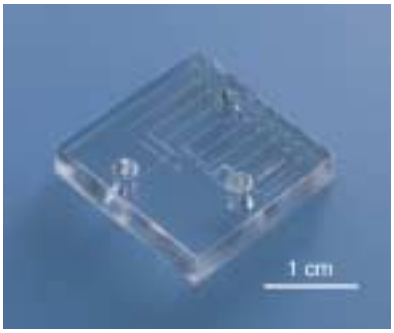

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Figure 2. A polydimethylsiloxane microfluidic chip designed for cell sorting. Diagram supplied courtesy of Fluidigm Corporation, San Francisco, CA, USA.

and then sized and sorted using a combination of pumps and valves before being pumped into a receptacle for further analysis.

The researchers have also carried out TaqMan™ assays using a similar device. Results were validated using conventional macroscopic analysis or the manufacturers' control protocols (Quake *et al.*, unpublished). 'The results have been very encouraging,' says Quake. 'We're quite optimistic that in the near future we will have a chip capable of performing single cell genetic and biochemical analyses.'

Users can design their own microfluidics systems using Fluidigm's computer-aided design (CAD) based software. Once a design is supplied, a mould can be printed and patterned within a day. Manufacturing is relatively easy because, unlike silicon, elastomers do not require clean room conditions for processing. Until now, innovation has been stifled by the length of time taken to produce new chip designs. 'A major benefit of this technology is that you can get very rapid turnaround times for prototypes,' Quake continues. 'You can find out quickly what works and what doesn't.' Standard polydimethylsiloxane adsorbs lipophilic compounds, but this problem can be overcome by either modifying the elastomer itself, or treating the device after manufacture to make it hydrophilic[5].

'This technology looks robust and easy to implement, even for non-experts,' says Sabeth Verpoorte of the University of Neuchatel's Institute of Microtechnology (Neuchatel, Switzerland). 'It will certainly open up routes to new microfluidic concepts based on pressure-driven flow. It will also be possible to expand the scope of applications to include many more types of solution matrices, since pressure-driven flow is not so sensitive to solution properties as electrokinetic flow.'

The next goal will be chips that are capable of more complex assays. Microfluidics is likely to find many more applications: these include genomic analyses; protein analysis, crystallization and purification; biochemical and electrophysiological assays; and gene expression and differential display analysis. It also has potential for use in drug delivery and diagnostics.

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Drug-eluting stents: flashy future or flash-in-the-pan?

Janet Fricker, Freelance writer

Recent data released from two clinical trials with different agents have highlighted the great potential for targeted drug delivery using drug-eluting stents in the reduction of clinical restenosis following angioplasty[1,2]. However, despite unprecedented results in some of these trials, some feel that the potential for these stents could be short-lived.

Restenosis

Percutaneous transluminal coronary angioplasty (PTCA) was first introduced in the late 1970s as an alternative to highly invasive coronary-artery bypass surgery to clear coronary vessels blocked by plaque. However, restenosis – the development of a new blockage from scar tissue – occurs in 30–50% of patients that are treated with balloon angioplasty[3].

Stents – cages of surgical-grade stainless steel resembling a scaffolding mesh – were then developed to eliminate elastic recoil and negative remodelling, and the widespread introduction of stents in the 1990s reduced the restenosis rate to 15–20%. However, restenosis continued

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in some patients because stents are not designed to address the process of intimal thickening that results from the cascade of events initiated by arterial injury. The restenosis process involves thrombus formation, inflammation and signal transduction, which mediates smooth-muscle-cell migration and proliferation.

'The rate of restenosis after stenting depends to a large extent on the lesion being studied', says Tony Gershlick, a consultant cardiologist from Leicester University Hospital (Leicestershire, UK). He continues, 'In a simple lesion it is ~10%, in a more complex lesion 15–20% and in a diabetic or small reference vessel, 40%.'

Stents for drug delivery

The rationale behind drug-eluting stents that are currently in development is to preserve the beneficial properties of stents (preventing vascular recoil and remodelling) and, at the same time, delivering therapeutic agents that inhibit intimal thickening. Drugs that prevent restenosis might act on inflammation, migration, proliferation and/or secretion of the extracellular matrix.

Sirolimus

A landmark clinical trial using drug-eluting stents was presented, by Marie-Claude Morice (Institut Hospitaller Jacque Cartier, Massy, France) at the *23rd Meeting of the European Society of Cardiology* (ESC) held recently in Stockholm, Sweden[1]. A study of the Sirolimus-eluting BXVelocity™ balloon expandable stent, developed by Cordis (Warren, NJ, USA), randomized 238 patients with *de novo* native coronary artery lesions to receive drug-coated or non-coated stent. [Sirolimus is a naturally occurring antibiotic marketed by Wyeth-Ayerst (St Davids, PA, USA) under the name of Rapamune®.] Restenosis with the rapamycin stent at six months was zero percent, compared with 26% in the control stents. Preliminary clinical data to 210 days, showed no deaths or target lesion revascularization in the rapamycin group, compared with two deaths and 26 revascularizations in the control group. A preliminary study of the Sirolimus stent on 45 patients, published earlier this year, had been the first to show zero rates of restenosis[4].

Wim Van der Giessen (Thorax Centre, Erasmus University, Rotterdam, The Netherlands), Chairman of the ESC session at which these results were presented, said: 'We've never seen anything like this before in interventional cardiology.' Cordis has finished enrolling 1100 US patients into the SIRIUS study (a US randomized multicentre study) and is currently enrolling 350 patients for a European study (E SIRIUS).

Paclitaxel

Other companies are coating stents with the drug paclitaxel. Cook (Bloomington, IN, USA) presented the first clinical trial results of its paclitaxel stent at the *11th Transcatheter Cardiovascular Therapeutics (TCT) Conference* in Washington (DC, USA)[2]. In the Asian paclitaxel-eluting stent clinical trial (ASPECT), the safety and efficacy of 3.1 μg mm^{-2} and 1.3 μg mm^{-2} dose densities of paclitaxel versus uncoated stents were assessed in 177 patients. After six months, investigators found that the restenosis rate was reduced from 27% in patients receiving control stents, to 12% in those receiving low-dose paclitaxel-coated stents, to 4% in those receiving higher dose paclitaxel-coated stents. (Presented by Alan Heldman from Johns Hopkins University, Baltimore, MD, USA.)

'Differences in the treatment protocols, ethnic backgrounds of patients, stents employed and methodologies of the clinical centres do not allow for valid comparisons of the relative abilities to inhibit restenosis between rapamycin- and paclitaxel-coated stents,' says David McCarty, Director of Cook.

Boston Scientific (Natick, MA, USA) also have a paclitaxel stent under development. This is a polymer-coated stent, whereas the Cook stent is polymer-free and uses a proprietary process to coat the stent with the drug. Cordis also uses a proprietary polymer coating.

Boston Scientific (Watertown, MA, USA) has recently undertaken a Taxus I safety trial (designed to assess the safety of a slow-release dose formulation paclitaxel-eluting coronary stent) involving 60 patients from three centres, but the results have not yet been published. However, they are planning a Taxus II study to look at *de novo* lesions.

Actinomycin D

Finally, Guidant Corporation (Santa Clara, CA, USA) started enrolling patients into their actinomycin-D-eluting stent improvement outcome (ACTION) trial in May 2001. In a trial involving 360 patients at 30 sites in Europe, Australia, New Zealand and Brazil, Guidant is comparing the outcomes of patients implanted with 2.5 μg cm^{-2} and 10 μg cm^{-2} of an actinomycin-D polymer-coated stent with a control uncoated stent.

Differences between the approaches

There is much debate over the relative benefits of polymer versus non-polymer stents. Proponents of polymer stents maintain that the coating allows the stent to control the release of the drug over a period of time and could enable the release rate to be varied. However, Neal Fearnot, President of MED Institute (West Lafayette, IN, USA), a subsidiary of Cook, says: 'There is a potential risk for polymers to deteriorate over time raising concerns that the polymer might cause inflammation.'

Cordis believes that the distinct mode of action of Sirolimus[5] offers significant advantages over other drugs under investigation. Sirolimus, a natural product of the macrocyclic lactone class, is a small, stable lipophilic molecule that can readily cross cell membranes. Once inside the cell it binds with high affinity

to a cytosolic receptor protein, FKBP12. FKBP is upregulated in response to vascular injury providing a unique targeting mechanism preferentially directing Sirolimus to injured cells.

Cordis says that, because Sirolimus inhibits the cell cycle at the early-stage G1–S checkpoint, it produces a safe cytostatic response, whereas inhibition of the cell cycle at later points will induce cell death and mutational changes. However, Fearnot says that, 'All drugs have the potential to be cytotoxic. It is the dose used that is important.'

Fearnot believes that paclitaxel shifts the equilibrium so that tubulin does not cycle between the insoluble and soluble forms because, for cells to divide, tubulin needs to be in the soluble form. Meanwhile, actinomycin D is a potent antibiotic that binds with DNA to inhibit RNA-polymerase-mediated transcription. It also causes single-strand breaks in DNA.

There are concerns that all of these drug-eluting stents could limit the growth of a layer of cells that is necessary to cover the stent and prevent bare metal from coming into contact with the blood, an event that could lead to clot formation. 'Another danger is that cells in the vessel wall could stop dividing resulting in vessel wall disruption and thrombus,' warns Karl Karsch, Chairman of Cardiology, University of Bristol (Bristol, UK). He adds that it could also be difficult to control the concentration of drug because the concentration in calcified vessels will be completely different from other vessels.

Future prospects

Stents could eventually be developed to deliver gene therapies and cholesterol-lowering agents, such as statins, to the vessel wall. But some predict that the stent's heyday will be short lived. 'My opinion is sophisticated molecular therapy, delivering genes and cellular modifiers in a more deliberate way, will be the future. In 10 years, mechanical interventions like stents are likely to be defunct,' says Karsch.

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Drug delivery through the keyhole

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A new biomaterial delivery system has been developed that could create novel opportunities for targeted drug delivery. The Celltran™ delivery system, developed by BioDelivery Systems (Portland, OR, USA), consists of a delivery instrument and proprietary gel matrix, which aim to solve the problems associated with current methods of delivering biomaterials and other surgical materials.

Existing technologies

Current methods for delivering biomaterials generally use needle syringes, but this has several drawbacks arising from the simple mechanics of injection. Turbulence, shear forces and the high pressure that is created as material is forced from the barrel through the needle can destroy a significant percentage of living cells in a formulation. When the implantation technique that was found to be optimal was used, as many as half of the implanted cells were not viable one hour after implantation[1]. Viabilities of 55–85% were recently reported following percutaneous testicular sperm aspiration through a 20 gauge needle[2]. It is also difficult to administer highly viscous solutions using a needle syringe. Additionally, problems can occur at the site of delivery, where the high pressure created during an injection can cause embolism[3] or leakage to other sites[3,4].

An alternative to needle syringe application is to place the biomaterial directly on the target site using invasive surgery. However, this has the usual problems associated with a more invasive medical procedure.

'When you start using it [a needle syringe] for purposes it wasn't designed for, that's when you run into problems,' commented Carl Wilcox, President of BioDelivery Systems. 'The needle syringe design delivers material to a site without relieving the delivery pressure… [and can] destroy up to 50% of cellular or

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