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

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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

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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

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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

Mycobacterium smegmatis lacking the MspA porin grow defectively due to the lack of glucose uptake but display increased resistance to several antibiotics and also to nitric oxide. Nitric oxide burst is a well described bactericidal mechanism in mouse macrophages and the inducible nitric oxide synthase is the enzyme responsible for NO release. In this study, we describe a novel putative outer membrane protein conserved between *M. tuberculosis* and *Mycobacterium bovis* BCG. We show that the absence of this protein limits bacterial growth *in vitro* but results in increased BCG survival within macrophages. We also demonstrate that although interferon-gamma stimulation of macrophages induces ten times increased killing of BCG, bacteria lacking this protein remain unsusceptible to this stimulation. Furthermore, quantification of iNOS and IL-1beta expression through qRT-PCR revealed that those genes were less upregulated during infection with the mutant bacteria compared to the WT strain suggesting that the increased survival of the mutants is due to lower macrophage activation and release of nitric oxide. We conclude that MtpA from Mtb complex is important to release virulence factors required for macrophage activation.

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In vivo phage display to identify peptides that target the brain

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The delivery of novel macromolecular therapeutics into brain parenchyma to treat central nervous system disorders (CNS) is hindered by the blood–brain barrier (BBB). The BBB is comprised of microvascular endothelial cells that line the capillaries traversing the brain. The existence of highly restrictive tight junctions and the relatively low abundance of morphologically evident endocytic vesicles restricts both paracellular and transcellular access to the brain of therapeutic proteins, peptides and nano-medicines [1]. As part of an ongoing programme to identify novel ligands that mediate endocytotic and transcytotic events within the BBB we report here the use of a Phage Display library to identify small cyclic peptides (-7mer) that traverse the *in vivo* rat BBB. A Phage Display Library (Ph.D.-C7C™ New England Biolabs) representing 1.2×10^9 unique genotypes encoding random-7mer disulphide constrained peptides genomically fused to the pIII coat protein of the filamentous phage M13 was utilised in all studies. A synchronous selection strategy [2] was employed to select for peptides homing to a range of organs before undertaking a final selection for peptides that home to brain grey matter. In this final selection the library was injected i.v. into a rat and circulated for 15 minutes before perfusion with saline to remove freely circulating phage and then glycine buffer (pH 2.2) to strip the vasculature of binding phage. The brain was removed and the white matter and capillaries depleted before the grey matter (brain parenchyma) was homogenised and phages recovered. The recovered phages were gene sequenced to determine the corresponding peptide library sequence displayed. From the sequenced population a conserved motif AC-SXTSSTX-CGGGS was identified at a frequency of 25%; secondary phage studies and bioinformatic analysis of a large population of sequenced clones (>500) corroborated this sequence. *In vivo* biodistribution studies of a clone displaying the conserved motif (AC-SYTSSTM-CGGGS) revealed a selective homing to brain grey matter as demonstrated by a 4-fold increase in AUC0-∞ and 3.5-fold increase in Cmax in brain grey matter compared to insertless phage (no displayed phage). Studies are addressing the molecular pathways of entry of this peptide phage into the CNS.

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A93

Phage display identification of a lung transduction peptide that affords enhanced macromolecule transport across the intact lung epithelium

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Evolutionary technologies based upon the screening of combinatorial libraries, for example, phage display, are used to survey the molecular diversity of target cell surfaces with the aim of identifying peptide motifs that promote target cell binding or internalisation [1]. Here, an M13 phage peptide library displaying cyclic 7-mer peptides was biopanned against the luminal surface of primary cultures of rat lung alveolar epithelial cells. 'Cell associated' phage were isolated after 4 rounds of biopanning, with the peptide library repertoire contracting from 1.2×10^9 clones to a maxium of 2×10^3 clones. DNA sequencing of 'cell associated' phage clones indicated peptide sequences to be largely composed of hydrophillic amino acids with isoelectric points approximating neutrality. The most frequent phage clone bore the peptide sequence C-TSGTHPR-C (termed LTP-1) and displayed enhanced (>1000-fold) transport (versus phage control vector) across restrictive *in vitro* alveolar epithelial monolayers [2]. When the LTP-1 phage clone (LTP-1) was administered as a coarse aerosol into the airways of an isolated perfused rat lung IPRL preparation [3] the extent of phage absorption across the pulmonary epithelium was 8.6% by 120 min, some 1500-fold greater than either the insertless vector control or a library clone that displaying a control peptide sequence (C-PLLAPGI-C, termed NB-3) that was isolated from the first biopanning round. When LTP-1 phage was co-administered with a 100-fold molar excess of the synthetic LTP-1 peptide sequence (syn-LTP-1) the extent of LTP-1 phage was competitively inhibited (LTP-1 phage absorption reduced to 0.1% by 120 min, $p < 0.05$). In contrast, the synthetic NB-3 peptide (syn-NB-3) displayed no inhibitory effect (7.6% LTP-1 phage absorbed dose absorbed by 120 min, $p > 0.05$). The syn-LTP-1 peptide sequence was grafted onto the surface of an anionic PAMAM G5.5 dendrimer