Developing Improved Nucleic Acid Delivery Vehicles

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In this presentation I will be talking about the development of improved lipid-based systems for the cellular delivery of nucleic acid. To achieve cellular delivery, viral-vector and non-viral based systems have been developed with the view to protecting the nucleic acid they carry from degradation, maximise delivery to on-target and minimize exposure to off-target cells. Although viral-based therapies had more initial success, it is now known that their effectiveness can be limited by pre-existing immunity, induced immunogenicity, payload size, inability to re-dose, difficulty in up-scaling, expensive vector production. These limitations have fuelled research into non-viral alternatives such that a number of lipid-based systems, including the intramuscularly administered lipid nanoparticle (LNP)-based mRNA COVID vaccine, that have been approved by the regulatory authorities.

Lipopolyplexes, consist of a core of nucleic acid complexed polymer, surrounded by a lipid bilayer composed of cationic and neutral helper lipid. Lipopolyplexes combine the advantages of lipoplexes and polyplexes, producing LNPs with better colloidal stability, decreased cytotoxicity and enhanced nucleic acid delivery. This presentation will detail work in our group whereby we have further improved LNP stability and the effectiveness of nucleic acid delivery by formulating lipopolyplexes with multi lipid bilayers as opposed to the usual single bilayer. The role of small angle neutron scattering in developing these improved LNPs will be described.

To deliver their nucleic acid payload to their target cells, the LNPs have to be internalised, after which they are generally taken up and trafficked via the endosome. It is essential, however that the LNPs (or their nucleic acid payload) escape from the endosome into the cells cytoplasm before it transforms into the lysosomes with its highly acidic microenvironment and high level of enzymes which degrade the nucleic acid. To study the release mechanisms of LNPs from the endosome we have prepared monolayers of the endosomal lipid on a Langmuir trough and using a combination of ellipsometry, Brewster angle microscopy and neutron reflection probed the interaction of LNPs prepared from ionisable cationic lipid, phospholipid, cholesterol, PEGylated lipid and RNA with the model endosomal membrane as a function of pH. These studies have been invaluable in enabling an understanding of the effect of pH on the interaction of the LNP and its constituent components with the model endosomal membrane, thereby enhancing our ability to design more efficient nucleic acid delivery.