent, tissues and biomaterials found in nature

• To combine biomaterials science, polymer science, nanotechnology and materials characterisation methods with cell culture experiments

KEY COLLABORATORS

Professor Jens-Petter Andreassen; Dr Berit Lakensgard Strand; Dr Therese Standal, Norwegian University of Science and Technology (NTNU) • Professor Jan E Brinchmann, Oslo University Hospital, Norway • Professor Liam Grover, University of Birmingham, UK • Professor Kenny Dalgarno, Newcastle University, UK • Professor Andreas K Süßler, Unfallchirurgische Klinik an der Eberhard Karls Universität Tübingen, Germany

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PROFESSOR PAWEŁ SIKORSKI obtained a PhD in Polymer Physics from the University of Bristol, UK, in 2002. Following a postdoctoral period at the Departments of Physics and Biotechnology, he started work as Professor in Biophysics and Medical Technology at the Department of Physics at NTNU.

DR DAVID BASSETT obtained a PhD in Dental Sciences in 2011 from McGill University, Montreal, Canada. Following a short postdoctoral appointment at McGill, he then joined Sikorski’s laboratory at NTNU as a postdoctoral fellow in 2013.

When incubated in an appropriate cell-compatible solution, the enzyme could produce phosphate ions within the gel which, together with calcium, could form new mineral crystals. This method was found to stimulate a more even CaP distribution in the alginate gel beads. Bassett elucidates how the method mimics natural processes: “The ALP enzyme-based method is in some ways more ‘biomimetic’ in the sense that it employs an enzyme that is naturally active in the bone turnover process to provide phosphate ions for the mineralisation process”.

The mineralised hydrogels were subsequently considered in relation to their compatibility with cells both encapsulated within the alginate hydrogel and growing on the surface of the beads. Various bone cell types and also stem cells derived from bone marrow and adipose tissue were tested and found to perform well. An important finding was that the mineralised alginate surface was excellent for cell attachment.

A MULTIDISCIPLINARY APPROACH

Ultimately, the research has yielded intriguing discoveries: the team was able to demonstrate that it is indeed possible to emulate natural biomineralisation processes and form mineral-alginate nanocomposites. By considering a variety of different approaches and utilising a range of experimental techniques, the researchers were able to predict the material properties of the resulting nanocomposites according to their preparation conditions.

These findings have formed a very strong foundation for future research, and allowed for the development of an impressive web of international collaborations crossing a range of disciplines. The project brought together a multidisciplinary team from NTNU, specialising in areas from mineralisation and crystallisation to stem cell biology, and an expert in cell biology from the University of Tübingen, Germany. Researchers from the University of Birmingham and Newcastle University, UK, were also involved, providing support with material characterisation and the 3D printing of scaffolds.

Sikorski illustrates the value of drawing on the specialist research backgrounds of a variety of international researchers: “It is our intention and a long-term goal to make best use of these collaboration opportunities and to build a close connection with potential users of our materials; for example, medical researchers working at the university hospital in Trondheim”. In addition, to keep the wider scientific community informed on the latest research developments, the team has been visiting a range of biomaterial and tissue engineering conferences arranged by organisations such as the Scandinavian Society for Biomaterials and the European Society for Biomaterials.

There is still much left to be achieved, but the advances made so far have been impressive and enabled the team to explore further innovations. The ultimate goal of the research is to create bioactive cell-gel composites suitable for injection into people requiring bone replacement or augmentation, such as in cases of reconstruction following trauma or disease, or bone loss conditions such as osteoporosis; when suitable for clinical use, such materials would aid the healing process.

One of the most recent developments in the project has been a new focus on the kinetics of hydrogel formation and mineralisation. In order to study these processes in more depth, numerical models have been applied to experimental observations of real-time mineral formation and gelling. "This will improve our understanding of the behaviour of our materials in vivo, and may aid us in future materials design," Sikorski enthuses.

The multidisciplinary team behind the project continues to innovate and approach the area of study from numerous angles. By working to achieve a more rounded and nuanced understanding of natural biomineralisation processes, and develop hydrogel-based materials capable of mimicking them, the researchers have been able to make significant progress towards the goal of more effective hydrogels for tissue engineering applications.