Novel engineered supramolecular enzyme induced self-assembled peptide (EISA) gels for sustained release of a corticosteroid targeting anterior segment inflammation in the eyes.

Shubhamkumar M Baviskar, Sreekanth Pentlavalli, Garry Laverty, Raghu Raj Singh Thakur.

[1] School of Pharmacy, Queen's University Belfast, BT9 7BL, Northern Ireland, UK

Background: Ocular drug delivery is a challenging task with the existence of its complicated physiological and anatomical barrier. Post-cataract inflammation is more common in patients having a past of inflammatory diseases. Current treatment includes topical eye drops to control inflammation with 5% drug targeting to the affected site. As self-assembled peptide sequence (SAP) hydrogels have intrinsic antibacterial activity, biocompatibility, and tunable biodegradability, EISA of SAP has been widely investigated over the last decade. Sub-conjunctival injections of the corticosteroid Dexamethasone (DEX), which has a low molecular weight and can penetrate biological membranes, were used to reduce inflammation in this study. In this work, the L and D enantiomers of the EISA peptide sequence were synthesised. While L enantiomers are obtained from nature, D enantiomers are synthesised chemically and are therefore more resistant to proteolysis, making them more biocompatible and bioavailable than L amino acids. This sequence forms a hydrogel in presence of enzyme. As, the enzyme cleaves the phosphate (dephosphorylation) from the tyrosine which enables hydrogelation. This work illustrates the need to develop EISA hydrogels, which can achieve sustained release over longer period and are resistant towards the enzymes present in the eye for greater bioavailability and stability.

Methods: L and D enantiomers of SAP sequence were synthesized *via* the solid phase peptide synthesis method. The sequence consisted of Naphthalene, phenylalanine, lysine, tyrosine phosphate and the drug was modified to DEX-NHS-ester and attached to the peptide sequence. The NMR, ESI-MS, FTIR and CD confirmed chemical structure and mass. Peptide hydrogelation was studied *via* an inversion assay, oscillatory rheology, SEM and TEM imaging. *In vitro* studies were conducted to study release in phosphate buffer (PBS) and proteinase K (PK) induced PBS. Degradation was studied in PK and injectability studies were also carried out. Scleral permeability was studied *via* an ex-vivo Franz cell model.

Results: The inversion assay and minimum gelation concentration demonstrated that SAP forms gels at a minimal conc. of 0.5% w/v and higher, which was confirmed for its mechanical properties by oscillatory rheology studies. *In vitro* release studies demonstrated that the D enantiomer release drug was more sustained compared to L enantiomer sequence. *In vitro* degradation kinetics studies in the presence of PK enzyme demonstrated that the D enantiomers show greater resistance to proteolysis than the L enantiomers. *Ex vivo,* scleral drug permeability demonstrated scleral permeation in the D enantiomer and in the L enantiomer peptide sequence formulations. Further, formulations were found to be biocompatible as cell viability was more in ARPE-19 cell lines.

Conclusions: All peptide sequences were synthesized in-house using solid phase peptide synthesis, which resulted in a 95% yield and 99.9% purity of the peptide sequence. L enantiomers undergo faster proteolysis resulting in low bioavailability compared to D enantiomer peptides. D enantiomer peptides have proved to be a potential motif for sustained ocular drug delivery application to control inflammation using EISA hydrogelation.