Cell Penetrating Peptides: Penetrating the Impenetrable

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The intracellular delivery of bioactive agents and subsequent therapeutic manipulation of sperm physiology poses a significant challenge. The sperm plasma membrane presents a static physical barrier and mechanisms such as endocytosis, which facilitate the movement of macromolecules into the majority of eukaryotic cells, are severely restricted in mature spermatozoa.[1] This limitation is further compounded by the fact that at the time mammalian sperm are released into the seminiferous tubules, the processes of transcription and translation have largely been silenced thus limiting therapeutic targets to protein-protein interactions and post-translational modifications.

We have previously established that cell penetrating peptides (CPPs) rapidly and efficiently enter bovine and human spermatozoa whilst being compatible with sperm physiology. Moreover, a range of structurally diverse CPPs demonstrate a propensity to accumulate in different sperm compartments, thus propounding their utility for the delivery of bioactive cargoes to specific intracellular *loci*.[2,3]

The development and application of bioportides, bioactive cell permeable peptides that mainly act by a dominant-negative mode of action, is a unique approach to the regulation of sperm physiology. Identified by QSAR prediction algorithms designed to recognize CPPs within entire proteins or sites of protein-protein interactions, bioportides possess the dual features of cellular penetration and biological activity and are therefore distinct from the more commonly used inert vectors such as tat and penetratin.[4] As an initial proof of concept we utilized bioportide technology to modulate Ca^{2+} signalling in human spermatozoa, which governs fundamental processes such as hyperactivation, capacitation and the acrosome reaction. Synthesis and evaluation of $STIM^{371-392}$, a candidate bioportide corresponding to the cationic region the STIM1-Orai1-activating domain of STIM1, an endoplasmic reticulum calcium sensor and regulator of $[Ca^{2+}]i$, sustained progesterone-induced $[Ca^{2+}]i$ transients in human spermatozoa.[3]

Our recent endeavours have focused on bioportides that control male fertility. Sperm motility, a prognostic parameter of human fertilization capacity, is regulated by the activity of a male gamete-specific isoform of phosphoprotein phosphatase 1 (PPP1), phosphoprotein phosphatase 1 catalytic subunit gamma 2 ($PP1\gamma2$) during epididymal transit. Bioportides designed to target the $PP1\gamma2$ interactome, discretely and rapidly inhibit human sperm motility and have proven unmatched potential for validation as a non-hormonal male contraceptive.[5] In conjunction with Margarida Fardilha at the University of Aviero Portugal, our strategy has been to selectively target post-testicular events with these peptide therapeutics and in doing so, avoid the many side effects associated with hormonal-based male contraception. Our current objective is to further develop these bioportides towards clinical utility as a potential contraceptive option.

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2. Jones S, *et al*. *Hum. Reprod.* **2013**, *25*, 1874-1889.
3. Morris J, *et al*. *Mol. Hum. Reprod.* **2015**, *21*, 563-570
4. Howl J, Jones S. *Expert Rev. Mol. Med.* **2015**, *17*, e1 doi.org/10.1017/erm.2014.24
5. PPP1CC2 interactome-derived bioportide technologies for the control of sperm motility and male fertility. UK Patent Application (No. 1711620.3) filed 19th July 2017.