The 2nd Annual Irish Drug Delivery Network Conference with UK and Ireland Controlled Release Society: advancing drug delivery

The Science Foundation Ireland-funded Irish Drug Delivery Network 2nd Annual Conference on Advancing Drug Delivery

School of Pharmacy at University College Cork, Ireland, 17 June 2010.

This meeting was part funded by Science Foundation Ireland and by the University College Dublin Seed-Funding program, and was an opportunity for the Irish Drug Delivery Network to invite selected internationally-recognized scientists from across Europe onto a program, together with some of its own principal investigators. The meeting was co-promoted by the UK and Ireland Controlled Release Society. Topics included fluorescent dyes for stability testing of proteins, engineering of nano-containers, peptide–polymer conjugates, designing novel biomaterials, oral liquid-emulsion drug delivery systems, barrier modulation for drug delivery to the eye using siRNA, cell-specific targeting in the lungs, hot-melt extrusion and modified cyclodextrins for delivery of siRNA. The conference was attended by 85 researchers and the Irish Drug Delivery Network co-chairs were Caitriona O'Driscoll (University College Cork) and David Brayden (University College Dublin).

Andrea Hawe (University of Leiden, The Netherlands) reviewed her research on how extrinsic dyes are being used to assess protein and peptide stability. This is an important technology as physical and chemical instability can potentially lead to loss of bioactivity, induction of toxicity and decay of stored peptide. While traditional methods including light scattering, size-exclusion chromatography and Fourier transform infrared microscopy (FTIR) provide useful information on structural changes in monomers and the presence of aggregates, many of these technologies lack sensitivity for early detection of structural changes. Hawe described how organic extrinsic dyes including Nile red and Thioflavin T can be used to assess aggregation changes induced by even short term temperature and viscosity changes. Importantly, the dyes do not interact with the native protein or with formulation excipients, for example, polysorbates. In one example she showed how the dye, 9-(2-carboxy-2-cyanovinyl)julolidine, can detect heat-induced changes in adalimumab (Humira®). When correlated with FTIR and size-exclusion HPLC data, extrinsic dyes are a powerful, inexpensive and sensitive tool to examine structural changes [1].

Woie-Ping Cheng (University of Hertford, UK) discussed the issue of poor aqueous solubility for an estimated 40% of small-molecule drugs in development and the issue of poor intestinal peptide permeability. Microemulsions, liposomes and cyclodextrins vary in their solubilizing capacity and many conventional excipients can be harsh on the payload. She described how, as an alternative, self-assembling grafted amphiphilic co-polymers can be used to formulate a range of poorly soluble drugs including paclitaxel, griseofulvin and prednisone. Her group has worked on the hydrophilic poly(allyl) amine polymer (PAA) grafted with hydrophobic pendant groups (e.g., dansyl, cholesteryl and palmitoyl) to yield amphiphilic polymers, which act as effective noncytotoxic solubilizers for oral and parenteral delivery of small molecules. These types of polymers are also being used to formulate anticancer agents in preference to the use of DMSO as the solvent. In one example, a novel anticancer agent complexed with cholesteryl–PAA killed pancreatic cancer cells to the same degree as the gold standard agent, gemcytabine, efficacy data that was confirmed in tumour-bearing mice induced by these same cells. Turning to peptides, she showed that when palmitoyl–PAA was complexed with either insulin or salmon calcitonin (sCT), it protected them from a range of peptidases and permitted bioactivity to be retained. An attractive feature of this versatile technology is the simple scalable process, although challenges remain in accurate estimates of entrapment [2].

Chris Morris (University of Cardiff, UK) reviewed the challenges for pulmonary peptide delivery in the post-Exubera® era. Focusing on potential lung epithelial targets for delivery, he

David J Brayden[1], Graham Armstrong[†1] & Caitriona M O'Driscoll[2]

[1]University College Dublin
[2]University College Cork
[†]Author for correspondence:
Tel.: +35 317 166 017
Fax: +35 317 166 219
E-mail: graham.armstrong@ucd.ie

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initially described Syntonix Pharmaceutical's technology (acquired by Biogen IDEC), whereby a potent fusion antibody hybrid of erythropoietin could be targeted to the neonatal antibody FcRn receptor in lung alveolar cells to enable systemic delivery. On the basis that this receptor was expressed by the rat-isolated lung model as suggested by immunohistochemistry [3], he outlined a phage display biopanning programme using primary cell cultures and the lung model. This has led to the identification of a highly permeable lung transduction motif peptide. The peptide is currently being used to target molecules to the apical membranes of pulmonary epithelia when attached in a 1:1 ratio to polyamidoamine dendrimers of molecular weight (MW) 5.5 kDa. Morris is also working on antimicrobial peptides from the airways and has discovered that toxicity to host cells can prevent their further development. To get around this problem, his team has have been synthesizing novel hybrid peptides and is using polyethylene glycolation (PEGylation).

Gavin Andrews (Queen's University, Belfast, UK) gave a pharmaceutical technology review of the hot melt extrusion process and how resulting solid dispersions are being used to assist delivery of the 90% of the new chemical entities that belong to the Biopharmaceutical Classification System Class II and IV. He described the principles of thermoplastic behavior, transition and degradation temperatures and amorphous versus crystalline formats, then went on to discuss some specific polymers currently being used in hot melt extrusion formulation based on methacrylates, PEG and pyrrolidones [4].

Monica Rosa (Sigmoid Pharma, Invent Centre, Dublin [101]) reviewed progress on the company's oral delivery technologies, SmPill® and liquid emulsion drug delivery system (LEDDS®). SmPill® is an emulsion embedded in a polymeric matrix with varying levels of surface coatings up to 40 µm and it is designed to be suitable for poorly soluble payloads. *In vitro* release data revealed 70% release of payload at 6 h and a residue at 12 h. Sigmoid is currently enrolling up to 100 inflammatory bowel disease patients in Irish and British hospitals for a Phase II study examining a cyclosporin formulation using SmPill®. Mouse studies show that cyclosporine from this formulation can reach the colon intact and that it is not metabolized in that region. Further rationale for colonic delivery of cyclosporine is that the formulation may enable topical rather than systemic delivery of the agent and this may reduce potential side effects through use of lower doses in an intestinal regional targeting system. SmPill® is also undergoing preclinical oral vaccine studies in which antibody levels to a common antigen were detected in mice when used with an adjuvant following two oral gavage administrations followed by an injected booster. Studies with LEDDS® have used sCT as the cargo and have been carried out as part of the company's role as an industry partner in the Irish Drug Delivery Network (IDDN). This formulation contains sCT in beads with a range of excipients, which are in turn inserted in a larger coated capsule. Studies showed that the excipients do not damage sCT and that it is bioactive when released into T47D breast cancer cells expressing the receptor. Techniques including circular dichroism, isothermal calorimetry and atomic force microscopy are being used to further examine the structure of sCT when formulated in LEDDS®. When the released material was instilled intrajejunally to anaesthetized rats, it was able to lower serum calcium levels and levels of sCT were detected in serum over many hours.

Peter Humphries (Trinity College Dublin, Ireland) presented on 'Barrier modulation: a process for systemic drug delivery to the brain and retina'. He argued that several approved low-molecular-weight drugs that are in current use in the treatment of non-neuronal conditions could be injected systemically for the prevention of neurodegenerative diseases, neuronal malignancies or retinal degenerations, but only if a means could be found to permit them to selectively access the brain or retina from the peripheral circulation. This can only be done in such a manner as to prevent access to these tissues of larger, harmful substances such as anaphylatoxins, antibodies, soluble enzymes and pathogens. Drug access to the brain and the eye however, is normally blocked by the tight junction architecture of the vascular endothelial cells of the blood vessels supplying the brain and retina, the blood–brain and inner blood–retina barriers (BBB and iBRB). Humphries' team has developed a method for periodically and reversibly rendering the BBB and iBRB transiently permeable to drug formulations up to 1 kDa (approximately the size of two DNA base pairs), leaving them closed to larger potentially harmful blood-borne materials. A very large number of drugs fall within this MW range. Barrier modulation was achieved either by systemic administration of siRNA downregulating transcripts encoding claudin-5 [5], one of a variety of protein components well expressed in the tight junctions of vascular endothelial cells. Data from mice were shown in which siRNA

against claudin-5 could open the tight junction, enabling marker drug access across the retina when administered by the intravenous route with the transfection agent, phenylethylimine. Importantly, while boosted transport of markers including Hoescht 33342 (MW 562 Da) and gadolinium (MW 742) could be enabled, larger molecules such as peroxidase (MW 1900 Da) were excluded, suggesting that tight junction opening could be limited to assisting drugs in a size range, against letting pathogens and toxins through. By incorporation of barrier-modulating machinery into an inducible adeno-associated virus-2/9 system for localized brain or retinal modulation, a potentially more practical system can be used. Downregulation of transcripts encoding other tight junction proteins may also enable endothelial cell barrier permeability to be further manipulated in a controlled fashion. According to Humphries, siRNA suppression of claudin 5 does not induce neuronal oedema, has no negative impact on retinal function, minimal effect on global neuronal transcriptional profiles and has been proven to be effective in a number of *in vivo* mouse models of retinitis pigmentosa and also in a choroidal neovascularisation model of age-related macular degeneration. Humphries went on to argue how systemic delivery would be better accepted by patients than local invasive delivery to the eye. Further applications might also be for treatment of intractable neuroblastomas through modulation of both the blood–brain and the blood–tumour barrier with siRNA for tight junction claudins. While these technologies may eventually represent a new and potentially powerful approach to molecular therapy for such chronic conditions, he emphasized that greater understanding of siRNA off-target effects and interactions with TOLL-like receptors is required.

Sally-Ann Cryan (Royal College of Surgeons, Ireland) presented on pulmonary delivery from a cell-targeting perspective. As a principal investigator in the IDDN, she has a program to target lung epithelia to achieve local and systemic siRNA delivery as well as attempting to target drugs in particulates to alveolar macrophages (AM) in order to treat inflammation and tuberculosis. She reviewed data showing that the optimum size for particle uptake by AM was 2.0 µm. When poly(lactide-co-glycolide) particles were loaded with the anti-TB drug rifampcin, more drug could be localized to THP-1 monocytes (a model for AM) due to increased particle uptake. Furthermore, siRNA could be delivered to THP-1 cells and primary cultures in microparticles and uptake was superior to that achieved with the gold standard transfection agent, Lipofectamine 2000®. Useful anti-inflammatory cargoes include TNF-α knock down and a 40% reduction was also achieved in cell culture models of AM using siRNA delivered in microparticles. She reviewed the potential of targeted PEGylated targeted particles and liposomes to achieve siRNA delivery both to alveolar epithelia and to AMs **(Figure 1)**. In Calu-3 lung-derived epithelial cultures, use of high content analysis and confocal microscopy showed that PEGylated polymeric particles and liposomes coated with a range of cel-penetrating peptides were taken up well. The challenge is to translate such data to validated animal models of pulmonary delivery and to work with delivery device technology so that deep lung delivery can be achieved [6]. To this end, her team are collaborating with the IDDN industrial partner in pulmonary delivery devices, Aerogen Ltd (Galway, Ireland).

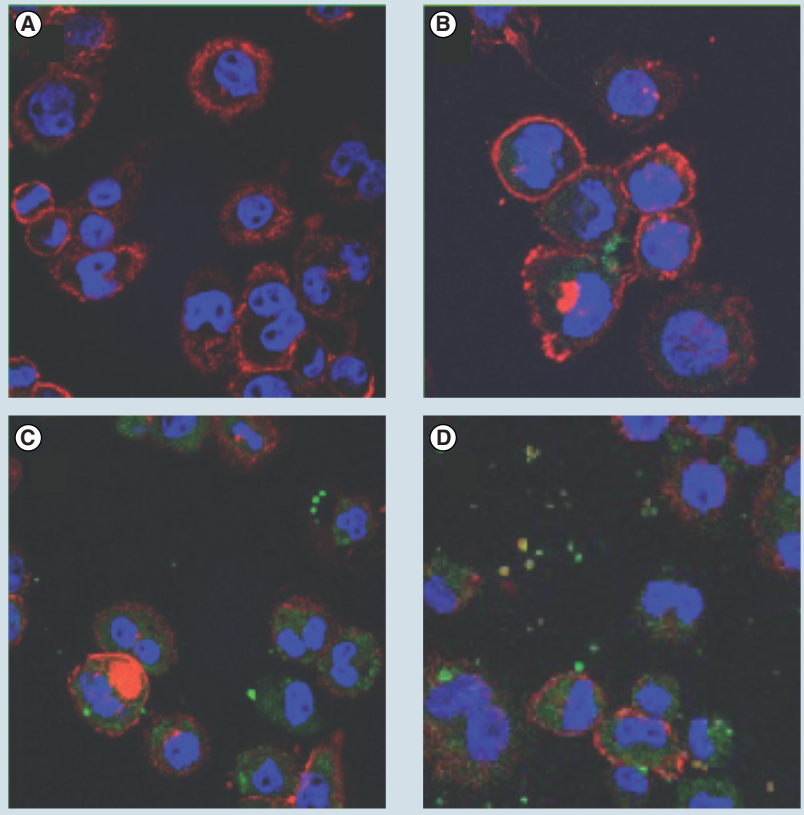

Figure 1. Uptake of siRNA into differentiated THP-1 macrophage-like cells using microparticles (siRNA-MP) analyzed by high content analysis.
(A) Untreated cells, **(B)** siRNA-treated cells, **(C)** siRNA in PLGA microparticles and **(D)** siRNA in PLGA/PEI microparticles.
Blue: Nucleus; Red: Cell membrane; Green: siRNA.
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Vitaliy Khutoryanskiy (University of Reading, UK) described his research on intestinal mucosa mimetics in order to discover new reliable assays for mucoadhesive contructs. 60% of studies, from an examination of 348 papers from 1998–2008, used animal-derived tissue from pigs or rat, so the work has an important element of reducing animal use. Secondly, typical assays are based on the principle of measuring the force required to remove a drug/tablet from a fixed mucosal surface and they are not very physiological or accurate. This research described how novel hydrogel co-polymeric materials [7] can be used in place of animal tissue, and can give data measuring the work of adhesion that is equal or superior to that achieved with fresh mucosae. Synthetic materials used to date to measure adhesion include PVC tape, wet glass and polypropylene plates. These are not ideal and do not mimic the mucosa. An ideal substrate should be soft, wet and porous, contain functional groups similar to mucosae and should be cheap and easy to handle. Hydrogels fulfil these criteria. Initial attempts used poly(acrylic acid)-methyl cellulose (PAA-MC) complexes thermally crosslinked and set on a glass slide, but unfortunately the hydrogel detached from the glass. When silane was coated on glass, detachment was prevented. However, the PAA-MC construct remained unsuccessful and the force required to detach the positive controls (Carbopol® and hydroxyethylmethyl cellulose tablets) from the ultrathin hydrogel poorly mimicked that of intestinal tissue. Using 3D co-polymerization of 2-hydroxyethylmethacrylate (HEMA) with various co-monomers led to comparable adhesion data being achieved with a set of high, medium and low adhesives on porcine buccal and stomach mucosae. A total of 45 hyrogels based on HEMA have been tested to date under the criteria of force of adhesion and detachment and promising data has been achieved with *N*-acetyl glucosamine as a co-polymer with HEMA in an attempt to mimic surface functional groups of intestinal tissue. Aspects being examined include degree of hydrogel swelling, surface topology measurement with atomic force microscopy and mechanical properties.

Caitriona O'Driscoll (University College Cork), a principal investigator in the IDDN, discussed University College Cork/University College Dublin research on modified cyclodextrins (mCD) for siRNA delivery. She reviewed a recent positive clinical trial from Calando Pharmaceuticals of systemic siRNA delivery to melanoma patients using targeted PEGylated CDs, in which the lipophilic agent, adamantine and transferrin receptor targeting played an important role [8]. CDs are approved in 33 injected products and have a good regulatory history; moreover they can be modified to reduce toxicity if present. She described how the alternative mCD vesicles of approximate diameter 100–200 nm are based on an entirely different construction **(Figure 2)** that utilizes click chemistry whereby hydrophobic pendant groups can be introduced, along with PEGylation to alter the charge profile, for example, folate for targeting the condensed siRNA [9]. Epithelial cell culture transfection studies were

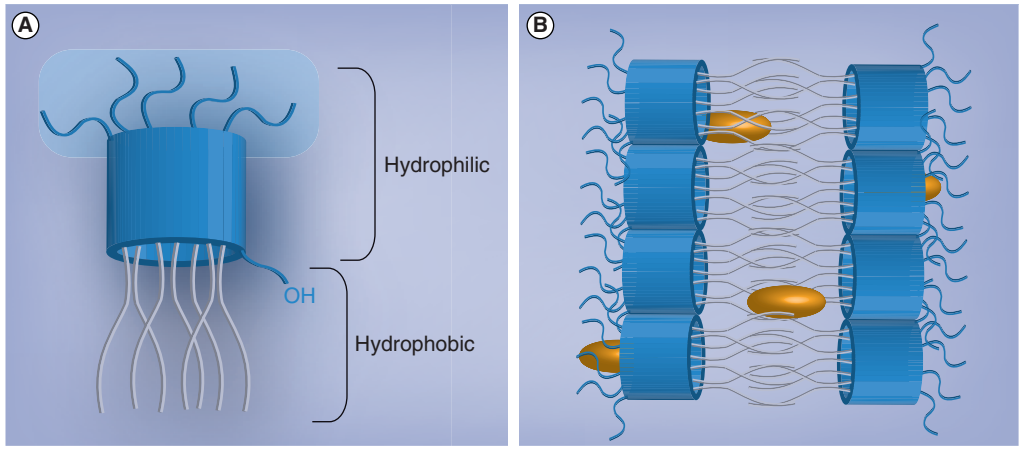

Figure 2. Synthesis of novel amphilic charged and uncharged cyclodextrins vesicles. These constructs have hydrophilic and hydrophobic groups on the alternative surfaces of the cyclodextrins. This allows lipid based cargoes to be concentrated in the oily phase, while siRNA-type cargoes are entrapped in the cyclodextrins cavity or close to the hydrophilic region.

described, in which a range of the mCDs successfully knocked down reporter gene expression as well as, if not better than, gold standard nonviral vectors including Lipofectamine 2000. Impressive knock-down data was achieved in neuronal primary cultures and also in fibroblasts from Huntington's disease subjects. *In vivo* data comprised intra-tumoral targeting for anti-VEGF siRNA in mice; the mCD data in reducing tumour volume was equal to that achieved with polyethylenimine. The IDDN is also currently setting up a recognized genetic mouse Huntington's disease model (R6/2) and a range of behavioural and motor studies are being validated at University College Cork. Future studies to demonstrate preclinical proof-of-concept will include intra-cerebral regional delivery of mCDs entrapping siRNA for huntingtin protein.

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