Innovation in formulation: Taking inspiration from physiology to restore pathology

The demand met by formulation scientists to design cutting edge drug delivery systems (DDS) has never been greater. The patient's responsibility to take multiple dosage forms throughout the day has been historically delegated to scientists to inherently build into a formulation. In the simplest context, this is achieved by providing a prolonged-release tablet which can be taken once a day rather than multiple immediate-release tablets daily. Problematic drugs which don't reach the target site at a sufficient concentration are handed to formulators to effectively "deliver". With increasing rates of cancer, there is an unmet supply of therapies that target the tumour without causing undue damage to other cells and tissues in the body. In response to the increased demand for targeted delivery and increased patient compliance, formulators must devise innovative strategies to revolutionize drug delivery today. Taking inspiration from the patient's physiology and thereby designing biologically inspired carrier systems is an innovative blueprint drawn up by formulation scientists to try to keep up with the relentless nature of human disease.

It is important that innovative therapies possess a degree of novelty but also realistic potential to clinically translate. Formulators can nowadays fine-tune delivery of therapeutics. This has been made possible by stimuli-responsive DDS coming to fruition. Regulated drug release at the pathological site has made possible advancements in treatment of local diseases. With stimuli-responsive polymeric systems, the physician can decide precisely when and what dose of drug should be released to a specific site. These stimuli-responsive systems also enable external control of dosing to establish optimum pharmacokinetics in cases where a narrow concentration of active is necessary to enhance therapeutic outcome.

At the basis of such responsive systems is the interplay of materials science with other sciences such as physics of sound or click chemistry. High intensity ultrasound provides a physical stimulus which can allow a drug delivery system to penetrate deep into human tissue thereby allowing control over release even deep within a patient. Mechanistically, ultrasound provides the shear force to physically induce rupture of polymer strands or alternatively, free radicals from local heating allow free-radical-mediated breaking of polymer strands [1]. It is unclear which mechanism is the predominant factor behind depot degradation. Electric-stimulated drug delivery is based on the physicochemical basis of the drug and ability of hydrogels to efficiently transport electrical ions in response to an electric field. Such responsive drug delivery is an example of the successful journey for innovative formulation principles to translate to the clinic.

It is always a mission of formulation scientists to design delivery systems that target disease from various points of view. One way to achieve this is by creating a delivery system with multiple inherent degrees of combatting a disease. Layer-by-layer (Lbl) assembly is a promising and innovative technique to incorporate various materials into a thin layer by means of sequential deposition of alternatively charged polyelectrolytes onto a colloidal template [2]. There is a plethora of advantages to such an innovative system, such as tuneable external layers with potential to become functionalised and interact with the physiological environment, implementation of active targeting moieties/approaches, a DDS with properties enabling loading of different types of "problem drugs" e.g. hydrophobic drugs. Through processes such as direct adsorption of charged biopolymers including proteins and incorporation of synthetic drug-loaded polymers, LbL have potential to incorporate therapeutics into the LbL film itself. LbL systems are highly sophisticated and require expert design. They can deliver genes and growth factors and can prove possible to be vaccines and deliver antigen and adjuvant via the externa.

Formulation scientists are seeking more inspiration from physiology to find carriers which deliver their cargo effectively while avoiding the immune system. One interesting example is the use of autologous red blood cell (RBC) ghosts. In fact, using RBCs as carriers can enhance pharmacokinetics, alter pharmacodynamics, and modulate immune responses. Berikkhanova et al. [3] investigated the wound healing potential of RBC ghosts loaded with IL-1ß and ceftriaxone. The authors showed a significant acceleration of the healing process in a group of animals when compared to those treated with local injection of free drugs or IM injection of the antibiotic in combination with topical application of ointment. The group confirmed that local application of immune-enhancing agents provided higher therapeutic concentrations of drugs at the site of infection, without associated toxic effects.

RBCs demonstrate clearly the plethora of advantages which biologically inspired materials may offer. Biocompatibility and abundance are attractive attributes which may be exploited in drug delivery. The natural mechanisms by which RBCs successfully deliver oxygen throughout the body enables drugs to be circulated for prolonged periods in the bloodstream, thereby facilitating redistribution in blood from plasma. This change to pharmacokinetics (PK) could in effect enhance drug bioavailability, leading inevitably to reduced doses and increased patient compliance.

Besides utilising autologous RBCs, efforts have been made to mimic RBCs to derive benefit from the inherent advantages associated with their use as carriers. A layer-by-layer (Lbl) particle templating of polyelectrolytes was used to create a synthetic RBC formulation composed of natural RBC proteins and synthetic polyelectrolytes created via LbL on hollow polystyrene spheres subjected to core dissolution [4]. While some challenges pose a barrier for clinical translation of RBC carrier utilisation such as dependence on blood typing, restriction to vascular space or inability to cross or penetrate physiological barriers, the potential exists for improved bioavailability of problem drugs, by utilising our own vascular weaponry to better deliver therapeutic agents.

Nucleic acid therapeutics such as siRNAs and miRNAs represent highly promising molecules for the treatment of a wide array of pathologies, since about 4000 diseases can be attributed to malfunctioning genes [5]. However, the predominant bottleneck in clinical translation of interfering RNA molecules is delivery of functional RNA molecules into the cell cytoplasm. One common method of stimulating drug delivery of nucleic acids is use of nucleic acids themselves as the stimuli [6]. Nucleic acid aptamers are single stranded DNA or RNA oligonucleotides that adopt conformational shapes to bind their targets. This specificity of recognition between interacting partners, along with potential to modify nucleic acids and eliminate the disadvantage of their poor stability, may represent an innovative solution to deliver life-saving nucleic acid therapeutics.

It is well established that carriers in the nano-size range are advantageous from a drug delivery perspective, owing to their evasion of the host immune system, targeting to the diseased site, among other features. A nanocarrier which resembles in structure an infectious pathogen such as Hepatitis B Virus would combine the plethora of advantages to carriers in the nano range with those of biomimetic approaches. Hepatitis B Virus is a double-stranded DNA virus which infects patients, causing hepatitis B. While there are treatments which dampen the virus from taking complete ownership of the patient's immune system when chronically infected, including antiviral therapy such as tenofovir, lamivudine, no treatment to date can clear the viral infection. The discovery and molecular study of the HBV virion to date has led to an evolution in understanding of the structure-function relationship of the virus. Somiya et. al [7] took to develop a virus-mimicking nanocarrier dubbed the "bio-nanocapsule" (BNC), taking direct inspiration from the structure of the Hepatitis B Virus, by overexpressing the L protein in yeast cells. The group successfully developed a BNC which targets human hepatocytes, and is functionalised with fusogenic, stealth, self-organising activities and is modified to deliver payload to the cytoplasm. In addition to directly mimicking the Hepatitis B Virus,

efforts have been made to fabricate artificial nanocarriers utilising the functional domains of the virus. Titanium dioxide nanoparticles modified with pre-S1 peptides have been shown to accumulate specifically to human hepatic cells in vivo. Coupled with the previously mentioned ultrasound form of stimulus for delivery, such NPs can generate radicals and consequently displayed cytotoxic effects when intratumorally injected into xenograft mice following ultrasound irradiation.

Biomimicry also proves a potential solution to modelling the most complex disease states which to date do not have successful treatments. Muscle fibrosis represents the end stage consequence of muscular inflammation associated with diseases such as muscular dystrophies, and results in profound changes in the architecture of muscle tissue and its properties such as stiffness and density [8]. The skeletal muscle is a dynamic tissue which responds to stimuli and injuries with a well-coordinated response to restore the tissue environment, involving a cascade with cells, growth factors. Muscle fibrosis features an increased deposition of extracellular matrix and collagens in connective tissue. This serves as a physical barrier for delivery of therapeutics and may represent an additional challenge to formulators, as the microenvironment is consequently highly hydrophobic. It is therefore necessary to design an appropriate model which considers this highly complex environment.

While it is difficult to replicate the main characteristics of the fibrotic environment, many efforts have been made to develop 3D cell cultures which allow cells to organise into complex structures and closely resemble their in-vivo counterparts, much more so than traditional 2D assays.

It has been demonstrated through multiple studies that contractile muscle fibres can be successfully generated for predictive toxicology studies. the main drawback of such a system is that muscle cells are cultured alone and lack the ability to reproduce key properties of the overall muscle environment, pertinent to studying dystrophies and fibrosis. This could be upgraded by introducing fibroblasts during muscle fibre maturation and by treating with pro-fibrotic TGF-ß1. Current in vitro models have not reached a sufficient quality standard to provide adequate drug delivery data, biomimicry may strengthen the quality of such models.

The applications of biomimicry not only encompass enhanced delivery of therapeutics and modelling complex diseases, biomimetics may also extend to diagnostic potential. Circulating tumour cells (CTCs) have been implicated as a biomarker for diagnosis and prognosis of multiple cancer types. However, the rare and heterogenous nature of CTCs render them a tremendous challenge to detect and isolate effectively. Myung et al. [9] demonstrated improved CTC detection by coupling biomimicry and nanotechnology. The group envisaged mimicking the concurrent cell rolling process, which occurs after CTC and endothelium initially surface bind with fast association/dissociation kinetics, using their biofunctionalized surface with E-selectin and aEpCAM. To increase firm adhesion kinetics, dendrimers of poly(amidoamine), PAMAM, were used to immobilise aEpCAM to mediate a strong multivalent binding effect. PAMAM dendrimers were selected as a reportedly excellent mediator for multivalent binding effect due to pre-orientation of ligands, polymer backbone topology and easy deformability of the macromolecules. The dendrimers are hyperbranched, flexible macromolecules with high numbers of peripheral functional groups thus enabling multifunctionalisation. The group finally engineered both E-selectin mediated cell rolling and dendrimer-mediated multivalent binding onto a single platform via micropatterning. The two biomimetic approaches synergistically enhanced surface capture and the engineered surface exhibited enhancement in capture efficiency by up to 7-fold, compared to surfaces with CTC-specific antibodies only. This innovative approach enhanced detection sensitivity, selectivity and purity of CTC capture compared to control surface (solely antibodies).

Biomaterials provide a promising strategy to mimic the natural extracellular matrix (ECM) to encourage pro-regenerative immune cell phenotypes and consequently enable tissue remodelling.

The optimal physical and chemical configurations of new biomaterials as they interact with living cells to produce tissue-engineered constructs are under study by many research groups. The host immune response to “foreign” implants can be targeted with “therapeutic-eluting” strategies. Implants with surface coatings or embedded particles to promote surrounding cells to secrete anti-inflammatory agents is a promising formulation approach to improve acceptability of biomaterials intended to replace functional tissue.

In one study [10], a cell-free bone tissue engineering system based on a silk fibroin (SF)/nano-hydroxyapatite (nHAp) scaffold was developed, in which two bioactive molecules, stromal cell derived factor-1 (SDF-1) and bone morphogenetic protein-2 (BMP-2), were embedded and released in a sequential and controlled manner to facilitate cell recruitment and bone formation, respectively.

The study also exemplified the advantage of sequential release of actives in the recapitulation of a spatiotemporal process such as bone tissue healing. The group hypothesized that compared to the concomitant release from scaffold with physically adsorbed actives, the sequential release of these two bioactive molecules from scaffold with physically adsorbed actives encapsulated into SF microspheres are beneficial for the promotion of bone regeneration. This is because the initial burst release of SDF-1 promotes mesenchymal cells (MSC) homing to the graft areas in the early time, whereas the sustained release of BMP-2 maintains an effective concentration of BMP-2 to promote the transformation of MSCs into osteoblasts. The group used the now standard microsphere approach to deliver high doses of BMP-2. Silk fibroin (SF) proteins are promising as they occur naturally as fibres which help to enable slow and sustained drug delivery, along with their mechanical hardness, processing malleability, self-assembly, biocompatibility [11].

The limited efficiency of conventional drugs has instigated the development of new and more effective drug delivery systems (DDS). This essay has highlighted that smart, stimuli-responsive polymeric delivery systems may potentiate deeper delivery of therapeutic than traditionally. Lbl is an innovative technique to target diseases from different perspectives based on a multitude of active agents and cues all in one film. Biomimetic approaches are increasingly built into formulations to fixate their targeting to the certain physiological site. Biomaterials render the DDS biocompatible and allow the system itself to ameliorate healing rather than to act solely as a vessel to elute a therapeutic agent. It seems that the future of innovation in formulation is largely focused on synergising two fields: human physiology and advanced technologies.

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