Theriogenology 85 (2016) 74–82

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Nanomedicine and mammalian sperm: Lessons from the porcine model

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ARTICLE INFO

Article history:
Received 7 April 2015
Received in revised form 5 May 2015
Accepted 20 May 2015

Keywords:
Nanotechnology
Nanomaterial
Drug delivery
Gamete
Assisted reproduction technology

ABSTRACT

Biomedical nanotechnology allows us to engineer versatile nanosized platforms that are comparable in size to biological molecules and intracellular organelles. These platforms can be loaded with large amounts of biological cargo, administered systemically and act at a distance, target specific cell populations, undergo intracellular internalization *via* endogenous uptake mechanisms, and act as contrast agents or release cargo for therapeutic purposes. Over recent years, nanomaterials have been increasingly viewed as favorable candidates for intragamete delivery. Particularly in the case of sperm, nanomaterial-based approaches have been shown to improve the efficacy of existing techniques such as sperm-mediated gene transfer, loading sperm with exogenous proteins, and tagging sperm for subsequent sex- or function-based sorting. In this short review, we provide an outline of the current state of nanotechnology for biomedical applications in reproductive biology and present highlights from a series of our studies evaluating the use of specialized silica nanoparticles in boar sperm as a potential delivery vehicle into mammalian gametes. The encouraging data obtained already from the porcine model in our laboratory have formed the basis for ethical approval of similar experiments in human sperm, thereby bringing us a step closer toward the potential use of this novel technology in the clinical environment.

1. Introduction

Reproductive science is an exciting and continuously developing field which aims to provide theoretical insight into the mechanisms of gametogenesis, fertilization, early embryo development, pregnancy establishment and to develop applied tools for the intentional manipulation of these processes to address fertility issues across different biological species. Assisted reproductive technology (ART) represents a collection of sophisticated laboratory techniques and approaches for the *in vitro* manipulation of gametes and embryos that were developed to enhance the chances of fertilization and development to full term, respectively. Today, ART is deemed essential for the production of livestock [1–3], the preservation of animal species [4], and also for the treatment of human infertility [5,6]. In addition, reproductive science investigates an array of fundamental biological processes underlying reproduction, including cell division and fusion, genetic recombination, maintenance of pluripotency, and cell migration and differentiation, which are very similar to the events involved in tumor biogenesis and tissue regeneration. Therefore, research into reproductive biology has an inherent translational component and permits advancement in our understanding of cell-based regenerative therapies for cardiovascular, renal, neurologic, and other diseases [7–9] and the fine mechanisms of cancer [10,11]. Over the past few decades, the field of reproductive science has expanded tremendously, now incorporating the principles of cell culture, micromanipulation, live cell microscopy, cryobiology, laser technology, proteomics, metabolomics, molecular genetics and biology. Such rapid

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0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.05.025

development and the continuous introduction of novel techniques into this biologically and ethically sensitive field [12] require thorough investigation of the potential risk/benefits ratio, including permanent effects on offspring. Such investigation traditionally includes a fundamental step which evaluates short- and long-term safety and efficacy in a variety of both vertebrate and invertebrate animal models [13].

In this short review, we provide a brief outline of the current state of nanotechnology for biomedical applications in reproductive biology and present highlights from a series of our studies evaluating the use of specialized silica nanoparticles in boar sperm as a potential delivery vehicle into mammalian gametes.

2. Nanotechnology as a versatile tool for targeted biological delivery

Nanotechnology is an innovative multifaceted discipline, combining the aspects of physics, chemistry, biology, mathematics, engineering and computer sciences, which designs and investigates the properties of small-scale (in general, 1–100 nm) structures for the variety of industrial, consumer, and scientific applications. In biomedicine, nanotechnology allows us to engineer versatile nanosized platforms that are comparable in size to biological molecules and intracellular organelles. These platforms can be loaded with large amounts of biological cargo, administered systemically and act at a distance, target specific cell populations, undergo intracellular internalization *via* endogenous uptake mechanisms, and act as contrast agents or release cargo for therapeutic purposes [14]. The ability to adjust the physical and surface chemical properties of nanomaterials, robustness, action at a distance, inherent "affinity" towards malignant cells in whole-body models, along with the potential for additional active targeting, has resulted in the rapid expansion of nanomaterial-based technologies for drug delivery [15]. To date, nanomaterials have been shown to facilitate selective delivery of a range of well-characterized and novel drugs into various target cells, including malignant lesions [16], immune cells [17], cells across the *in vivo* blood–tissue barriers, e.g., the blood–brain barrier [18], and resistant bacterial cells [19,20]. Nanomaterials reduce the systemic toxicity of pharmacologic agents because they deliver cargo precisely into the cell population of interest and allow the novel application of previously known, but rarely used, molecular compounds with poor bioavailability due to either rapid degradation or high nonselective uptake by other cells in the body. Such compounds include small molecules [21] and agents for gene-based therapies such as DNA, messenger RNA, microRNA, small interfering RNA, and antisense oligonucleotides [22]. Nanomaterials have also been applied as contrast agents for the targeted early-stage detection of pathologic lesions in whole-body models [23] and are capable of combining targeted detection and drug delivery capacity *via* the same platform, thus forming prototypes for highly adjustable personalized "nanotheranostic" agents [24]. In future, these agents could be engineered to the needs of the individual patient and could be administered systemically to target early-stage pathologic lesions, improve their visualization during diagnostic imaging, and, at the same time, deliver therapeutic agents into affected cells under real-time control.

3. Nanotechnology: Potential uses for ART

The revolutionary benefits of nanotechnology, especially from the perspective of improved intracellular delivery, have resulted in a growing number of studies applying the principles of nanomaterial-mediated delivery to help internalize molecular cargo into gametes (reviewed in [14,25]). Indeed, studies of gamete structure and physiology across a variety of animal species can be compromised by the relatively poor physiological uptake capacity of mature sperm [26,27] and oocytes [28] under *in vitro* conditions, which restricts the delivery of molecular probes into these cells unless aggressive membrane-permeating and fixation agents are applied [29–31]. Such techniques, interfering not only with the developmental capacity of gametes but also with their viability, can severely restrict functional studies and downstream applications of treated gametes. Therefore, over recent years, nanomaterials have been increasingly viewed as favourable candidates for intragamete delivery, particularly in the case of sperm. Such approaches have improved the efficacy of existing techniques such as sperm-mediated gene transfer (SMGT) [32–34], loading sperm with exogenous proteins while preserving their viability [35], and tagging sperm for subsequent sorting based on sex chromosomes [36] or functional status [37]. Apart from the intracellular delivery, nanomaterials have also been used to tag preimplantation embryos [38], promote embryonic growth and development [39], and assist in the bioimaging of sperm [40]. All such studies have been carried out in animal models, including the boar, bull, mouse, and chicken.

4. Livestock sperm as a model for nanotechnology studies

Interestingly, the majority of nanomaterial-mediated delivery studies in sperm have used bull and boar sperm—a phenomenon with a rather simple explanation. As discussed previously, the use of ART, specifically artificial insemination, for breeding livestock is continuously expanding [1,2,4], and the potential of novel "accessory" techniques, including superovulation, sperm separation, *in vitro* handling and fertilization of oocytes, embryo transfer, cloning *via* embryo splitting, reproductive cloning *via* nuclear transfer, and genetic selection (reviewed in [41,42]), to improve the efficacy of ART is being continuously investigated.

The increased demand for animals with carefully selected and/or engineered genetic traits, both for livestock breeding and research purposes, and the inconsistent success rates of SMGT [43–46], which, otherwise, represents a straightforward and inexpensive alternative to micromanipulation techniques, has led to the clear need for increased sperm "transfection" efficacy. For example, in the case of the porcine model, the introduction of desired genetic traits into embryos *via* the microinjection of genetic constructs into preimplantation embryos could be