Electroporation and ultrasound for gene and drug delivery

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New gene transfer technologies based on physical processes could eventually prove a safe and effective way of administering gene therapy. Techniques based on electroporation and ultrasound have shown promise in preclinical studies, the results of which were presented at the *American College of Cardiology* meeting in Atlanta, GA, USA, 17–20 March 2002.

The lack of a safe and efficient way to get new genes into cell nuclei is still the greatest obstacle to making gene therapy for human disease a reality (Box 1). The most efficient method known is to use viral vectors, but these have raised serious safety concerns [1]. Non-viral gene transfer has so far been less efficient, but is the subject of much research because of its potential safety advantages.

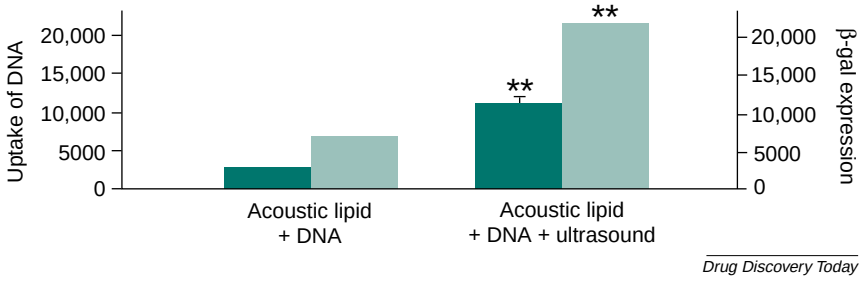

Figure 1. Uptake (dark green) and expression (light green) of β-galactosidase DNA from acoustic liposomes in vascular smooth muscle cells and baby hamster kidney (BHK) cells is enhanced by ultrasound. ** p <0.05

Electroporation

One well-established way of getting DNA and other macromolecules into cells is electroporation. High-intensity electrical pulses create aqueous pores in the cell membrane through which molecules can pass. Electroporation is routinely used with cell cultures, but researchers led by Luyi Sen at UCLA (Los Angeles, CA, USA) are applying it to whole organs in large animals. The aim is to conduct *ex vivo* gene therapy on human donor hearts and other organs before transplantation, to deliver genes for immunosuppressive cytokines, such as IL-10. This has been shown to decrease allograft rejection and to prolong the survival of transplanted hearts in animal models [2].

In standard electroporation, the voltage used is proportional to the diameter of the cell or tissue sample being treated, typically 200–700 mV cm^{-1}. For whole organs, it was believed that the high voltage needed would kill the tissue. But Sen challenges this widely-held assumption. 'You cannot multiply the diameter of the cell to decide how many volts you need,' she says. 'If you can get very close to the cells you want to treat, you need a very low voltage.'

She designed a device in which an array of positive electrodes is applied to the outside of the myocardial wall and negative electrodes are arrayed on a basket-like structure that is inserted into the heart via a catheter and 'expanded' *in situ*. The maximum distance between opposite electrodes is ~1 cm. The device was found to be both safe and effective in a rabbit heart transplant model when compared with liposomal and adenoviral transfection techniques [3]. IL-10 gene expression was fivefold higher than in the liposome group and 1.25-times the level in the adenovirus group ($p < 0.01$), and there was a parallel increase in IL-10 protein expression. Transgene expression was also much more uniform in the electroporation group, and there was no difference in the haemodynamic and electrophysiological properties of the hearts compared with untreated transplanted controls six days after transplantation.

'Low voltage electroporation has the same or higher gene transfer efficiency as viruses,' says Sen. 'You could also use it to deliver drugs, antibodies or peptides.' Preclinical studies with dogs are about to begin. If the apparent safety profile is confirmed, Phase I clinical trials could start by the end of the year.

Ultrasound

Researchers at Northwestern University (Evanston, IL, USA) are using ultrasound to improve the efficiency of liposomal gene transfer. They have developed cationic acoustic liposomes whose composition and structure enables them to reflect ultrasound [4]. *In vitro* studies with cultured cells showed that uptake of the β-galactosidase gene from the liposomes was enhanced up to fivefold and transfection efficiency up to fourfold by exposure to 30 sec pulses of 1 MHz

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Box 1. Other gene therapy advances

Three other potential advances in gene therapy have been reported recently.

(1) Introgen Therapeutics (Austin, TX, USA) immunized tumor-bearing mice with mouse dendritic cells treated with Advexin®, which incorporates the p53 tumor suppressor gene in an adenoviral delivery system. This completely prevented tumor development when the cells were activated, with no evidence of toxicity or autoimmune complications.

(2) Researchers at Stanford University Medical Center (Stanford, CA, USA) used a genetically engineered live adenovirus to attack gastrointestinal cancers that had spread to the liver. The attenuated virus is engineered to infect only cells with an abnormality in the tumor suppressor gene, p53. It was found to be safe in a Phase I trial in patients with advanced cancer, and those receiving the highest dose showed increased median survival time. Phase II studies in combination with chemotherapy are due to start this year.

(3) DNA compaction technology developed by Copernicus Therapeutics (Cleveland, OH, USA) is being tested in cystic fibrosis (CF) patients. Compacted genes are small enough to pass through the cell membrane and then into the nucleus. A compacted version of the normal CF gene is administered to the nasal passages via a saline drip. Gene uptake and expression in the nasal tissue will be monitored to see if the approach is effective.

ultrasound at 0.5 W cm^{-2} [5] (Fig. 1). The ultrasound increases DNA release from the liposomes at much lower power than is required to produce the same effects with standard liposomes. The frequency used is similar to that used in diagnostic ultrasound, so cell damage is not expected to occur. However, safety has not yet been evaluated *in vivo*.

Acoustic liposomes conjugated to antibodies have been shown to target sites of vascular disease. 'Using this technology it becomes feasible to both identify a disease site and to activate a therapeutic agent *in situ*,' says Shaoling Huang, a postdoctoral research fellow at Northwestern University.

'Gene transfer is still very much an open field,' says Ijeoma Uchegbu, Senior Lecturer in Drug Delivery at the University of Strathclyde (Glasgow, UK). 'No technology has yet emerged as a problem-free winner, so anything that works has merit. Electroporation has produced impressive data and is promising for *ex vivo* work or superficial tissues, but it would be difficult to apply to tissues that are not accessible. The same applies to other physical techniques – you need to get the tissue close to the device being used.'

References

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Vaccinating against ticks

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Anti-tick vaccines would provide a much needed alternative to current methods for the control of these blood-feeding parasites. Recent molecular approaches are now raising fascinating possibilities.

Ticks feed several times during their life cycle and can become infected with many pathogens. These can be transmitted to their host – humans and a wide range of animals, including pets and livestock – and can cause potentially serious diseases (Fig. 1). Sarah Randolph, a tick expert at the University of Oxford (Oxford, UK), says that in Africa, most farmers will identify ticks as the single biggest problem they are presented with in terms of livestock pests (Table 1). There are ~850 tick species and 30 major tick-borne diseases. These diseases generally affect the blood and/or lymphatic system and cause symptoms

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