be of a major interest for future development. Given the biological response yielded through this approach, we propose that non-covalent, peptide-based delivery technologies hold a strong promise for therapeutic administration of siRNA.

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A86
High-efficient transfection using cationic lipids with programmed biodegradability

Asier Unciti-Broceta [1,*], Loredana Moggio [1], Kevin Dhaliwal [2], Laura Pidgeon [1], Keith Finlayson [1], Chris Haslett [2], Mark Bradley [1]
[1] *School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK*
[2] *MRC Centre for Inflammation Research, University of Edinburgh, 47 Little France Crescent, EH9 3JJ Edinburgh, UK*

*Corresponding author.
E-mail: asier.ub@ed.ac.uk (A. Unciti-Broceta).

Delivery of nucleic acids into cells has an ever-increasing number of applications with outstanding advances in both gene therapy and biotechnology, highlighting the induction of pluripotency in somatic cells. While the use of viral vectors is currently the most efficient transfection method, their antigenicity along with the risk of potential mutagenesis, among other inconvenients, are important limitations that hinder its application in medicine. Non-viral delivery systems (cationic lipids and polymers) represent an attractive alternative, particularly because of their low-cost, tuneable design and procedural simplicity. However, the *in vivo* efficacy of these carriers needs to be increased for both research purposes and clinical application. As repetitive dosing would be required in any gene therapy treatment, the cytotoxicity due to the use of these chemicals needs to be reduced, ideally by regulating their metabolic fate. To address these issues, a tripodal cationic lipid [1] was specifically designed to undergo complete intracellular metabolisation into naturally occuring compounds aiming to minimise the toxicity associated with its cytoplasmatic residence. Besides the toxicity issue, the incorporation of hydrolysis-prone linkages was addressed to enhance the cationic lipid-DNA dissociation once the lipoplexes have entered the cell by endocytosis. The novel compounds showed remarkable transfection efficiency along with reduced toxicity in a variety of immortalized cells and stem cells. Moreover, preliminary *in vivo* studies underlined the potential applicability of these non-toxic reagents for the delivery of DNA into mouse lung. These reagents, contrary to the most of chemical carriers commercially available, might offer a viable chemical alternative to viral transfection.

Reference

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A87
Immune stimulation following microneedle delivery of influenza virus-like particle (VLP) vaccines to human skin

Marc Pearton [1], Sang-Moo Kang [2], Jae-Min Song [2], Yeu-Chun Kim [3], Fu-Shi Quan [2], Matthew Ivory [1], Mark R. Prausnitz [3], Richard W. Compans [2], James C. Birchall [1,*]
[1] *Welsh School of Pharmacy, Cardiff University, UK*
[2] *Department of Microbiology and Immunology, Emory University, UK*
[3] *School of Chemical and Biomolecular Engineering, Georgia Institute of Technology, UK*

*Corresponding author.
E-mail: birchalljc@cf.ac.uk (J.C. Birchall).

Virus-like particles (VLPs) possess a number of features that make them attractive vaccine candidates for immunization against infectious disease. Efficient intra-epidermal delivery of VLP vaccines would exploit the abundance of Langerhans cells (LCs) that reside within the skin epidermis to generate an efficient host immune response. Microneedles (MNs) are currently being developed for the convenient and pain-free delivery of drugs and vaccines across the skin barrier layer. Whilst MN-based vaccines have demonstrated proof-of-concept in mice, it would be extremely valuable to understand how MN targeting of influenza VLP vaccines to the skin epidermis affects activation and migration of LCs in the real human skin environment. MNs with lengths of 700 μm were laser-etched from stainless steel sheets and surface-coated with either influenza H1 (A/PR/8/34) or H5 (A/Viet Nam/1203/04) VLPs. The coated MNs easily and reproducibly penetrated freshly excised human skin, depositing approximately 80% of the vaccine load within 60 s. Experiments conducted in cultured human skin showed that H1 and H5 VLPs, delivered via MNs, stimulated LCs causing morphological changes and a significant decline in total LCs number in epidermal sheets at 24–48 hours compared to untreated skin at the same time points. Histological sections showed that LCs in VLP treated samples were more dispersed throughout the epidermis with substantial numbers in the vicinity of the basement membrane. The response made by LCs was more manifest in human skin treated with H1 VLPs, compared with H5 VLPs. These findings corroborate observations in mouse studies, where H1 VLPs were shown to be significantly more immunogenic than H5 VLPs. Our data provide strong evidence that MN-facilitated delivery of influenza VLP vaccines initiates a stimulatory response in LCs in human skin epidermis. The results complement and support data gained from animal models, suggesting dendritic cells (DCs), including LCs, targeted through intra-epidermal or intra-dermal deposition of the vaccine generates immune response. This study also emphasizes the value of cultured human skin alongside animal studies for informative preclinical testing of intra-dermal vaccines.

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A88
Electrically based transdermal techniques for delivery of therapeutic macromolecules

Rakesh Kumar Tiwari [1,*], Ritesh Kumar [2]
[1] *University of Wales Instituture Cardiff, UK*
[2] *Ravishankar College of Pharmacy, Bhopal 462010, Madhya Pradesh, India*

*Corresponding author.

Advances in molecular biology have given us a wide range of protein and peptide based drugs that are unsuitable for oral delivery because of their high degree of first-pass metabolism. Though parenteral delivery is successful for developed and commercially available protein and peptide based drugs, chronic and self administration formulations are not the ideal choice through this route. Transdermal delivery is emerging as the biggest application target for these agents, however, the skin is extremely efficient at keeping out such large molecular weight compounds and therapeutic levels are never going to be realistically achieved by passive absorption. Therefore novel transdermal drug delivery systems have been developed with the aim to achieve the objective of systemic medication through topical application to the intact skin surface with benefits of deliver therapeutic macromolecules in desire therapeutic doses to overcome the difficulties associated with the oral route, namely poor bioavailability of drug and the tendency to produce rapid blood

level. Some newly active rate controlled electrically based transdermal techniques including: iontophoresis, electroporation, ultrasound and photomechanical waves have been developed and commercialized for the delivery of troublesome therapeutic protein and peptide based macromolecular drugs. This study covers the development of different electrically based transdermal techniques for delivery of therapeutic protein and peptide based macromolecular drugs, current status and assesses the pros and cons of each technique and summarises the evidence-base of their drug delivery capabilities.

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Molecularly imprinted polymers: macromolecule recognition

Marc Kelly*, Jenna Bowen, Mark Gumbleton, Chris Allender
Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3NB, UK

*Corresponding author.
E-mail: kellyma1@cf.ac.uk (M. Kelly).

Molecular imprinting is a technique used to engineer synthetic antibody mimics by the polymerisation of so-called functional monomers and cross-linkers around a target (template) species. Following removal of the template from the polymer matrix, cavities remain which display both chemical and steric selectivity for the imprinted molecule. To date the imprinting of biologically relevant macromolecules has been somewhat unsuccessful due to the inherent complexity of imprinting such moieties in aqueous media. Unlike small, organic molecules that are typically employed as templates, macromolecular structures such as peptides and proteins can exist in a multitude of conformations which leads to the development of heterogeneous binding sites as opposed to the well defined cavities formed during the regular imprinting process. The proteins will denature in traditional imprinting environments due to the presence of organic solvents and elevated temperatures. Additionally, the size of these biomolecules means that removal from the polymer matrix and subsequent re-binding is often inefficient. As a consequence, molecular imprinting has yet to achieve its true potential as efficacious, robust, reliable and cost-effective alternatives to the currently used antibody-based recognition systems. Projects currently underway within our laboratories aim to utilise target-selective peptides, derived from a phage display library, as a high affinity 'functional monomers' in a hybrid peptide-polymer molecularly selective system. Targets include lipopolysaccharide (LPS), the major pathogenic determinant of Gram negative bacteria and prion protein which is believed to be the causative agent of a group of invariably fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). Work to date has focused on optimisation of surface chemistries. Bifunctionalised polystyrene resin and glass surfaces have been synthesised to facilitate the independent immobilisation of peptide moiety and an initiator species. Polymer growth from the surface has been monitored by Fourier transform infra-red spectroscopy and atomic force microscopy. Future work will involve optimising a number of polymerisation variables and incorporating the phage-display derived peptide into the system to fully evaluate its potential as an antibody mimic.

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A90
Interference of mycobacterium tuberculosis with the endocytic pathways on macrophages and dendritic cells from healthy donors: role of cathepsins

D. Pires[1], P. Bettencourt[1], N. Carmo[1], T. Bergant[2], L. Jordao[1], E. Anes[1,*]
[1] *Centro de Patogénese Molecular, Fac. Farmácia, Univ. Lisboa e Instituto de Medicina Molecular, Lisboa, Portugal*
[2] *Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana, Slovenia*

*Corresponding author.
E-mail: eanes@ff.ul.pt (E. Anes).

Antigen-presenting cells (APC) such as macrophages and dendritic cells (DCs) play a pivotal role in tuberculosis pathogenesis. Macrophages are also key effector cells in mycobacteria killing. In order to survive inside the host immune cells mycobacteria developed different strategies. Among them blocking of phagosome-lysosome fusion and consequential reduced phagosome acidification assumes a crucial role allowing mycobacteria to escape acidic pH and destruction by proteolytic enzymes present in phagolysosomes. Since phagosome acidification varies between macrophages and DCs this may allow different kinetics of acquisition and activity for the enzymes involved. The aim of the present study was to compare the distribution of two key cathepsins: the exopeptidase cathepsin B and the endopeptidase cathepsin S inside human monocyte derived macrophages and DCs infected with *Mycobacterium* spp. Infected immune cells were collected after 3 hours and 1 day post-infection and prepared either for immunofluorescence confocal microscopy or for immunogold electron microscopy on ultrathin cryo sections. In macrophages we did not observe significant co-localization between either BCG or Mycobacterium tuberculosis and cathepsins B or S indicating that phagosome-lysosome fusion was strongly hindered. Similar results were observed for Mycobacterium tuberculosis after infection of DCs. In DCs the acquisition of cathepsin B into the phagosomes containing BCG was different from the acquisition of cathepsin S. Cathepsin S content was decreased by 30% after 1 day of infection whereas cathepsin B content inside BCG-positive phagosomes was increased. Our data indicate that cathepsins might be involved in differential mycobacterial persistence in macrophages compared to dendritic cells.

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A91
Role of mycobacterium tuberculosis outermembrane porins in bacterial survival within macrophages

D. Pires, P. Bettencourt, N. Carmo, M. Niederweis, E. Anes*
Centro de Patogénese Molecular, Fac. Farmácia, Univ. Lisboa e Instituto de Medicina Molecular, Lisboa, Portugal

*Corresponding author.
E-mail: eanes@ff.ul.pt (E. Anes).

Mycobacterium tuberculosis (Mtb) is the etiologic agent of tuberculosis a major worldwide health concern. One important feature in Mtb virulence is the ability to withstand the detrimental conditions of the phagosome within macrophages. Most of the virulence factors of Mtb are PAMPS from the outer membrane of the bacilli. Outer membrane porins participate in the inflow of hydrophilic compounds and we have shown that they are important for mycobacteria intracellular survival. Several porins have already been described as a means for nutrient acquisition but also as a possible pathway for antibiotic inflow. Previous studies showed that mutant