Impact Objectives

- Discover how to effectively control protein loading in clay gels
- Investigate the mechanisms of nanoclay bioactivity
- Gain a better understanding of local tissue responses to implanted clay gels

Harnessing clay for regenerative medicine

***Dr Jonathan Dawson** and **Professor Richard Oreffo** from the **University of Southampton** discuss their work using clay nanoparticles to enhance regenerative medicine*

Dr Jonathan Dawson

Professor Richard Oreffo

Can you tell us a little about your background and how your interest in the use of nanoparticles in tissue regeneration has developed?

JD: I am a biologist and began working with nanoclays during my PhD with Richard. Our interest lay in the interesting gelation properties of the nanoclay, Laponite, which, when dispersed in water, forms a gel that behaves as a fluid under shear. Due to these properties, we hoped that Laponite could serve as an injectable carrier for delivering bone stem cells to injury sites. We found that Laponite spontaneously set into a gel, potentially forming a stable environment for stem cells to promote bone regeneration.

We found that clay gels are extremely effective at binding and sequestering biological molecules. We realised this property could be very useful for delivering and retaining key biological signals to direct stem cell behaviour in the body. Harnessing this potential of clay nanoparticles to create injectable microenvironments able to stimulate tissue regeneration is now a central focus of my group.

RO: I have been working in the field of skeletal biology for almost three decades. I am particularly interested in the application of bone stem cells and regenerative medicine strategies for the repair and regeneration of damaged skeletal tissue. Interest in nanoclays began more than a decade ago with a Biological and Biotechnological Sciences Research Council (BBSRC) PhD studentship that had Jon looking at the potential of a nanoclay, Laponite, to deliver cells and factors for bone repair. This has developed into an exciting research approach for tissue reparation and regenerative medicine application.

Who are the key stakeholders in this study and what has their role been in achieving your research goals?

JD: At this stage, beside potential commercial partners, we have principally reached out to orthopaedic surgeons who would be applying the technology to patients. Through these interactions, it has become very clear that harnessing bone inductive agents safely using an injectable approach that could help avoid surgery would be extremely beneficial in a range of orthopaedic applications.

RO: Our orthopaedic community have been pivotal in explaining needs, advantages, issues and most importantly keeping us grounded in the whole process.

How have you collaborated with other researchers or groups at different sites?

JD: We have various collaborators around the UK and internationally. The field of tissue engineering and regenerative medicine is extremely interdisciplinary, drawing in materials scientists, stem cell biologists, clinicians and engineers. This makes collaboration an essential part of any project in this area. Sometimes this creates challenges as we seek to communicate effectively and work productively at the interface of the various disciplines, but normally it is simply great fun and exposes you to new places, people and ideas.

RO: Regenerative medicine is necessarily collaborative with a need to harness expertise across many disciplines - we are fortunate to have wonderful collaborators across the world to aid our research goals.

What do you think is the most important hurdle to overcome in order to move your laboratory's research forward?

JD: Communication can be a challenge. This is a very new area of research that cuts across several ingrained practices in the field. Nanoclay gels are very different from conventional hydrogels and require new approaches to characterisation and application. Effectively communicating key concepts to tissue engineering practitioners is critically important if the benefits of our approach are to be adopted more widely within the field.

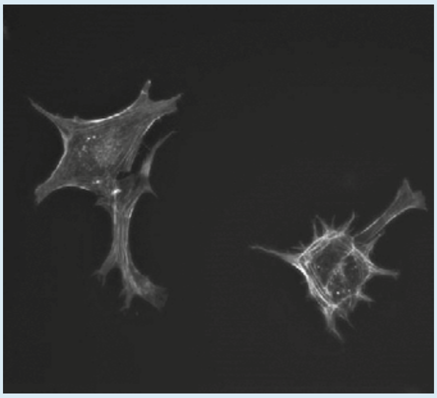
RO: Funding! - Translation to large preclinical studies are a key component in our research. However, these studies are expensive, time consuming and, as often not hypothesis-driven, can be less attractive to funding agencies. Our research has been facilitated by our university spin-out, Renovos, offering an additional research approach to deliver nanoclay platform technology for regenerative medicine.

Regenerative clay

*Researchers at the **University of Southampton** and **Renovos Biologics Ltd.** are harnessing the structural properties of clay nanoparticles for regenerative medicine*

The field of regenerative medicine aims to completely regenerate a tissue or organ rather than merely preventing the worst damage or relying on donated tissue. Ideally, this would be accomplished using stem or progenitor cells derived from the patient themselves – dramatically reducing the rate of rejection. In theory, this could result in medical procedures with longer-lasting results that leave little negative impact on the body. Both the basic and clinical research in the field have made great advances in recent years; however significant blocks to regenerative medicine remain. The most important of these include the stimulation of reliable differentiation of stem and progenitor cells, the medium-to-long term persistence of the treatment and the ability to provide the right cellular environment for regeneration *in vivo*.

The search for the right material for the delivery and creation of a supportive cellular environment is complex. The perfect material depends on the context of the regeneration and must be able to withstand the physical pressures in that part of the body. It also needs to be flexible enough to readily adapt its shape to the unique size and shape of the particular person. Equally it needs to be sufficiently biologically active to help promote and direct growth yet at the same time, not provoke an immune response and subsequent rejection. Dr Jonathan Dawson and Professor Richard Oreffo, of the University of Southampton, have been working on developing a robust regenerative carrier for over a decade and have identified clay nanoparticles as a strong candidate to fulfil this role. Dawson explains the basic properties of clay nanoparticles that give them an edge: 'Due to their self-gelling properties and their ability to localise and stabilise biological molecules, clay nanoparticles are excellent candidates for providing regenerative microenvironments for stem cells. We are currently exploring their application in bone and cartilage regeneration and in wound healing applications.'



Human skeletal stem cells growing upon clay gels

CLAY STRUCTURES

Clay has a long history of medical use. Historical and archaeological evidence suggest that clay has been used to treat a wide variety of ailments as well as wounds and bleeding. Clay has been both ingested and applied topically for medicine purposes. Indeed, various clays are still used today as active ingredients in a variety of drugs such as antacids and anti-diuretics. Furthermore, clays frequently have properties that make them useful as additional ingredients in pharmaceuticals. The emulsifiers used in cosmetics and pharmaceutical preparations, for example, are often clays. Clays are, therefore, already well established as safe and useful for medicinal purposes.

Individual clay particles are about 1nm thick. They are each composed of a central octahedral sheet formed of metal ions such as aluminium or magnesium, sandwiched between two tetrahedral sheets of silica. Laponite, a synthetic clay, forms individual particles of around 25nm in diameter. Dawson and his team use these nanoparticles to generate injectable gels that – due to their charges – are responsive to tissue and blood. Dawson explains, 'Nanoparticles have many useful properties often attributable to the large surface area they display relative to their volume. Clays in particular have a negative electrostatic charge on their surface and a positive charge on their rims. These two simple features: their small size and their electrostatic charges, give them a surprisingly wide range of uses relevant to stem cells and tissue regeneration.'

INJECTABLE BONE

As Dawson highlights; 'Electrostatic interactions between clay particles in water cause them to form reversible gels that can pass through a needle and then set in the body in response to the proteins and ions present in blood.' The formation of these gels is essential to Dawson's work, making them ideal as scaffolds for delivering stem cells and templating new tissue formation. In addition to these useful physical properties, the charges on the clay units make them ideal to coordinate biologically active molecules. This means that such molecules can be incorporated into the gel and remain highly localised to the site of healing and regeneration.

Dawson and Oreffo have demonstrated the efficacy of the delivery of biological molecules by clay nanoparticles for stimulating bone growth. Bone morphogenic protein 2 (BMP2) is well-known to have a strong ability to stimulate the growth of bone in the body. It is used in medicine to encourage the growth of bone in order to fuse vertebrae in the spine. Though one of the most effective therapies available, its use is associated with various

> *‘The principle again seems to be that, by forming gels able to stabilise proteins, clay nanoparticles can establish a local environment conducive to repair’*

dangerous side effects due to the very high doses needed for efficacy and the difficulty in restricting its effects exclusively to the site of implantation. The clay gels are able to localise BMP2 and maintain the majority of the molecule at the site. At the same time, they have shown that the molecule remains biologically active, as Dawson elaborates: ‘We have shown that clay gels can bind BMP2 very effectively to allow highly localised bone induction at greatly reduced doses (about 3000 times lower than currently used in the clinic). We hope this could greatly improve the safety of BMP2-based therapies and open up a wide range of new applications.’

A GROUNDED FUTURE

The successes Dawson and Oreffo have had with growth factor delivery such as BMP2 are just the beginning for the field. Dawson and his colleague Dr Nick Evans are already investigating the potential of clay gels to help skin wound healing, as he describes: ‘The principle again seems to be that, by forming gels able to stabilise proteins, clay nanoparticles can establish a local environment conducive to repair.’ He envisages that this will be used to treat chronic wounds such as diabetic leg and foot ulcers.

Many intriguing questions surrounding clay nanoparticles remain. There has been little investigation, for example, as to what actually happens to clay once in the body. How does it get degraded? Is it excreted? How and why is clay bioactive? These questions are essential to understand in order to properly utilise this exciting material.

In order to properly develop the potential of clay nanoparticles, Dawson and Oreffo have spun off a company – Renovos Biologics Ltd. – through the University of Southampton, to develop the technology further. Renovos aims to market and introduce into the clinic the current findings. Dawson explains how the business and academic fronts can work together: ‘From a basic science perspective, we are exploring how to more effectively control protein loading and localisation within clay gels, seeking to understand local tissue responses to implanted clay gels and also trying to gain a deeper mechanistic understanding of nanoclay bioactivity. From a translational perspective, Renovos is developing nanoclay gels as a regulated clinical grade product with the potential to make currently licensed growth factor-based therapies better, safer and more cost effective.’ This two-pronged approach should ensure that clay nanoparticle technology improves and becomes a standard method employed in regenerative medicine. As Richard elaborates: ‘Renovos aims to translate innovative growth factor and cell delivery technologies for an increasing, ageing population, using our unique synthetic nanoclay biomaterial for bone augmentation in bone repair.

Project Insights

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BIOS

Dr Jonathan Dawson is an EPSRC-funded early-career research fellow developing nanoclays for tissue regeneration. His PhD initiated research into clay gels for cell and protein delivery. He has worked on projects exploring stem cell and material strategies for regeneration. He is a co-founder of Renovos Biologics Ltd., a new regenerative medicine spin-out company.

Professor Richard Oreffo holds the Chair of Musculoskeletal Science and is co-founder and Director of the Centre for Human Development, Stem Cells and Regeneration at Southampton University. Over the last 20 years, he has brought together teams of clinicians and life scientists to develop strategies to regenerate bone and cartilage for orthopaedic application with translation a key driver. He is the lead founder of Renovos Biologics Ltd., a spin-out providing orthopaedic regenerative medicine solutions.

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