also be controlled. 'We have observed that the destruction of biocompatible capsules by enzymes can be varied from nearly indestructible to rapidly destroyed,' Voigt continues. 'It depends on the components used and the medium conditions, like pH, salt, temperature and light.'

If the properties of the drug make it impossible to encapsulate directly, colloidal particles or even cells can be used as templates[2] (Fig. 1). Once the polyelectrolyte shell has been constructed, the core template is dissolved away to leave a hollow structure. This can be reversibly opened, for example, by a change in pH, enabling even large drug molecules to enter. Alternatively, the drug can be precipitated in by generating nucleation centres in the capsule lumen.

Controlled release

Controlled-release delivery is likely to be an important application. The PEM capsules are semi-permeable and the wide range of possible encapsulation materials gives plentiful scope for adjusting permeability properties. Researchers at the Max-Planck Institute created simple ibuprofen PEM microcapsules using polysaccharide layers as the coating[3]. Ibuprofen has low solubility in water. Drug release was studied *in vitro* in simulated gastric (pH 1.4) and intestinal (pH 7.4) fluids. The ibuprofen crystals dissolved and the drug diffused out of the intact capsule. The rate of release was found to depend on the size of the crystal, the thickness of the capsule wall and the solubility of the drug in the given medium. There was no rapid initial burst of drug release or incomplete release of the drug dose from the carrier, both of which are problems that are usually associated with the use of microcarriers.

Future prospects

Voigt is optimistic about the versatility of PEM capsules. 'We have found that all the limitations we thought would exist have vanished after a few months' work,' he says. 'I don't see any limitation to the type of molecules we can encapsulate.' However, the technology has not yet been tested *in vivo*. Capsulution NanoScience hopes to start animal studies with an encapsulated drug in early 2002. Toxicity is not expected to be a problem because all the polymers used will be taken from the generally recognized as safe (GRAS) list.

'I am confident that we will begin to see several applications of the layer-by-layer coating technology over the next five years, including drug delivery,' says John Lally, Head of Polymers and Interfaces at Ciba Vision Corporation (Duluth, GA, USA). 'Its main advantage is the ease of fabrication and the ability to incorporate a wide range of entities. However, more work is needed in defining the toxicity profile of the nanocapsules, especially to determine their fate and distribution in the body through animal studies. In addition, some model drugs may need to be studied to demonstrate a tangible benefit for drug delivery applications. Depending on the route of administration, the stability of the nanocapsule wall could become an issue'.

The direction of future work will depend on client requirements: Capsulution NanoScience hopes to attract interest from companies wishing to formulate a range of new and existing drugs. The technology is also being developed for use in diagnostics, cosmetics and plant protection products.

References

1 Donath, E. *et al.* (1998) Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes. *Angew. Chem. Int. Ed. Engl.* 37, 2202–2205

2 Sukhorukov, G.B. *et al.* (1998) Layer-by-layer self assembly of polyelectrolytes on colloidal particles. Colloids and Surfaces A: physicochemical and engineering aspects. 137, 253–266

3 Qiu, X. *et al.* (2001) Studies on the drug release properties of polysaccharide multilayers encapsulated ibuprofen microparticles. *Langmuir* 17, 5376–5380

Budding new HIV therapies?

Kathryn Senior, Freelance writer

The first insights into the molecular mechanism underlying the process of viral budding in HIV-infected cells are suggesting a whole new range of drug targets that could prove useful in the suppression of AIDS in HIV-positive patients. Wesley Sundquist's group at the University of Utah School of Medicine (Salt Lake City, UT, USA), working in conjunction with collaborators at Myriad Genetics (Salt Lake City, UT, USA), have recently revealed that HIV-1 uses cellular machinery to bud from infected cells and the protein Tsg101 is an essential requirement of this process. Blocking Tsg101 could, therefore, prevent HIV-1 budding.

The life cycle of HIV-1

The life cycle of HIV infection is known to consist of six major events: attachment

 1359-6446/01/$ – see front matter PII: S1359-6446(01)02054-2

of the virus to the host cell; reverse transcription of HIV RNA; integration of HIV DNA into the host cell; transcription of viral proteins; translation and viral assembly; and, finally, budding and maturation. HIV-1 assembly is driven by the viral Gag protein, which is actively transported to the cell membrane. Once there, it forms enveloped, spherical particles that bud from the cell. The budding event itself occurs when the cell membrane is broken and then resealed to create discrete viral and cellular membranes[1].

Although the Gag protein is an essential requirement for viral budding, HIV-1 does not have the genes to code for proteins to break and reseal the cell membrane, and it is thought to recruit cellular proteins for this job. A potential docking site for these proteins has been identified on the Gag protein, in the p6 domain. Mutations within the *gag* gene at this point allow viral assembly to occur normally but the cell membrane cannot be disrupted, causing abnormal, severely attenuated viral release[2].

Looking for a cellular Gag-binding protein

'One of the main aims of our research was to identify cellular proteins that could bind to this area of the p6 domain of the HIV-1 *Gag* gene – an area termed the PTAP motif. We did this using ProNet™ technology developed by Myriad,' explains Sundquist. ProNet is a yeast two-hybrid-based system for discovering new protein-binding partners for a protein of interest. In initial experiments performed at Myriad, HIV-1 p6 was used as a 'bait' to screen for potential binding partners in a human spleen cDNA library. Genes encoding nearly full-length Tsg101 were isolated twice, and this was the only gene detected in the screens. 'Subsequent experiments in our laboratory, that were planned in consultation with Kenton Zavitz and Scott Morham from Myriad, showed that full-length Tsg101 bound wild-type p6 in directed two-hybrid liquid culture assays. The same experiment performed with three different p6 mutants, all with different point mutations in the PTAP motif, failed to show binding,' reports Sundquist[3].

A molecular mechanism for budding

Further studies showed that depleting Tsg101 in the cell arrested HIV-1 maturation at a late stage (Fig. 1). 'We think that Tsg101 can bind to the p6 domain and also to ubiquitin, another protein involved in viral budding. One attractive molecular model put forward by the team suggests that ubiquitination of HIV-1 Gag during viral assembly creates high-affinity binding sites that recruit Tsg101 to assist in the final stages of budding,' says Sundquist. The choice of Tsg101 by HIV-1 could be explained by the usual role of Tsg101 in a normal cell process called vacuolar protein sorting (Vps). The Vps pathway sorts membrane-bound proteins for eventual degradation in the lysosome or vacuole, and involves the breaking and resealing of membranes.

A new drug development strategy?

Although these results are exciting, there is much work still to be done. 'In my view this is just the beginning,' stresses Sundquist, who adds that the mechanics of membrane breakage and resealing are not yet understood. 'Tsg101 is only part of the machinery that is taken over by HIV-1 – now we need to find the rest of it and show how it fits together,' he says. In the meantime, Myriad researchers are investigating Tsg101 and other cellular proteins that have been identified as important in viral budding and are actively seeking to develop drugs that target them. 'We have already constructed high-throughput drug screens around several proteins and some of the 'hits' have been tested for anti-HIV activity in human white blood cells in culture,' explains Kenton Zavitz, Senior Investigator at Myriad. Many of these hits show potent anti-viral activity with no apparent toxicity. One, MPI49839, is due to enter pre-clinical safety and evaluation in animals. 'This will be done mainly from a toxicological and pharmacokinetic point of view since there is not a good animal model for HIV, so it is not possible to test for efficacy in animals,' points out Zavitz. Once these studies have been completed successfully, the first candidates can enter Phase I clinical trials, but 'it is difficult, if not impossible, to predict the timeline that this research will take,' he stresses.

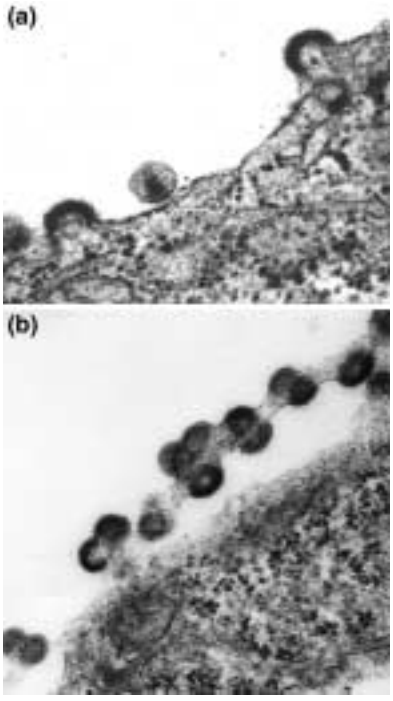

Figure 1. An electron microscope image of HIV particles budding from a cell (a). Following prevention of Tsg101 production, which is necessary for viral budding, the HIV particles 'clump' together in a chain (b), leaving them unable to spread and cause the infection of neighbouring cells. Figures credited to Uta von Schwedler, University of Utah (UT, USA; http://www.utah.edu/unews/releases/01/oct/aidsbud.html).

Avoiding drug resistance

All current anti-HIV drugs inhibit the reverse transcriptase enzyme encoded by

HIV genes. The use of protease inhibitors, together with nucleoside analogues, is relatively successful at reducing the viral load to undetectable levels, but HIV exhibits a high level of antigenic variation leading to the increased generation of drug-resistant strains. In fact, ~25% of the 3000 new cases of HIV diagnosed in the UK each year are infected by drug-resistant strains. A potential anti-HIV drug based on the inhibition of Tsg101 and other targets is, therefore, particularly attractive, as Myriad's president Adrian Hobden points out. 'The search for drugs to block cellular proteins that are taken over by the virus has one potential major advantage; the target here is a host protein, not a viral protein, so we hope that the virus will not find a way to get around it quite so easily,' he predicts.

References

1 Freed, E.O. (1998) HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology* 251, 1–15

2 Huang, M. *et al.* (1995) p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease. *J. Virol.* 69, 6810–6818

3 Garrus, J.R. *et al.* (2001) Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107, 1–20

Holey chips for drug delivery

Sharon Kingman, Freelance writer

Small pellets of porous silicon have been developed that could make targeted drug delivery and diagnosis a much simpler process. Implanted under the skin, these pellets could monitor blood levels of an administered drug, release new doses when necessary and then completely dissolve once empty.

pSiMedica (Malvern, UK) was set up as a joint venture in August 2000 by the UK's Defence Evaluation & Research Agency (DERA; Malvern, UK) and Sumich Group (Perth, Australia) and has already patented a form of porous silicon (BioSilicon™), which is both biodegradable and biocompatible. The company hopes this invention could help to solve many of the existing problems with drug delivery: these include noncompliance, painful injections and adverse effects resulting from the systemic bioavailability of the drug.

Leigh Canham, Chief Scientific Officer of pSiMedica, said: 'We are optimistic that this type of porous silicon gives us a material platform that is very flexible. We can tune the rates at which it degrades by altering its microstructure and varying pore size and pore density. The pore size distribution and the porosity that you achieve can be varied by changing anodization parameters like silicon resistivity, electrolyte composition and current density.' He emphasized that, unlike biodegradable polymers, the chemistry of porous silicon does not have to be altered to achieve different rates of degradation – a distinct advantage when it comes to applying for regulatory approval.

Porous silicon

Silicon is, of course, the raw material for the silicon chip and semiconductors. It can be produced to a high level of purity and is relatively cheap: even a highly pure form of silicon (99.99%) costs less than US$30 per kilogram. Canham began studying silicon while working at DERA, where he was involved in a project to develop a silicon laser.

However, after extensive reading around the subject, Canham noted some similarities between porous silicon and bioactive ceramics, which were being investigated for orthopaedic applications. This led him to investigate whether silicon could be biocompatible. To his surprise, *in vitro* tests showed that thin layers of highly porous silicon could completely dissolve away in simulated body fluids[1]. Subsequently, a six-month study using guinea pigs showed that solid silicon implants could persist in the body without rejection, whereas implants of porous silicon continuously decreased in weight over the study period[2] (Fig. 1). Canham, together with collaborators at St Thomas's Hospital (London, UK), have shown that porous silicon will break down in the body into the harmless compound silicic acid, which is present in many foods and drinks (Canham *et al.*; unpublished data).

Drug delivery

Silicon can be made porous by either electrochemical or chemical etching[3] and these techniques can be used for both silicon wafers and silicon powders. However, Canham pointed out that 'For many drug delivery applications, you do not necessarily want chips or segments or wafers – you want microparticles or nanoparticles. You can either start with a silicon film, porosify it and process it into a powder, or you can begin with silicon powder of the required size and shape and porosify that. We are investigating both options.' Powdered porosified silicon could, he said, be incorporated into ordinary capsules for oral delivery, into patches for transdermal drug delivery or into microparticles covered with the appropriate antibodies that could be injected into the bloodstream, for example, to lodge in tumours.

1359-6446/01/$ – see front matter PII: S1359-6446(01)02056-6