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Effects of mesoporous silica nanoparticles upon the function of mammalian sperm *in vitro*

Natalia Barkalina, MD, MSc[a], Celine Jones[a], Junaid Kashir, PhD[a], Siobhan Coote, MBiochem[a], Xinyue Huang, MEng[b], Rachel Morrison, MEng[b], Helen Townley, PhD[b], Kevin Coward, PhD[a,*]

[a]*Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Level 3, Women's Centre, John Radcliffe Hospital, Headington, Oxford, UK*
[b]*Department of Engineering Science, University of Oxford, Parks Road, Oxford, UK*

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Abstract

Nanomaterial-mediated delivery represents a promising technique for reproductive biology with a potential to improve the safety and efficacy of existing methodologies, including experimental gene therapy and sperm-mediated gene transfer. Mesoporous silica nanoparticles (MSNPs) have been characterised as a powerful and safe delivery tool, rendering them an excellent candidate for use in reproductive research. However, their effects upon mammalian gametes with highly specialised structure and functionality remain untested. Here, we show for the first time, that spherical MSNPs with hexagonal pore symmetry, functionalised with polyethileneimine and aminopropyltriethoxysilane, and optionally loaded with two common types of cargo (nucleic acid/protein), form strong associations with boar sperm following incubation *in vitro* and do not exert negative effect upon the main parameters of sperm function, including motility, viability, acrosomal status and DNA fragmentation index. Our findings provide a rationale for the use of MSNPs for the transfer of investigative, diagnostic and/or therapeutic compounds into mammalian sperm.

Key words: Nanoparticles; Mesoporous silica; Sperm; Toxicity; Delivery

Recent progress in nanoscience has transformed the concept of targeted biological delivery, allowing the engineering of complex biocompatible organo-inorganic platforms with enormous loading capacity, stability, highly selective affinity, and potential for multiple, simultaneous applications; all within the nanometre scale.[1,2] The key benefits of these engineered nanocarriers include protection and controlled release of payloads, reduction of systemic toxicity, and the promotion of non-invasive internalisation into target cells via endocytotic pathways.[1,3]

Nanomaterial-mediated delivery represents a highly promising technique in reproductive biology. The specialised structure and functional role of reproductive tissues and gametes require the use of minimally-invasive research tools that do not interfere with fertility or affect the development in resulting offspring.

Nanotechnology can improve the biological safety and efficacy of many existing experimental methodologies.[2,4] In particular, the versatility of nanocarriers greatly facilitates their use as delivery systems for most types of biological compounds, including small molecules, peptides, proteins and nucleic acids (DNA/siRNA). One of the most anticipated applications of nanomaterials in reproductive science is gene transfer. Research into mechanisms of infertility, a medical condition affecting nearly 48.5 million couples worldwide,[5] is increasingly showing the role of aberrant gene expression and genetic polymorphisms in various forms of previously unexplained reproductive failure, including certain types of testicular insufficiency,[6] failure of

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*Corresponding author at: Nuffield Department of Obstetrics and Gynaecology, Level 3, Women's Centre, John Radcliffe Hospital, Headington, Oxford, United Kingdom.

E-mail address: kevin.coward@obs-gyn.ox.ac.uk (K. Coward).

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fertilisation,[7] ovarian dysfunction,[8] and recurrent pregnancy loss.[9] Therefore, targeted gene transfer can evolve into a powerful research tool to study the fine pathophysiological mechanisms underlying infertility, but also a potential therapeutic strategy. Spontaneous internalisation of nanovectors into target cells is associated with a number of benefits, compared to conventional electro- and viral transfer. Nanomaterials combine the main advantages of viral vectors, particularly high specificity and non-invasiveness of delivery, with the key benefit of electroporation: a total avoidance of viral integration into the host genome. Several studies show that testicular and ovarian gene transfer using viral vectors can restore production of functional gametes in mouse models of genetic gonadal failure; however data regarding safety of this methodology remain highly contradictory.[10-12] Therefore, the availability of a technique, which is devoid of the biological risks associated with traditional gene therapy, could support research into reproductive gene transfer as a possible treatment for specific forms of infertility in future.[13]

Another promising application of nanomaterials in reproductive biology is enhancement of the efficacy of sperm-mediated gene transfer (SMGT). During SMGT, the sperm spontaneously incorporates exogenous DNA and acts as a 'natural transfection vector', delivering the construct into the oocyte at the time of *in vitro* fertilisation (IVF).[14,15] SMGT is often applied in the breeding of transgenic animal models for human diseases and xenotransplantation studies.[16] In many species, SMGT is more cost-effective than conventional genetic modification through complex micromanipulation procedures.[16,17] However, the overall reproducibility of the technique remains suboptimal, with large variations in the rates of exogenous DNA uptake by sperm and transgene expression in the offspring.[18] Several studies show that the use of nanomaterials to carry exogenous DNA increases the efficacy of SMGT in livestock production,[19-21] although the range of tested nanomaterials and animal models remains very limited. Following the same principle, nanocarriers could be used to facilitate the loading of sperm with alternative molecular compounds (peptides, proteins, fluorescent markers, or, in broader sense, any agents designed to inhibit, augment, or detect endogenous functional activity) for delivery into the oocyte and targeting of physiological pathways in early-stage embryos for investigative purposes.

Mesoporous silica is a special class of synthetically modified colloidal silica with highly ordered pores in the scale between 2 and 50 nm.[22] Favourable biomedical properties of mesoporous silica include chemical inertness, ease of production, robustness, adjustable surface chemistry and unique porous architecture.[23] This architecture increases the effective surface area, and allows compartmentalisation of different types of cargo on one nanocarrier through binding on the surface and inside the pores. The surface of mesoporous silica can be modified with functional groups or coatings for covalent or non-covalent linking of cargo, and improvement of internalisation into target cells.[24] Mesoporous silica nanoparticles (MSNPs) have been well-characterised as powerful tools for targeted drug and gene delivery, bioimaging, and tissue engineering, and a solid body of evidence supporting low cytotoxicity of MSNPs in most cell types has been accumulated.[24-26] These features make MSNPs an excellent candidate for use in reproductive research, including delivery into gametes and early-stage embryos.

Although MSNPs represent a robust, versatile, and non-toxic delivery system in somatic cells, their effects upon mammalian gametes with highly specialised structure and functionality remain largely untested. In this pilot study, we show that spherical MSNPs with hexagonal pore symmetry, functionalised with Polyethileneimine (PEI) and aminopropyltriethoxysilane (APTES) and optionally loaded with two common types of cargo (nucleic acid or protein), form strong associations with boar sperm following incubation *in vitro* and do not exert negative effect upon the main parameters of sperm function. Our findings, therefore, provide a rationale for the use of MSNPs as a platform for the transfer of investigative/diagnostic/therapeutic compounds into mammalian sperm.

Methods

A summary of types and surface modifications of MSNPs, and types of cargo used in this study is presented in Table S1 (see the Supplementary Data).

Synthesis and functionalisation of MSNPs

Synthesis of non-fluorescent MSNPs was performed in a surfactant-templated base-catalysed sol-gel reaction, as previously described by Hom et al (2010).[25] To synthesise fluorescent MSNPs, fluorescein isothiocyanate (FITC) was introduced into the silica framework in the form of FITC-(3-aminopropyl)triethoxysilane (FITC-APTES) conjugate during the main reaction.

The surface of synthesised MSNPs was functionalised with Polyethileneimine (PEI, MW 1.3kD, Sigma-Aldrich, UK) or APTES (Sigma-Aldrich, UK). Coating of MSNPs with PEI aimed to reduce particle agglomeration, improve interaction with cells and provide positively charged surface for electrostatic binding with lamin A/C siRNA (siGLO Lamin A/C Control siRNA (human/mouse/rat), D-001620-01-05, Thermo Fisher Scientific, CO, USA).[26] Coating with APTES aimed to provide amine groups for covalent cross-linking with carboxyl groups of mCherry fluorescent protein.[27] For detailed description of the procedure and a list of specific reagents, please refer to the Supplementary Data.

Characterisation of MSNPs

Synthesised MSNPs were characterised using a combination of conventional analytical chemistry tools. Physical size, mean hydrodynamic size, and electrokinetic (ζ) potential of synthesised MSNPs were evaluated using transmission and scanning electron microscopy (TEM and SEM), disc centrifugation, and electrophoretic light scattering, respectively. A detailed description of the procedures is provided in the Supplementary Data.

Loading of MSNPs with cargo

Non-fluorescent PEI- and APTES-coated MSNPs were loaded with fluorescent lamin A/C siRNA and mCherry protein, respectively. These two types of payloads were chosen as the 'prototypes' for the two classes of biological cargo, which represent a particular interest for delivery into mammalian