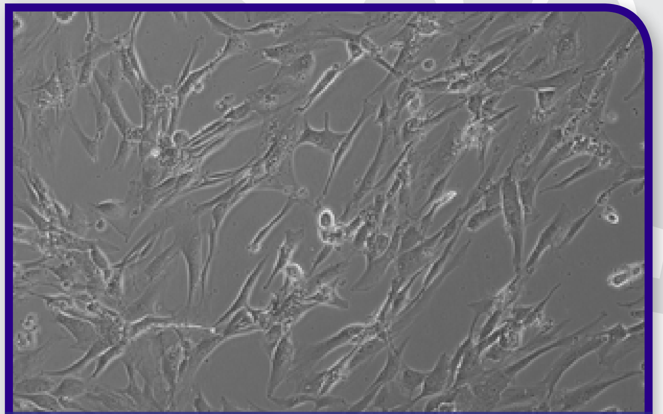
Hydrogels: a unique solution for stem cell storage and transport

BBSRC innovation funding for research at the University of Reading had led to the development of a new method for transporting living cells such as stem cells. The technology, where cells are encased within a 'hydrogel', allows clinicians and the stem cell industry to store cells at room temperature for up to two weeks.

Hydrogels provide the cell therapy industry with an alternative to freezing cells for transport, which can be complex and expensive, greatly increasing the number of places that could make use of such therapies.

BBSRC support, including a Pathfinder grant[1], funding from the Bioprocessing Research Industry Club (BRIC)[2], and a Sparking Impact award played an important role in financing the development of the technology, alongside an MRC grant and funding from the University of Reading.

The research is led by Che Connon, Professor of Tissue Engineering at Newcastle University and formerly Team Leader for Tissue Engineering and Cell Therapy Laboratory at the University of Reading, where much of the hydrogel work took place, and builds on his background in tissue engineering of the cornea.



Adipose derived stem cells following release from storage in hydrogels after two weeks. Image: *Dr Che Connon/University of Reading.*

So far Connon has established Non-Disclosure Agreements with 25 companies interested in using the technology, allowing them access to Connon's research to assess the potential commercial applications. The researchers are also discussing with clinicians how hydrogels could benefit stem cell therapies currently undergoing or soon to enter clinical trials. Finally, the researchers have an evaluation licence in place with a veterinary medicine company to explore the use of hydrogels for transporting livestock semen for artificial insemination.

Stem cells by post

Cell therapies, where patients are treated with cells taken from themselves or from a donor, have been used since the 1960s when the first bone marrow transplants were conducted. The number of cell therapies is likely to increase in future, as researchers explore the potential of stem cells to treat a wide range of illnesses. One market research report suggested that the global stem cell market is likely to grow from $3.8Bn in 2011 to around $6.6Bn by 2016, and is increasing annually by 11.7 %[4].

The rapidly expanding stem cell industry faces a number of challenges, however, including how best to store and transport fragile living cells without damaging their ability to treat disease. At the moment, stem cell manufacturers and clinicians must freeze cells in a process known as 'cryopreservation'. Frozen cells are expensive to transport, as they must be kept cold, and complicated to use, requiring facilities to thaw and culture the cells at their destination.

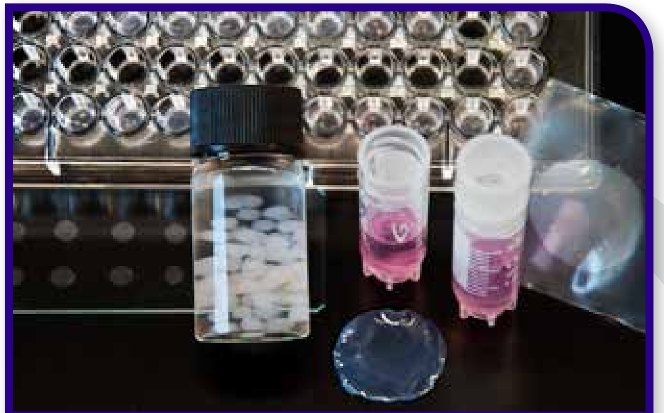
IMPACT SUMMARY

BBSRC-funded research by Dr Che Connon at the University of Reading has led to the development of hydrogels for storing and transporting living cells such as stem cells.

The stem cell industry has expressed an interest in the technology. As a result, the researchers have:

- **Signed 25 Non-Disclosure Agreements with interested companies, and 12 material transfer agreements.**
- **Established an evaluation licence with a veterinary medicine company to use the hydrogels for transporting livestock semen for artificial insemination.**
- **Discussed the hydrogels with clinicians, with a view to incorporating their use into stem cell therapies currently under development.**

The hydrogels used by Connon allow cells to survive outside the laboratory without the complex and bulky infrastructure required for cryopreservation[5]. The hydrogels used are produced from a natural compound called alginate, extracted from seaweed, which is already widely used in food production and in medicine. "Out of the lab, you don't need to change the media or do anything to it. You can leave [the cells in the hydrogel] in a container of any sort, at room temperature, for two weeks. They'll sit there quite happily, and you get 80 % viability," says Connon.



A variety of applications for the hydrogels, including gel discs, beads and so-called 'ready plates' - 96 well plates in which cells are stored and used when required for experimental purposes.
Image: *Dr Che Connon/University of Reading*

"It's not very technologically challenging – it's quite simple, actually."

As a result, the technology would allow stem cells to be shipped from their point of manufacture to the places where they are needed. In many of these places, such as clinics in rural Africa, it is difficult, if not impossible, to maintain the 'cold-chain' of refrigeration to keep cells frozen. In addition, these clinics may not have the cell culture facilities and technicians required to make use of frozen cells. Cells encapsulated in hydrogels do not need to be kept cold during transport and can be used straight away.

"You can send them out to all parts of the world, which is personally quite exciting," says Connon. "With this technology you could start to see cell-based therapy being used in remote clinics in India, Africa, basically anywhere that can receive post."

From cornea to clinic

Connon made his hydrogel discovery while carrying out research supported by an MRC-funded grant to investigate whether it was possible to take corneal epithelial cells from a donor, encase them in a hydrogel and apply that directly to the surface of a patient's eye[6,7]. There is a pressing need for new treatments for diseases of the cornea, as the increasing prevalence of laser-eye treatment to correct eyesight is reducing the supply of donated corneas available for transplants. Corneal stem cells could offer an alternative, and were one of the earliest areas of stem cell research alongside bone and blood.

During the MRC-funded research "I noticed the cells within the hydrogel were not respiring greatly." Connon explains. "Would that allow them to remain viable in less hospitable environments and, specifically, outside the cell culture medium?"

Further investigation showed that, because the hydrogel suppressed respiration in the cells, they could survive in the hydrogel outside normal laboratory conditions for up to two weeks.

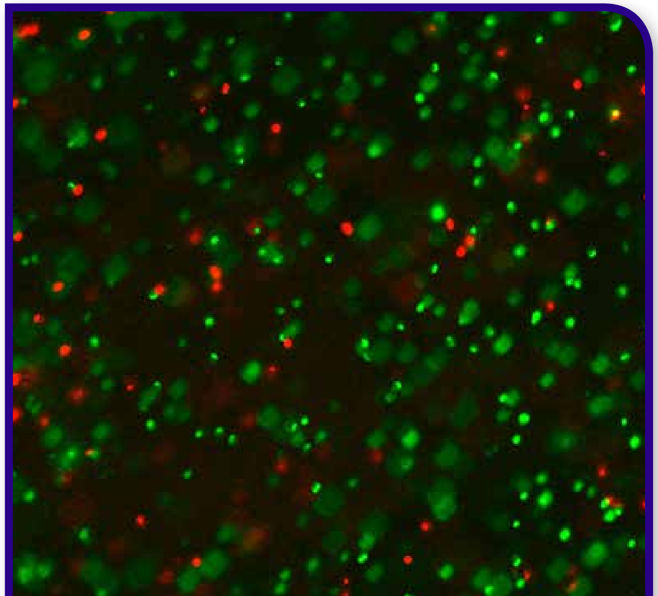
BRIC by BRIC

Following the MRC grant, Connon sought funding from BBSRC to further develop the hydrogel technology. This led to a BBSRC Pathfinder grant in 2009 to conduct a market analysis of the current state of cell transportation and cell storage. The analysis found that there was no current alternative to cryopreservation and indicated that the hydrogels could be commercially valuable.

Connon then received £30K from the University of Reading to improve his proof of concept, which enabled a post-doctoral researcher to expand on the initial discovery. In

BRIC

BRIC was established in 2005 by BBSRC, EPSRC and industry to support and develop the UK bioprocessing research community, and enable knowledge transfer between the science and engineering base and industry. BRIC includes 15 industry members, as of June 2012. The first phase of BRIC awarded £13.7M of funding to 25 research projects. The second phase, which began in 2010 and will run for five years, will award a total of £10M.



Live stem cells (green) and dead stem cells (red) stain following release from hydrogels after two weeks in storage.
Image: *Dr Che Connon/University of Reading.*

particular, the researchers were able to look at how different cell types responded to encapsulation in the hydrogels, and how the cells fared over time. This led to Connon's first grant from the Bioprocessing Research Industry Club (BRIC), in 2011, co-funded by BBSRC and EPSRC.

From BRIC, Connon received a one-year enabling award, which helped him put limits on the technology – to find out how long cells could be stored for, what density of cells could be stored in the hydrogel, and how these varied for different cell types. With that knowledge, Connon won a full BRIC grant, which began in October 2013.

"More importantly, BRIC gave me access to key industrial contacts, primarily in bioprocessing, but a lot of the people there are also interested in cell culture and products revolving around cell culture more generally," says Connon.

Talking to industry representatives has enabled Connon to ensure his research takes account of their needs, for instance by allowing him to see whether processes could be scaled-up to industrial quantities.

In parallel, Connon also used a database managed by the Cell Therapies Catapult[8] to identify clinicians planning or running stem cell clinical trials. The researchers contacted these clinicians to explain the hydrogel technology and to discuss how the clinicians could incorporate it into their methods once their therapies were well-advanced.

Sparking Impact

A BBSRC Sparking Impact Award in 2013 provided a small amount of funding for a post-doctoral researcher in Connon's lab to demonstrate their hydrogel technology to industry. This resulted in 25 Non-Disclosure Agreements with interested companies, and around 12 material transfer agreements. It was also a valuable training exercise for the post-doctoral researcher involved, as she developed experience in talking to industry representatives.

The Sparking Impact award also led to an evaluation licence with a veterinary medicine company interested in using hydrogels to encapsulate sperm cells from livestock. These are used to artificially inseminate farm animals such as cattle, avoiding the cost of transporting animals for breeding, as well as allowing farmers to use the sperm from high quality males for their herds.

At the moment they rely on cryopreservation, but it is not always possible to maintain a reliable cold chain on the farm. According to Connon, "anything that can simplify the delivery will be of benefit to [farmers] and, hopefully, have a greater success rate."

What next?

The hydrogel technology is still being developed, and Connon's research (supported by the BRIC grant awarded in 2013) is now focussing on two areas. The first encompasses the biological questions around the effects of the hydrogel on the encapsulated cells; in particular, what mechanism suppresses the cells' respiration? Connon's group are also focussing on a single type of stem cell - adipose-derived mesenchymal stem cells – to establish clear limits to the technology.

In parallel, Connon is working with Dr Andrzej Pacek at the School of Chemical Engineering in Birmingham to scale-up the technology using a stirred bioreactor system to create beads of gel containing the therapeutic cells. "It's early days," says Connon, "but we should end up with a way of processing billions of cells in beads that could then be shipped at ambient temperature."

REFERENCES

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3 Dr Che Connon

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8 Cell Therapies Catapult: https://ct.catapult.org.uk/
The Catapults receive core funding from Innovate UK, the Technology Strategy Board.