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Review Article

The Peep of Nanotechnology in Reproductive Medicine: A Mini-review

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A B S T R A C T

Nanotechnology has opened a new field in medicine as well as in other sciences. The aim of this study was to seek the capability of nanotechnology for the treatment of various reproductive diseases. In this study, we analyzed all articles about "nanotechnology and reproductive medicine" published in 2000-2015, indexed in Google Scholar, PubMed and Science Direct. This study indicated that nanotechnology has been extensively used for different reproductive applications, e.g. disease detection, drug delivery, diagnostic imaging, etc. particularly in cancer diagnosis and treatment. The available evidences regarding the use of nanomaterials as experimental tools for the detection and treatment of reproductive diseases are summarized here. Nanoparticles have potential and promising applications in reproductive biology. Treatment and imaging of reproductive system-related cancers can be performed by engineered nanoparticles. Also, some non-cancerous diseases can be treated by nanotechnology, e.g. endometriosis. The benefits and concerns associated with their use in a highly delicate system of reproductive tissues and gametes have been investigated. Nano-based methods are innovative and potentially controversial approaches in the clinical settings, and give us the opportunity for better understanding of mechanisms underlying reproductive diseases.

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Introduction

Diagnostic and therapeutic approaches based on nanomaterials have been investigated for a number of reproductive applications. Most of the articles report the use of nanoparticles in the detection and therapy of reproductive cancers. However, a growing number of studies assess the use of nanotechnology in the diagnosis and treatment of non-cancerous diseases, including endometriosis [1, 2], uterine fibroids [3], ectopic pregnancy and trophoblastic diseases [4], drug delivery systems [5-8], and testis artificial scaffolds [9]. Other articles focus on the use of nanoparticles as research tools in reproductive biology for gene expression in offspring [10, 11], molecular pathways in gametes and early-stage embryos, or selection of specific gametes [12]. Although there are some valuable articles showing the application of nanotechnology in reproductive medicine, there is no comprehensive review article to show all of them. In this study, we analyzed all articles about "nanotechnology and reproductive medicine" published in 2000-2015, indexed in Google Scholar, Pub Med or Science Direct.

Applications of nanotechnology in reproductive medicine

Detection, imaging and treatment of cancer

Reproductive cancers are commonly diagnosed malignancies in the majority of geographic regions of the world. In the case of developed countries, prostate cancer represents the most important malignancy in males and one of the most common causes of cancer-related deaths [13, 14]. Based on global data, reproductive cancer, e.g. ovarian, uterine, and cervical malignancies remain among the twenty most prevalent lethal diseases in the world [14]. Early diagnosis of cancer is crucial for adequate treatment success and acceptable survival rates. However, as mentioned previously, many current screening approaches particularly those that are biomarker-based fail to yield accurate, reproducible and, most importantly, quick and cost-effective results. These concerns arise as a result of the sub-optimal sensitivity and specificity of commonly applied biomarkers, as well as sophisticated design strategies and the high costs of common laboratory immuno-analyzers. The use of nano-biosensors which rapidly and directly transform the large number of simultaneous biological events (binding and/or reactions) into electronic signals without additional labelling steps has been proven advantageous in various fields of bio-detection including the identification of antigens, proteins, nucleic acids, reactive oxygen and nitrogen species [15]. Recently, this approach has successfully been attempted in reproductive oncology. Today, nanoparticle (NP)-based biosensors are being widely introduced for the detection of well-characterized and novel cancer biomarkers including cancer antigen 125 (CA-125), human epididymis secretory protein 4 for ovarian cancer and prostate-specific antigen (PSA) for prostate cancer [16]. Current evidence universally supports the advantages

of these label-free, straightforward, quick, portable, and cost-effective detection techniques which can be used directly at the 'point of care', in preference to conventional immunoassays [17]. Diagnostic imaging techniques including CT, MRI and ultrasound scanning form the first-line approaches for the visualization of cancer which aim to establish the location of primary tumors along with the detection of regional and systemic metastases in order to stage the disease. In this field, nanomaterials are being increasingly considered as the next generation of image enhancers. Their unique optical properties, along with the potential for active targeting towards malignant cells and improved detection limits, result in major benefits compared with previous contrast agents. A number of reproductive uses of nanoparticles for diagnostic and therapeutic aims are introduced in Table 1.

The use of nanomaterial-based formulations, for instance super-paramagnetic iron oxides and aptamer-conjugated gold NPs, has been extensively described in the treatment of prostate cancer patients, particularly for detection of small tumors and lymph node staging, both of which represent a significant challenge for standard imaging approaches [18, 19].

Table 1. Some applications of nanotechnology in reproductive medicine

Reproductive oncology	Non-cancer conditions
Cancer detection: • Nanobiosensors for cancer biomarkers • Contrast agents for clinical diagnostic imaging	• Endometriosis ○ Contrast agents for MRI ○ Targeted delivery of experimental treatment agents, including gene therapy
Cancer treatment: • Targeted delivery for improved efficacy and decreased toxicity • Combined therapy: simultaneous delivery of therapeutic payloads • Reversal of resistance to chemotherapy • Nanosensitisation: potentiation of anti-tumor effect of chemotherapy by simultaneous exposure of cancer cells to nanomaterials • Effective delivery of drugs with poor biodistribution profile • Experimental gene therapy	• Uterine fibroids ○ Nanosensitisation during minimally-invasive surgery ○ Targeted delivery of experimental treatment agents • Ectopic pregnancy and trophoblastic diseases ○ Targeted delivery of chemotherapy drugs • Drug delivery systems ○ Topical ○ Transdermal ○ Transplacental ○ Intravaginal • Reproductive infections ○ Detection ○ Targeted treatment ○ Prevention: microbicides

More recently, a similar technique has been developed and tested for the imaging of ovarian cancer [20]. Over the last few years, NP-guided imaging of prostate cancer has progressed into the concept of image-guided therapy, which utilizes nanomaterials with contrasting properties additionally loaded with a chemotherapeutic agent, for precise and traceable drug delivery and release [21]. Nanomaterial-mediated delivery of chemotherapeutics permits marked improvements in their efficiency, and also helps reduce systemic toxicity. Many studies have demonstrated the potential of various

types of NPs such as derivatives of poly-lactic acid and poly lactic-coglycolic acid (PLGA), bovine serum albumin, magnetic iron or gold functionalized with different targeting ligands such as follicle-stimulating hormone receptor-binding peptides, folates, and aptamers [22] to facilitate the delivery of chemotherapeutic agents into ovarian, endometrial and prostate cancer cells, which have significantly shown potentials for their anti-tumor effects compared with their respective free molecules.

The use of nanomaterials (Fig. 1) for intracellular delivery can reverse the resistance of tumors to chemotherapy via active targeting and the activation of alternative mechanisms of cellular uptake. Liang et al (2010) described the potential of metallo-fullerene NPs to overcome the resistance of human prostate cancer cell lines to cisplatin via the reactivation of dysfunctional endocytosis [23]. Similar effects have been reported for a specific formulation of cisplatin, encapsulated in F3 peptide-functionalized polyacrylamide (PAA) NPs, and targeted specifically to ovarian tumor vessels in an in vivo mouse model of human tumor vascularization. The use of this formulation resulted in strong anti-tumor activity, attributed to vascular necrosis, both in platinum-sensitive and in platinum-resistant cell lines [24].

In another study, Nair et al. (2011) reported that the encapsulation of letrozole, an aromatase inhibitor used to suppress oestrogen biosynthesis in post-menopausal patients with hormone-responsive breast cancer into hyaluronic acid-bound polymeric (PLGA-PEG) NPs restored drug sensitivity in letrozole-resistant cells in vitro and in vivo via the interaction of hyaluronic acid with CD44 receptors on the tumor cell surface [25]. Collectively, these findings demonstrate that nanomaterial-mediated delivery can effectively alter the pharmacokinetics and pharmacodynamics of common drugs for chemotherapy to overcome mechanisms of drug resistance. In addition, the efficiency of anti-tumor therapies can take effect by the simultaneous independent exposure of reproductive cancer cell lines to certain nanomaterials. The use of such targeted sensitizers would allow us to use milder regimens of chemotherapy or irradiation thereby improving the safety and tolerability of these procedures without compromising their anti-tumor efficacy. Nano-sensitizers, such as nano-particulate magnetic iron oxide, gold nano-shells and gold NPs have been widely applied for selective thermal ablation and radiotherapy in patients with prostate cancer since the mid-2000s [26]. More recently, Geng et al. (2011) [27] have reported that thio-glucose bound gold NPs (Glu-GNPs) exhibit a similar radio-sensitizing effect upon a human ovarian cancer cell line (SK-OV-3). The use of these NPs resulted in an approximately 30% increase in the inhibition of cell proliferation compared to the irradiation alone, primarily due to the elevation of reactive oxygen species (ROS) production. Similarly, Zhang et al. (2012) observed a sensitizing effect of carbon nanotubes to the chemotherapeutic agent paclitaxel in the human ovarian cancer cell line OVCAR3

mediated through the increase in apoptosis [28].

Nanomaterials allow the simultaneous targeted intracellular delivery of various types of payloads absorbed on one type of nano-carrier thus providing an effective means of combined therapy for cancer. In a study by Qi et al. [29], PLGA NPs were used to simultaneously deliver a pro-apoptotic human PNAS-4 (hPNAS-4) gene and cisplatin into mouse ovarian carcinoma cells. The authors reported significant anti-tumor activity associated with PLGA NPs loaded with hPNAS-4, mediated through the induction of apoptosis and the suppression of cell proliferation and angiogenesis. The anti-tumor property of hPNAS-4-carrying PLGA NPs further took effect by the simultaneous loading with cisplatin, thus highlighting the feasibility of using this combined approach for cancer treatment.

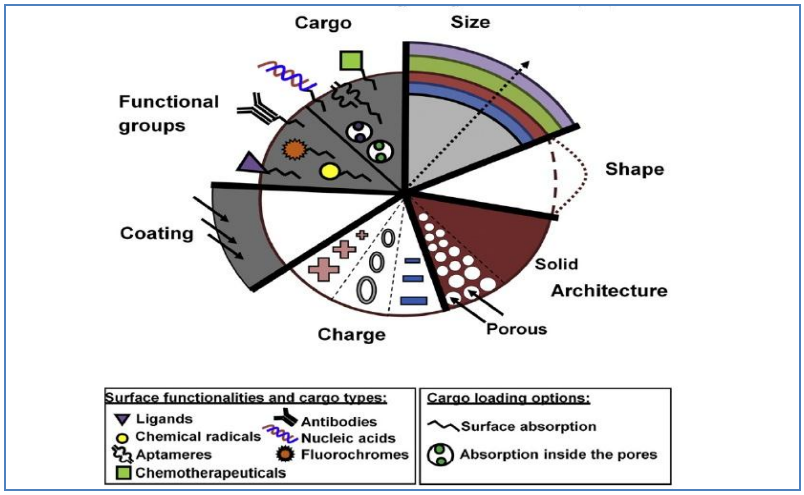

Fig. 1. The structure of nanomaterials, modified or coated with different ligands.

Nanomaterial-mediated delivery can also increase the efficacy of anti-cancer chemo-therapeutics and preventative agents, the previous use of which has been limited by poor bio-distribution and lack of specific affinity towards the malignant tissues [30]. Several studies have described the successful encapsulation of the chemo-preventive agent - epigallocatechin 3-gallate into PLA-PEG or polysaccharide NPs, and subsequent delivery of the formulation into prostate cancer cells [31, 32]. In a study by Long et al. (2013), a PEGylated liposomal formulation of quercetin, a flavonoid compound with anti-free radical and proposed anticancer activity, demonstrated significantly-improved ability to suppress tumor growth in in vitro and in vivo models of human ovarian cancer resistant to conventional treatment with cisplatin compared with the free quercetin [33]. Finally, nanomaterials are being increasingly investigated for targeted intracellular gene delivery in experimental gene therapy for cancer. The spontaneous uptake of nano-carriers by target cells is associated with a number of benefits compared with conventional electro- and viral transfer.

Nanomaterials combine the main advantages of viral vectors (such as selectivity, non-

invasive delivery, and stable expression of genetic constructs) with the main benefit of electroporation using plasmid DNAs (total avoidance of viral integration into the host genome). In reproductive oncology, the use of nano-vectors has been investigated for the experimental gene therapy of ovarian, cervical and prostate cancer, pre-cancer cervical lesions, and choriocarcinoma. Numerous publications describe the use of dendrimers, polymeric and lipid NPs for the delivery of suicide DNAs, tumor suppressor genes (p53), and short interfering RNAs (siRNAs) against genes expressed in malignant phenotypes (heat shock protein 27, androgen receptors, cofilin-1) into prostate cancer cells resulting in anti-tumour activity [34]. Several groups have utilized PLGA and chitosan NPs to transfer short hairpin ribonucleic acids (shRNAs) and siRNAs into ovarian cancer cells in order to interfere with the expression of target genes associated with cancer phenotypes and metastatic progression including focal adhesion kinase (FAK), CD44, claudine-3 and Jagged1. In all cases, target genes were successfully knocked-down resulting in a pronounced anti-tumour effect attributed to a reduction in cell proliferation and angiogenesis and a concomitant increase in apoptosis [35]. A similar approach was attempted by Yang et al. (2013) [36] who also used chitosan NPs to deliver siRNAs against the E6 and E7 onco-proteins of human papillomavirus into a cervical cancer cell line. Inactivation of these proteins which are currently held responsible for malignant epithelial transformation and preservation of the malignant phenotype resulted in significant induction of apoptosis in target cells. Another study described the synthesis of magnetic iron oxide (Fe_3O_4-dextran-anti-β-human chorionic gonadotropin (hCG) NPs and their successful use in chorio-carcinoma tissue in an in vivo mouse model of disease highlighting the potential use of this system as a nano-vector for gene therapy in trophoblastic diseases [37].

There is an increasing awareness that treatment approaches in reproductive medicine should be as fertility-sparing as possible since in the case of conditions with high recurrence rates, for example endometriosis and uterine fibroids, repeated surgeries can reduce the chances of spontaneous and assisted conception in the future, occasionally requiring third-party reproduction [38].

Currently, several experimental nanomaterial-based approaches have been investigated as a safer and less invasive alternative to standard diagnostic and therapeutic techniques for the management of several traditionally ‘surgical’ reproductive diseases.

Non-cancer applications

Endometriosis, a chronic gynaecological condition associated with the presence of endometrial-like tissue outside of the uterine cavity, affects nearly 2-22% of women of reproductive ages [39] and manifests with mild to severe pelvic pain and/or infertility. In several recent large-scale epidemiological studies, endometriosis has been proven to have a profound negative impact upon health-related quality of life and work productivity, exacerbated by an average delay in diagnosis in all healthcare levels of 7 to 10 years [40].

Early non-invasive diagnosis of this disease remains highly challenging due to the lack of sensitive serum biomarkers and limitations associated with the imaging techniques such as ultrasound and MRI in peritoneal endometriosis [41]. Therefore, for more than 50 years, the gold standard of detection of ectopic endometrial lesions has been laparoscopy [42], an invasive procedure with inherent surgical and anaesthesiological risks. In a recent study, Lee et al. [1] evaluated the performance of intravenously-administered ultra-small superparamagnetic iron oxide NPs (USPIO-NPs) (Fig 2) as MRI-signal enhancers in a rat model of surgically-induced endometriosis. The authors hypothesized that the affinity of USPIO-NPs towards macrophages could allow these NPs to be used as novel contrast agents and extend applications of MRI to the detection of ectopic endometrial lesions without a prominent hemorrhagic component such as pelvic adhesions or intraperitoneal implants. Results of this study demonstrated that the use of USPIONPs increases the diagnostic accuracy of MRI for detection of non-haemorrhagic ectopic endometrial lesions, and therefore represents a promising strategy for the non-invasive diagnosis of endometriosis. Endometriosis represents not only a diagnostic but also a therapeutic challenge. Current treatments including surgical and medical hormonal approaches are primarily symptomatic and have a temporary effect with pain recurrence rates reported to range from approximately 25% to nearly 50% [43].

A number of alternative approaches for the management of endometriosis are currently being investigated including anti-angiogenesis and anti-inflammatory agents, anti-cytokines, and gene therapy [44] with several recent publications describing the use of nanomaterials in the delivery of these experimental compounds. In particular, Zhao et al. (2012) reported the use of lipid grafted chitosan micelles loaded with a therapeutic gene encoding pigment-epithelium derived factor, a multifunctional protein with anti-tumor and anti-angiogenic properties in gene therapy approaches for endometriosis.

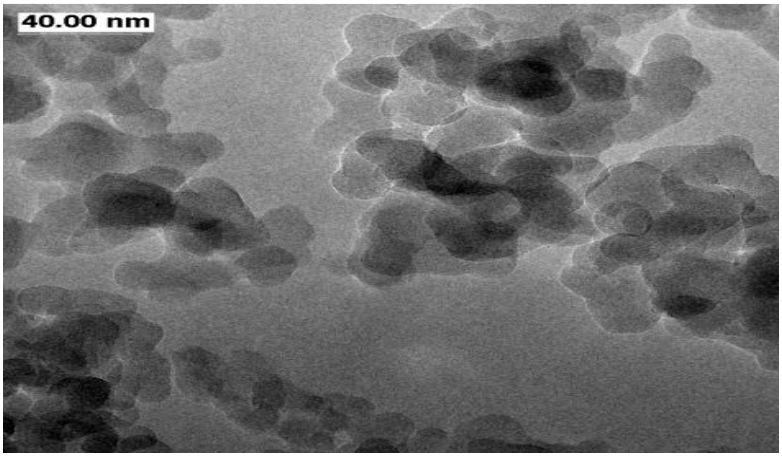

Fig. 2. Superparamagnetic iron oxide NPs used as a contrast agent

The intravenous administration of micelles to rats with surgically-induced endometriosis resulted in similar therapeutic efficiency as the commonly-used agent danazol, in terms of reducing the volume of ectopic lesions and inhibiting the endometriod cyst growth, and demonstrated a significantly stronger effect than placebo. In another study, Chaudhury et al. [45] described the beneficial effects associated with the intra-peritoneal administration of cerium oxide NPs with proven anti-inflammatory, anti-angiogenic and anti-free radical properties in a mouse model of endometriosis. These authors reported a reduction in the systemic levels of ROS and angiogenic factors (vascular endothelial growth factor (VEGF) and adrenomedullin (ADM), along with a reduction in CD34 expression and endometrial gland density in ectopic lesions, compared with an active control (N-acetyl-cysteine) and placebo.

Uterine fibroids

Uterine fibroids (leiomyomas) are benign hormone-dependent tumors originating from the myometrial smooth muscle cells. Leiomyomas represent the most prevalent type of pelvic tumors in women of reproductive age, and affect 60% to 80% of the population [46]. Clinical manifestations of the disease include abnormal uterine bleeding, anemia, genitourinary symptoms and infertility which collectively lead to a reduction in the health-related quality of life [47]. Current approaches to the treatment of uterine fibroids are primarily surgical, comprising a range of techniques with an increasing degree of invasiveness: focused ultrasound ablation, uterine artery embolization, myomectomy and hysterectomy. In view of the fact that the prevalence of uterine fibroids increases dramatically in women aged over 30 years [48] which coincides with the time of first parenthood, the need for minimally-invasive and fertility-sparing treatment approaches is being increasingly recognized. Nanomaterial-mediated delivery of anti-tumor cytokines has been investigated for the improvement of selectivity and potentiation of the effects of minimally-invasive surgical removal of fibroids. Jiang and Bischof reported the use of nano-gold-conjugated tumor necrosis factor alpha (TNF-α) as a nano-sensitizer during cryosurgery for uterine leiomyoma in a mouse model of the disease. Although an improvement of anti-tumor efficacy and post-surgery relapse time was observed after administration of both the nano-particulate and free form of TNF-α compared with cryosurgery alone, the nano-particulate formulation demonstrated a superior safety profile than intra- or peri-tumoral injections of free TNF-α [49]. An alternative approach was reported by Ali et al [3] which involved the use of PLL-PLGA NPs for the delivery of 2-methoxyoestradiol, a biologically-active metabolite of oestradiol with anti-tumor and anti-angiogenic activity into a human leiomyoma cell line as an investigational fertility-preserving alternative to hysterectomy. Similar to other researches investigating the effects of nanomaterial-mediated delivery, these authors observed an increase in anti-tumour activity of the nano-particulate formulation of the drug compared

with the free molecule. Medical management of early-stage ectopic pregnancies (EPs) is being increasingly adopted in clinical practice particularly for patients with rare locations of extra-uterine pregnancy such as the cervix, interstitial part of the fallopian tube, or caesarean scar [50]. In these cases, fertility preserving surgical treatment often represents a significant challenge requiring the use of alternative methods. Methotrexate, an anti-folate agent for chemotherapy with proven affinity towards the trophoblast, is the treatment of choice for the medical management of EP [51]. However, treatment with methotrexate is effective only in early gestational age, and often requires prolonged monitoring of human chorionic gonadotropin (hCG) levels and multiple administrations of the drug. To overcome the existing limitations in the medical management of EP and trophoblastic diseases, Kaitu'u-Lino et al. engineered bacterial-derived nano-spheres (EnGeneIC Delivery Vehicles (EDVs)) (Fig. 3) actively targeted toward epidermal growth factor receptors (EGRF), which are abundantly expressed in human placenta, and loaded the EDVs with doxorubicin [4]. EDVs represent a novel class of easily synthesized organic NPs with high loading capacity, versatility and low toxicity, and are currently undergoing a phase I clinical trial. This engineered delivery system was characterized by high specific affinity towards the trophoblast that allows it to be used for the treatment of both ectopic pregnancy and trophoblastic diseases. The administration of doxurubicin-loaded EGFR-targeted EDVs resulted in more pronounced anti-tumor activity in a mouse model of human choriocarcinoma compared to non-targeted doxorubicin-loaded EDVs and the free drug. An increase in anti-tumor activity was attributed to the increased intracellular uptake of EGFR-targeted EDVs and a higher rate of apoptosis compared to non-targeted EDVs. Based on the results of this study, the authors hypothesized that this new delivery system can not only increase the efficacy of the medical management of ectopic pregnancy and trophoblastic diseases in future but is also relevant for the delivery of placental specific drugs in general.

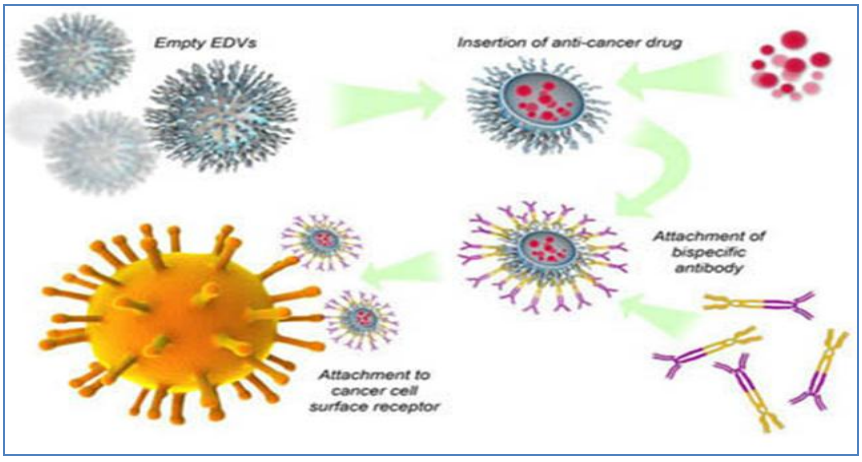

Fig. 3. Nano-spheres (EnGeneIC Delivery Vehicles, EDVs)

Discussion

Several studies report positive outcomes from the use of NPs in investigational drug delivery systems for hormonal replacement therapy (HRT), medical treatment of foetal diseases in utero, and topical intravaginal therapeutic agents [52]. Tomoda et al. described the advantages of encapsulating oestradiol into PLGA NPs for delivery through the stratum corneum of the skin. The same research group had previously attempted a similar approach to the transdermal delivery of indomethacin with encouraging results. In both studies, nanoparticulate formulations of the products demonstrated greater permeability compared to the free drugs, which was further enhanced by the simultaneous application of physical factors (iontophoresis) [5]. Ali et al. (2013a), presented a prototype of a versatile trans-placental delivery system for the first time for the medical therapy of foetal diseases [7]. Previously, trans-placental penetration of nano-carriers was repeatedly found to be regulated by their physicochemical properties including size, shape and surface chemistry, which create immense opportunities for manipulation of their pharmacokinetics [53]. Ali et al (2013a) applied PLGA NPs loaded with dexamethasone in an in vitro model of human placental trophoblast cells as an experimental treatment for congenital adrenal hyperplasia, and observed an increase in permeability of the nano-encapsulated drug compared to the free form from the maternal to fetal compartment [7]. Further research in this direction can aid the development of a trans-placental delivery nano-platform for the targeted medical therapy of foetal diseases in uterus. Several other studies describe the use of a polyamidoamine (PAMAM) dendrimer-based topical intravaginal delivery system to restrict the effect of antimicrobial drugs to the vaginal compartment which prevents the passage of drugs through the fetal membranes in pregnant women with ascending genital infections thus minimizing the risk of foetotoxicity [54] or genital infections. A significant number of publications address the use of nanomaterials for the detection and treatment of genital infections, including *Chlamydia trachomatis* [55], *Neisseria gonorrheae*, *Candida spp*, and *Herpes simplex virus* (HSV). The inherent antibacterial and antiviral properties of polyanionic and peptide dendrimers arise from their ability to competitively bind to particular receptors of the pathogens involved in cellular adsorption, and thereby reduce the likelihood of subsequent infection [56]. These findings have triggered the development of a series of microbiocidal formulations to target infection with HIV, human papillomavirus and HSV including a specific dendrimer-based vaginal gel for the simultaneous prevention of HSV and HIV, which is currently undergoing phase I clinical studies [8]. Dendrimers have also been shown to improve the delivery of azithromycin, the gold standard for treatment of *C. trachomatis* infection into intracellular chlamydial inclusions in vitro as well as the transfer of genetic constructs to knockdown gene expression in chlamydial cultures as an experimental therapeutic tool [57]. A unique

life cycle of this pathogen can involve the formation of intracellular microbial inclusions which remain remarkably resistant to antibacterial treatment and can account for long-term consequences of chlamydial infection including tubal infertility [58]. These research approaches of conjugating azithromycin with neutral PAMAM dendrimers or gene knockdown in cultures using PAMAM dendrimer-mediated transfection, described by Mishra et al., resulted in specific targeting of affected cells within the cell culture without the induction of infection and increased antimicrobial activity of the conjugates compared with free molecules of azithromycin. Highly satisfactory delivery properties into chlamydial inclusions have also been shown for PLGA NPs loaded with rifampin and azithromycin [59]. A growing body of evidence now supports antifungal effects of silver NPs and carbon nanotubes against *Candida spp* including suppression of formation of fungal films highlighting their potential to improve the efficacy of existing antifungal treatments. Other works [60, 61] describe the use of biodegradable PLGA NPs as carriers for the major outer membrane protein (MOMP) of *C. trachomatis*, a principal component of experimental subunit vaccines against chlamydial infection. MOMP is abundantly present on the outer surface of *C. trachomatis* and is capable of triggering strong immune response in the host. In its free form, MOMP is remarkably fragile, and demonstrates poor cellular internalization which decreases its immunogenic properties. However, the use of PLGA NP-based delivery system has substantially increased the immunogenicity of recombinant MOMP in vitro partially due to independent potentiation of the effects by PLGA, and has triggered a highly satisfactory Th1- immune response in vivo making this investigative nano-vaccine against *C. trachomatis* a strong candidate in search for effective anti-chlamydia immunization [62]. Research into the specific mechanisms underlying reproduction is increasingly demonstrating an association between various forms of previously unexplained infertility such as specific types of gonadal insufficiency, failure of fertilization and recurrent pregnancy loss, abnormal gene expression and/or genetic polymorphisms. In view of these findings, targeted gene transfer into reproductive tissues, gametes and embryos can evolve into a powerful tool with which to study and manipulate the fine pathophysiological mechanisms underlying infertility. Still other studies describe successful in vivo intra-testicular gene transfer in rodents using electroporation and viral vectors resulting in the production of transgenic epididymal sperm [63]. Several studies report that intra-testicular and intra-ovarian gene transfer using viral vectors restores gametogenesis in mouse models of genetic gonadal failure;, data regarding the safety of such methodology remain highly contradictory [64].

The feasibility of nanomaterial-mediated gene transfer into foetal tissues in utero for investigating gene therapy for monogenic diseases was demonstrated by Yang et al. [65]. In this pilot study, the authors utilized intra-

amniotic injections of chitosan NPs conjugated with enhanced green fluorescent protein (EGFP) gene into mouse embryos, and observed expression of the transgene in the alveolar epithelium of the lungs and the luminal intestinal epithelium, coinciding with penetration of NPs through respiratory and gastrointestinal pathways. Expression of the transgene, however, was temporary and localized exclusively to these tissues thus justifying that further research is required to optimize the techniques and timings of this procedure in order to achieve more stable outcomes [66].

Conclusion

It can be concluded that nanotechnology is a novel multi-disciplinary field being addressed by biotechnology, pharmaceutical synthesis and clinical medicine. There is an increasing anticipation that nanotechnology can dramatically alter routine approaches to the detection and treatment of a variety of diseases, although the revolutionary and controversial nature of this discipline remains a matter of discussion. In reproductive medicine, the use of investigational nano-biotechnological tools has already resulted in encouraging outcomes in the treatment of several high-impact conditions opening significant opportunities for alternative non-invasive or minimally-invasive treatments for several traditionally 'surgical' pathologies. In the current era of assisted reproductive technology as a seemingly ultimate answer to the problem of sub-fertility, an overall sub-optimal efficacy of in vitro fertilization and the need for fertility-sparing attitude to preceding treatments are becoming matters of growing concern. With this in mind, the use of dedicated systems to deliver pharmaceutical products for the targeted non-invasive treatment of chronic reproductive pathologies instead of surgery, which can interfere with natural fertility per se, can clearly optimize the chances for conception in future.

Conflict of Interest

There is no conflict of interest to declare.

Acknowledgement

There is no acknowledgment to declare.

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