Temperature-sensitive gels: from tissue engineering to drug delivery

Martina Habeck, Freelance writer

Temperature-sensitive polymers with unique gelling properties for both drug delivery systems and tissue engineering purposes have recently shown promise in *in vitro* and *in situ* studies. These thermoreversible hydrogels, designed by scientists from the Department of Energy's Pacific Northwest National Laboratory (PNNL; Richland, WA, USA), could eventually help in the targeted delivery of medical isotopes and chemotherapy drugs as well as serve as a method to deliver chrondrocytes for the repair of articular cartilage damage.

Thermoreversible hydrogels are aqueous polymer solutions that undergo a transition to a gel state in response to changing temperature. The gels are crosslinked by weak and heat-reversible secondary forces such as hydrogen bonding, hydrophobic interaction and van der Waals forces.

Due to differences in the mechanism of gel formation, some thermoreversible hydrogels gel in response to cooling, while others gel in response to an increase of temperature. Well-known examples of the former are aqueous solutions of gelatin or agarose. By contrast, Anna Gutowska, Byeongmoon Jeong and Karol J. Krzyminski work with polymers that flow freely at room temperature or lower, but form soft gels at body temperature as water stops being a solvent.

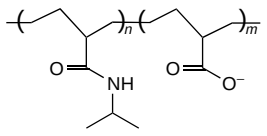
Polymer scaffolds to support chondrocyte growth

A main focus of PNNL research in this field is to develop materials for repairing damaged cartilage. Articular cartilage covers the end of bones in joints and provides a smooth gliding surface. Its smoothness and thickness determine the load-bearing characteristics and mobility of the joint. However, injury or pathological processes can result in lesions on the chondral surfaces, which interfere with the smooth motion of the joint and cause pain, instability and stiffness.

Unlike other tissues, articular cartilage does not have the capacity to repair itself. Instead of normal hyaline cartilage, fibrocartilage is produced, which has inferior biomechanical properties, and can eventually lead to osteoarthritis. Surgical approaches to deal with articular cartilage defects include drilling through the subchondral bone to allow vascular tissue and mesenchymal cells to invade the damaged site and repair the defect. Another common strategy is to harvest chondrocytes from the knee of the patient, culture and multiply them *in vitro* and reimplant these fresh chondrocytes into the damaged joint.

However, while these approaches can provide symptomatic relief, there is no evidence that the repair cartilage produced is of the durable (hyaline) type. The scientists at PNNL have two families of polymers in the pipeline that might improve this situation.

To obtain a solid 3-dimensional matrix in which chondrocytes can be seeded, Gutowska designed a gel made of random co-polymers of *N*-isopropylacrylamide (NiPAAm) with hydrophilic co-monomers (Fig. 1)[1]. Crosslinked gels of polymers based on NiPAAm are well known and were originally developed for the delivery of therapeutics. Gutowska's material is not chemically crosslinked, is thermodynamically stable and exhibits



Drug DiscoveryToday

Figure 1. Co-polymer of poly(*N*-isopropylacrylamide-co-acrylic acid) (NiPAAm/AAc); n and m denote sequences of NiPAAm and AAc, respectively. Length and distribution of the NiPAAm and AAc sequences vary randomly along the co-polymer chain.

no syneresis, i.e. no water is expelled from the polymer matrix on gelation. Once formed, the gel can revert to the liquid state only in response to a reduction in temperature.

The NiPAAm co-polymers are synthesized by mixing the components and initiating a polymerization reaction, after which they are purified, sterilised and, at room temperature, mixed with a cell culture medium. The chondrocytes are then added, the mixture gelled by raising the temperature to 37°C, and the cells are left to grow in the gelled polymer culture medium. A major advantage of Gutowska's invention is that the cells can be harvested again by simply lowering the temperature and centrifuging.

To test their system, isolated chondrocytes from rabbits were seeded into the gelling cell culture medium and incubated for 2 weeks. Gutowska says, 'We showed that the cells regained their chondrocyte phenotype. They had a round shape and were producing collagen II, a marker for articular cartilage. By contrast, control cells grown in monolayer cultures were more fibroblast-like,' (Fig. 2). The scientists have now started

1359-6446/01/$ – see front matter PII: S1359-6446(01)01824-4

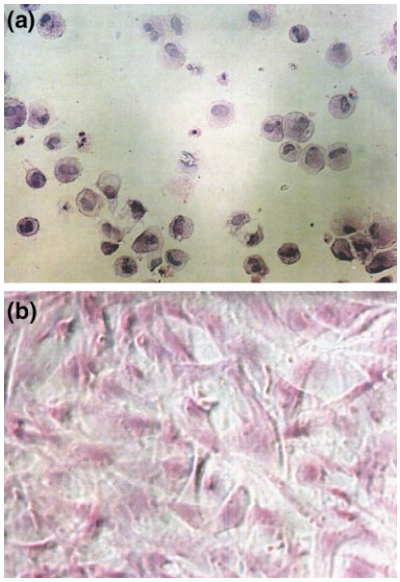


Figure 2. Chondrocytes (cartilage forming cells) cultured in **(a)** 3D gel and **(b)** in a monolayer. Scale: 20× magnification in both pictures.

in vivo experiments in rabbits to demonstrate that similar improvements in the type of chrondrocytes produced are retained on transplantation into a damaged joint.

Supporting *in situ* cartilage growth

The scientists also hope to support *in situ* growth of cartilage by mixing chondrocytes with aqueous solutions of polymers designed by Jeong. His novel graft polymers consist of poly(ethylene glycol) (PEG) and co-polymers of poly(lactic acid) and poly(glycolic acid) (PLGA) (i.e. PLGA-g-PEG and PEG-g-PLGA)[2] and, in contrast to the copolymer designed by Gutowska, they are biodegradable. On injection into the damaged joint, the body temperature would cause immediate gelling of the chrondrocyte–polymer mixture.

The scientists have teamed up with colleagues at the Medical University of South Carolina (Charleston, SC, USA) to test that this matrix will help chondrocytes maintain their phenotype and form 'normal' hyaline cartilage *in situ*. If all goes well, this treatment would avoid open surgery, usually required for implantation of the cells or for removal of the scaffolding material.

Kyriacos Athanasiou (Department of Bioengineering at Rice University, Houston, TX, USA) is involved in similar research. He says that, 'Their idea of injecting cells in a gel into a joint defect is a good approach, in principle. But there are some technical issues that make this approach difficult to implement. If you take a piece of gel with cells and put it in a damaged joint, you have to bear in mind that the first step the patient takes will expose the gel to significant stresses and strains. The material will collapse immediately.' He says it might help to immobilise the joint for a while, although that would probably cause other problems.

Targeted drug delivery

Jeong originally designed the biodegradable polymers for use as a drug delivery system, an area that the PNNL scientists are also investigating. The rate of gel degradation depends on several factors, including the lactic acid/glycolic acid ratio, the MW and the topology of polymers. As a result, it is possible to tailor the degradation rate according to the desired release pattern of the protein or drug to be incorporated. Gutowska says, 'This is a very important practical issue. We intend to design delivery systems with a broad range of drug delivery times, from 1 week to 3 months.'

One promising application is the targeted delivery of medical isotopes or chemotherapy drugs to treat inoperable tumours. The medical agent could be mixed with the polymer solution before injection directly into the tumour. The body heat would cause instant gelling of the injected mixture, thus holding the therapeutic agent at the target site, and hopefully reducing side effects.

Preliminary experiments have confirmed that the gel holds therapeutic isotopes in place and the gel seems to be compatible with both β- and γ-emitting isotopes. The PNNL scientists are now looking for additional funding to conduct further studies. If all goes well, they can start clinical trials for both gels in 2–3 years.

References

1 Gutowska, A. *et al.* [PNNL] (2000) Thermogelling biodegradable polymers with hydrophilic backbones: PEG-g-PLGA. US06103528

2 Jeong, B. *et al.* (2000) Reversible gelling culture media for *in vitro* cell culture in three-dimensional matrices. *Macromolecules* 33, 8317–8322