ADVANCED NANOBIOMED RESEARCH Open Access
www.advnanobiomedres.com

Sex-Based Differences in the Biodistribution of Nanoparticles and Their Effect on Hormonal, Immune, and Metabolic Function

*Maria Poley, Gal Chen, Noga Sharf-Pauker, Aviram Avital, Maya Kaduri, Mor Sela, Patricia Mora Raimundo, Lilach Koren, Sivan Arber, Egor Egorov, Janna Shainsky, Jeny Shklover, and Avi Schroeder**

Males and females respond differently to medications due to physiologic, metabolic, and genetic factors. At times, sex-related differences cannot be mitigated by dose adjustment to body mass, and are evident from the tissue level to the single cell. The rising number of clinically approved nanotechnologies calls for assessing how their activity is affected by the patient's sex. Herein, sex differences in nanotechnology are scoped, with emphasis on molecular considerations. Sex-specific pharmacokinetics of nanocarriers is influenced by the nanoparticle's composition, its size, and architecture. The biodistribution and immune response to nanoparticles in males and females, and the influence nanoparticles have on hormones, fertility, and toxicity, are discussed. Despite its importance, the effect of sex on the design and implementation of nanomedicines is underresearched. Herein, it is aimed to raise awareness of sex differences in the preclinical and clinical evaluation of nanotechnologies.

1. Introduction

Sex-related response to some medications is associated with genetic, anatomical, and molecular differences between females and males.[1] New nanotechnology applications in medicine[2] have added another level of complexity in assessing how sex affects nanoscale drugs in the body. Despite the increased awareness for including sex as a biological variable, the influence of sex on nanotechnologies' fate in the body is not well understood.[3] From a historical perspective, only in 1993 the US Food and Drug Administration (FDA) lifted restrictions that excluded females from participating in most clinical trials.[4] As a result, it was found that some drugs affect females differently than males, at times, resulting in severe adverse effects seen primarily in females.[5] This was followed by changing the dosing regimens, or even withdrawal, of drugs, which imposed risks to females' health.[6] For example, the antihistamine terfenadine and the drug cisapride monohydrate, which increases gastrointestinal contractions, were both found to cause potentially fatal arrhythmias in females.[7] This prompted the study of how physiological differences between males and females impact pharmacological responses.[8] In 2013, the doses of zolpidem-based products were lowered for females, but not for males, following unexplained car accidents involving female drivers that fell asleep at the wheel.[9] Although sex-related differences encouraged a change in drug dosing between sexes, they were mainly addressed by merely adjusting the dose according to the patient's body mass or body surface area.[10] However, not all sex differences can be addressed in this manner. More specifically, hormonal, genetic, immunological, and other molecular differences also govern sex-specific responses and pharmacokinetics (PK) profiles.[11]

Sex differences also affect the biodistribution and clearance of nanoparticle drugs (**Figure 1**, **Table 1**). Generally, drug delivery using nanocarriers can alter the PK profile,[12] lower drug toxicities,[13] protect delicate biological cargos, and prolong circulation time, thereby expanding the therapeutic window.[14] The biodistribution and PK of nanoparticles in the body are primarily affected by the nanoparticle's properties (composition, size, charge, surface modifications, and stability), rather than the loaded drug.[12,15] Nonetheless, sex plays an essential physiological role in influencing nanoparticles' fate in the body as well. A recent article published by our group reveals that the ovulation cycle affects nanoparticles' accumulation in the female

M. Poley, G. Chen, M. Kaduri, M. Sela, P. M. Raimundo, L. Koren, S. Arber, E. Egorov, J. Shainsky, J. Shklover, A. Schroeder
Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies
Department of Chemical Engineering
Technion – Israel Institute of Technology
Haifa 32000, Israel
E-mail: avids@technion.ac.il

N. Sharf-Pauker, A. Avital
The Norman Seiden Multidisciplinary Program for Nanoscience and Nanotechnology
Technion – Israel Institute of Technology
Haifa 32000, Israel

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/anbr.202200089.

DOI: 10.1002/anbr.202200089

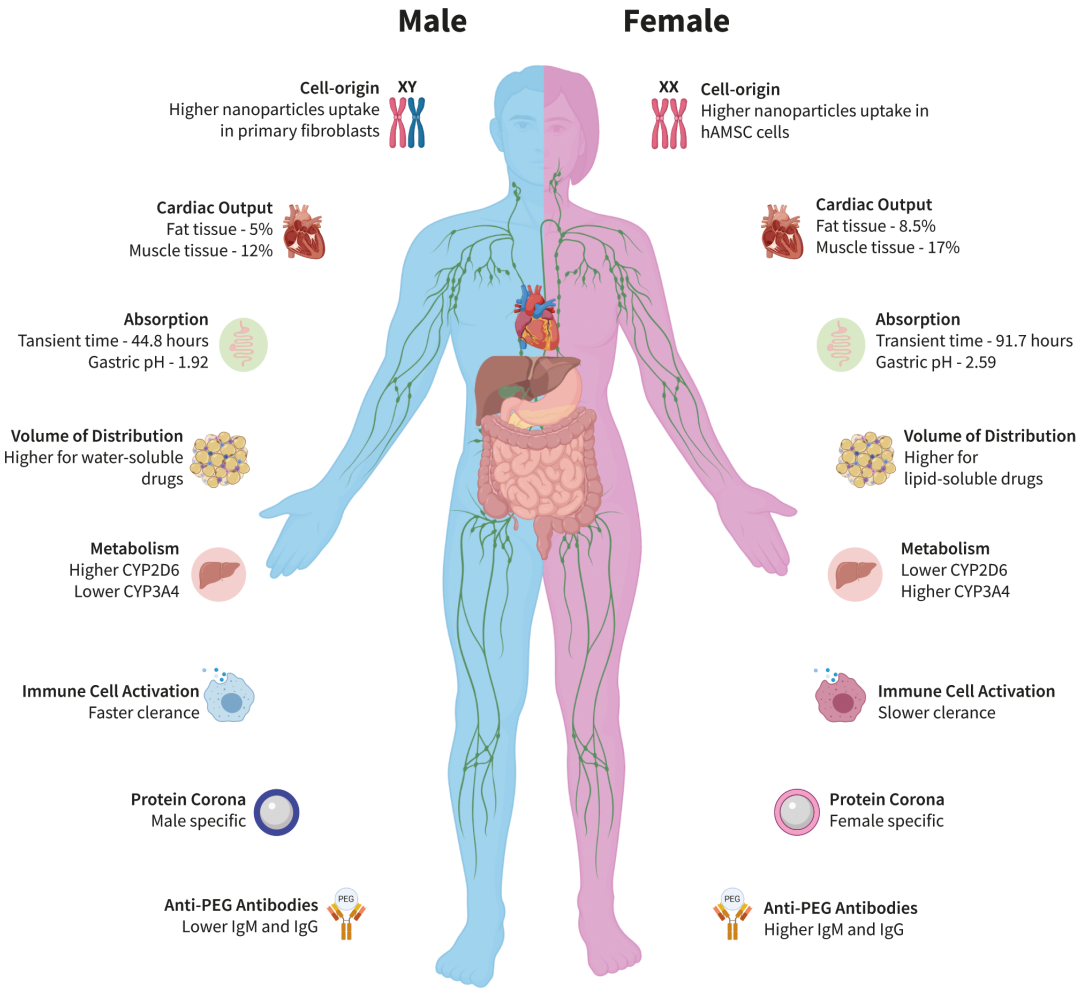

Figure 1. Sex differences affecting PK of nanocarriers.

reproductive system.[16] Furthermore, there are several indications for sex differences in the literature, for example, liposomes loaded with topoisomerase-I inhibitor (CKD602), or topotecan, for cancer treatment of neuroblastoma and carcinoma, had slower clearance rates in female rats compared to male rats, postintravenous (i.v.) administration.[17] Similarly, the administration of liposomal doxorubicin resulted in slower blood clearance rates in female rats and in humans compared to males.[18]

Alternatively, nanotechnology can help mitigate sex differences associated with the PK of free drugs. For example, cannabidiol (CBD), delivered orally in its free form, had higher bioavailability in females compared to males.[45] However, when CBD was delivered using 40–50 nm micelles, composed of vegetable oils and fatty acids, the sex differences were less evident.[46] Contrarily, although the PK of lipid nanoparticles containing siRNA targeting both vascular endothelial growth factor (VEGF) and kinesin spindle protein (a motor protein involved in the mitosis process) showed similar PK in both sexes, the intravenous administration resulted in higher liver toxicity in male rats compared to female rats.[40] In this case, sex-related metabolic differences induced difference in toxicity after the siRNA was released from the nanoparticles regardless of the similar PK profile.[40] The most up-to-date example is the lipid nanomedicine-based COVID19 vaccine. It was reported that females have a stronger immune response which leads to increased vaccine efficacy and that there are sex differences in several adverse reactions.[47] A proof-of-concept study showed that the sex-dependent response to the vaccine is related to differences in the uptake of the nanoparticles by female and male natural killer (NK) cells.[48]

Previous reviews in the field have shed some light on how biological sex is considered in clinical trials of nanomedicine and their design,[49] and provide a detailed comparison between male and female differences at a molecular level.[50] Here, we address the main findings that are reported in the literature regarding sex differences in nanomedicine and derive conclusions based on pooling all the available data together. We discuss how sex affects the PK and toxicity of nanoparticles (Figure 1, Table 1), key sex-related proteins (**Table 2**), and hormonal differences (**Figure 2**, **Table 3**) and main sex-related considerations regarding nanoparticles' design (**Figure 3**, **Table 4**).

Table 1. Sex-related PK and toxicity differences by nanoparticle type.

Nanoparticle type	Average size [nm]	Model	Administration route	Sex-related PK	Sex-related toxicity	Ref.
Metallic nanoparticles						
Silver nanoparticles (AgNPs)	54.5	Zebrafish	Oral	Male gut microbiome showed decrease in richness and diversity while the female's remained unchanged	N/A	[19]
	60	Rats	Oral	Higher accumulation in female kidneys, urinary bladder, and adrenal glands compared to males	N/A	[20]
	5–150	Rats	Inhalation	Higher accumulation in female kidneys. Gene expression in the kidney was sex-specific	N/A	[21]
	21.8	Mice	IV	Slower clearance in females. Higher accumulation in lungs and kidneys of female mice compared to males	N/A	[22]
AgNPs coated with albumin	14.9 and 37.7	Rats	IV	Higher accumulation in male liver	Female liver showed higher oxidative stress reaction	[23]
AgNPs coated with polyvinylpyrrolidone	8.6			N/A	Female liver showed oxidative stress reaction that was not seen in males	
AgNPs coated with polyvinylpyrrolidone	50	Smallmouth bass fish plasma	In vitro	NPs gained sex-specific protein corona	–	[24]
PEGylated gold nanoparticles AuNPs	4.4, 22.5, 29.3, and 36.1	Mice	IP	Female had higher kidney clearance	Male mice suffered from increased liver damaged compared to females, while females suffered from increased kidney damage. Immune response only in male mice.	[25]
PEGylated AuNPs (with and without amine modification)	14	Male mice only	IV	Amine-modified NPs induced increase in testosterone levels	N/A	[26]
AuNPs	1.4, 18, 80	Female tats only	IV	Higher accumulation in the reproductive system of pregnant rats compared to nonpregnant rats	N/A	[27]
Titanium dioxide nanoparticles	<25	Rats	Oral	Significant hormonal changes in males and females	Inflammatory reaction reported in female rats	[28]
	6	Female mice only	Oral	Imbalance in progesterone and estrogen production	Ovarian damage	[29]
	N/A	Female mice, male rats	N/A	N/A	Disrupt female follicles causing reproductive dysfunction; reduce sperm motility in males	[30]
Aluminum oxide nanoparticles	40	Mice	Inhalation	Sex-specific gene expression	N/A	[31]
Copper nanoparticles	23.5	Mice	Oral	N/A	Greater liver, kidneys, spleen, and gastric toxicity in males than females	[32]
Nickel nanoparticles	90	Rats	Oral	Male rats had lower levels of FSH and testosterone and, and females showed from lowered estrogen levels and high levels of FSH and LH	Decreased fertility for both males and females; males damaged sperm motility	[33]
Cerium dioxide nanoparticles	2–5	Male mice only	Oral	Elevation in testosterone and LH levels	N/A	[34]
Semiconductor nanoparticles						
Carbon black nanoparticles	14, 56, 95	Male mice only	Intratracheal	Elevated serum testosterone levels only after exposure to NPs under 95 nm	N/A	[35]

Table 1. Continued.

Nanoparticle type	Average size [nm]	Model	Administration route	Sex-related PK	Sex-related toxicity	Ref.
Carbon nanotubes	Diameter - 20–30 Length - 500–2000	Male mice only	IV	Carbon nanotubes accumulated in the testes without affecting sex hormone levels, sperm health and male mice fertility.	Accumulation of carbon nanotubes in the testes results in oxidative stress and reduced the thickness of seminiferous epithelium in the testes; damage was reversible.	[36]
Quantum dots	2–3	Human amniotic stem cells and salivary gland primary fibroblasts	In vitro	Uptake is cell-origin (XX or XY) dependent. Female human amniotic stem cells (hAMSC) displayed higher Qdot uptake compared to male cells. The opposite was found for primary fibroblasts derived from salivary gland.	–	[37]
Silicon oxide nanoparticles	46, 432, mesoporous 466	Mice	IV	N/A	Porosity of silica NPs altered the maximum tolerated dose (MTD), and higher values of MTD were observed in female mice versus male mice for all sizes of silica NPs.	[38]
	70	Zebrafish blood plasma	Incubation	NPs gained sex-specific protein corona. Leukocytes preferentially accumulated on female-specific protein corona covered NPs.	–	[39]
Lipid nanoparticles						
ALN-VSP (siRNA in liposomes)	80	Rats	IV	No differences in PK	More severe liver toxicity for male rats	[40]
S-CKD602 (PEGylated liposomal formulation of CKD-602)	100	Rats	IV	1.2-fold slower clearance in female rats compared to male rats	N/A	[17]
TLI (liposomal topotecan)	100	Rats	IV	1.4-fold slower clearance in female rats compared to male rats	N/A	[17]
IHL-305 (liposomal irinotecan)	100	Humans	IV	1.5-fold higher distribution volume of the drug in males compared to females	N/A	[41]
Polymeric nanoparticles						
PEG-*b*-PLA (polylacticacid) nanoparticles	50	Neonatal female rats only	IP	Increased progesterone levels	Disruption in ovulation cycle	[42]
Doxorubicin-loaded poly(lactic-*co*-glycolic) (PLGA) nanoparticles	94	Mice, rats, and rabbits	IV	N/A	Decreased leukocytes count and faster weight loss for females compared to males	[43]
Protein-based nanoparticles						
Abraxane nanoparticle albumin-bound paclitaxel (ABI-007)	130	Rats and humans	IV	Significantly higher renal extraction in female rats compared to male rats. These finding were not observed in humans.	N/A	[44]

2. Factors Affecting Sex-Specific Nanoparticle Biodistribution

The biodistribution of nanoparticles greatly depends on their physicochemical properties and stability after encountering the biological environment.[15b] Therefore, differences in biological parameters between males and females, such as biochemical and physiological differences including immune differences, sex-specific protein coronas, polyethylene glycol (PEG) antibodies, and even cell-origin (XX or XY), can all impact nanoparticle biodistribution.

2.1. Sex-Dependent Immune Cell Activation

The mononuclear phagocytic system (MPS), composed of phagocytic cells such as blood monocytes and Kupffer cells of the liver, encounters nanoparticles in the body following intravenous or other administration routes. The MPS cells engulf nanoparticles by recognizing complement activating proteins (opsonins) and other adherent proteins on the nanoparticles' surface after entering the systemic circulation.[57] Females have been shown to have lower MPS function than males, resulting in slower clearance rates of liposomes in females after i.v. administration.[18a,41,54]

Table 2. Sex-specific proteins that affect nanoparticles' PK.

Type	Function	F/M	Effect of nanoparticles' PK	Ref.
Metallothionein	Metal-binding protein, transport protein	Higher in F	Silver nanoparticles had higher accumulation in female rat kidneys compared to male rat.	[20,51]
Apolipoprotein E (ε2/ε3 genotype) and hepatic lipase	Lipid metabolism	Higher in M	Lipid carriers can be trafficked by Apolipoprotein E and metabolized by lipases	[52]
Myeloperoxidase	Peroxidase enzyme in neutrophils	Higher in F	Degradation of carbon nanotubes	[53]
Vitellogenin	Egg yolk precursor protein in vertebrates	–	Female-specific protein corona on SiO_2 nanoparticles and silver nanoparticles	[24,39]
Zona pellucid	Egg-related protein	–	Female-specific protein corona silver nanoparticles	[24]
Fetuin	Binding and transport protein	–	Male-specific protein corona silver nanoparticles	[24]

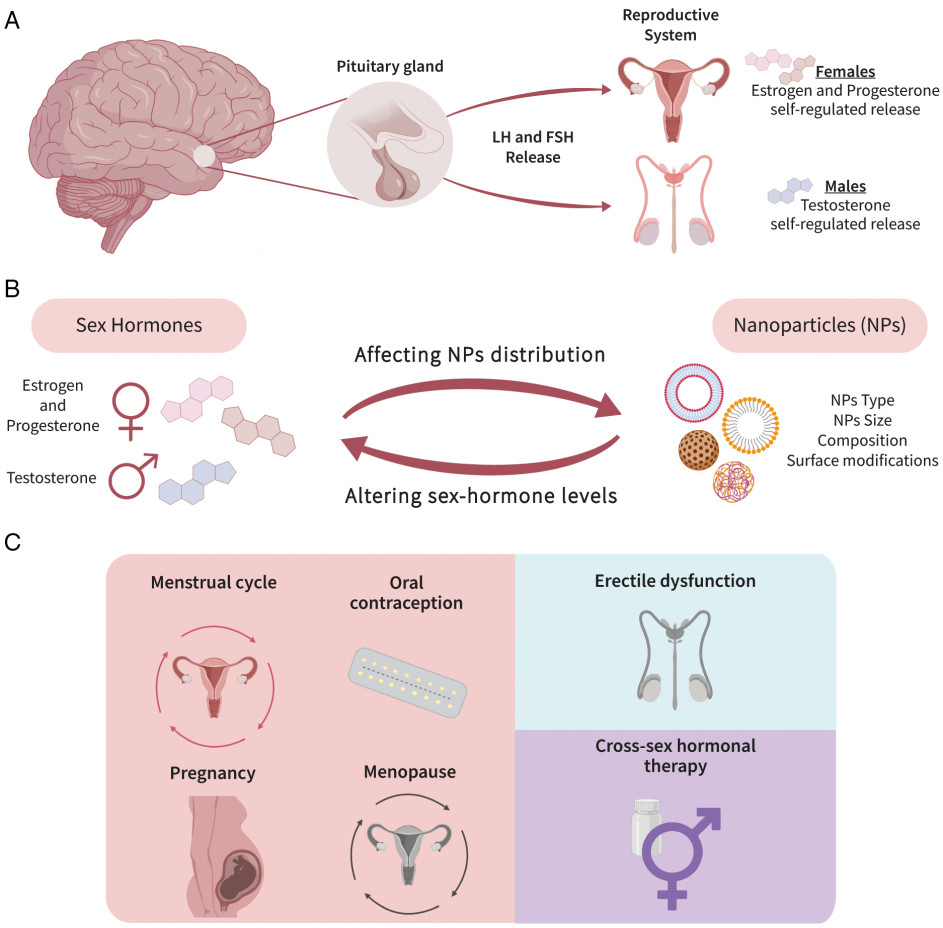

Figure 2. Influence of sex hormones in nanotherapeutics. A) Sex hormones route in males and females. LH and FSH are released from the pituitary gland in the brain resulting in sequential release of estrogen and progesterone in females and testosterone in males. B) The mutual influence between sex hormones and nanoparticle administration. C) Representative sex-specific conditions that should be taken into consideration with nanotechnologies.

In accordance, testosterone levels, which are high in males, positively correlated with MPS levels and enhanced clearance rates of PEGylated liposomal doxorubicin from the blood.[58] Furthermore, female sex hormones, such as estrogen, were found to reduce transcription of central activating receptors in monocytes, thus suppressing their activity in vitro.[59] On the other hand, the phagocytosis and macrophage activation is higher in females,[11a] possibly sequestering the nanoparticles for prolonged periods in an inactivated form.[60]

In addition to MPS function, other immune differences occur between sexes. For example, neutrophil levels in females were increased after i.v. administration of silver nanoparticles while

Table 3. Sex-specific hormones affected by nanoparticles.

	Nanoparticle type	Hormone level	Ref.
FSH	Titanium dioxide nanoparticles	F↓	[29]
	Nickel nanoparticles	F↑ M↓	[33]
Testosterone	Nickel nanoparticles	M↓	[33]
	Cerium dioxide nanoparticles	M↑	[34]
	Titanium dioxide nanoparticles	F↓ M↑	[28]
	Carbon black nanoparticles (below 95 nm)	M↑	[35]
	PEGylated amine-modified gold nanoparticles	M↑	[26]
Estrogen	Nickel nanoparticles	F↓	[33]
	Titanium dioxide nanoparticles	F↑	[29]
LH	Nickel nanoparticles	F↑	[33]
	Titanium dioxide nanoparticles	F↓	[29]
	Cerium dioxide nanoparticles	M↑	[34]
Progesterone	Titanium dioxide nanoparticles	F↓	[29]
	polymeric PEG-*b*-PLA (polylacticacid) nanoparticles	F↑	[42]

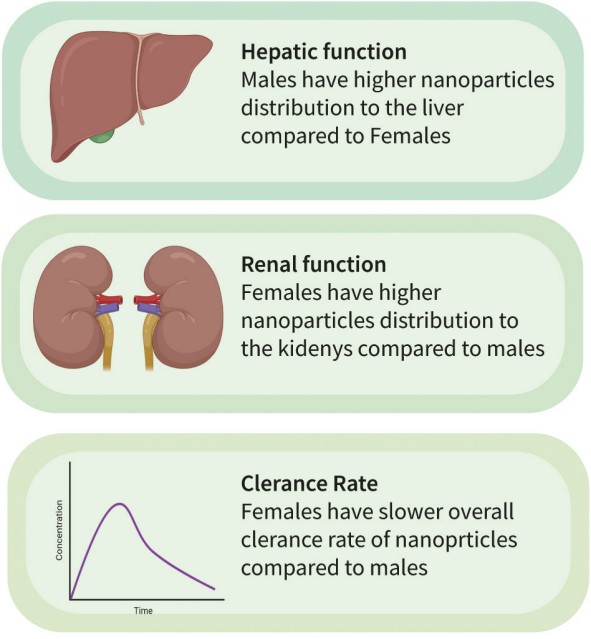

Figure 3. Main considerations of sex-differences in in nanotechnology implementation and design.

neutrophil counts in males decreased.[61] Intravenously administered mesoporous silica nanoparticles instigated sex-specific immune response in the form of helper-T-cell activation, which was influenced by sex hormones, leading to a higher maximal tolerated dose in male mice.[38] PEGylated gold nanoparticles (sized 4–36 nm) induced inflammatory reactions in male mice only, leading to increased white blood cell count.[25] In contrast, 25 nm titanium dioxide nanoparticles increased white blood cell counts exclusively in females.[28]

2.2. Sex-Specific Protein Corona

Once a nanoparticle enters the bloodstream, it encounters proteins that form aggregates on its surface, coined "protein corona," affecting biodistribution, PK, trafficking, and nanotoxicity.[62] Sex-specific protein coronas arise due to differences in the composition of serum proteins between males and females. Proteome profiling of healthy individuals reveals abundant sex-specific serum proteins such as estrogen regulators and breast cancer-related proteins in females, and male sex hormone regulators in males.[63] The protein corona can promote the type of interaction a nanocarrier will have with different cells in the body[64] For example, a study performed on zebrafish showed that SiO_2 nanoparticles had different protein coronas after incubation with female versus male plasma.[39] Female protein coronas included vitellogenin—an egg yolk precursor protein, while the male protein corona included fetuin, which is a binding protein.[39] These biological tags triggered leukocytes to preferentially accumulate on female specific protein coronas.[39] Similarly, when silver nanoparticles were introduced into female plasma, aggregation of zona pellucid and vitellogenin (egg-related proteins) occurred on the nanoparticles' surface. It was suggested that this led to nanoparticle accumulation in the reproductive system, resulting in higher toxicity to female fish.[24]. Interestingly, proteomic analysis of silver nanoparticles incubated in human plasma demonstrates that 70% of the proteins comprising the corona were shared between sexes compared to only 40% of shared proteins after incubation with fish plasma.[24]

Sex-specific protein coronas slowed the blood clearance rate of silver nanoparticles in female mice compared to males.[22] Furthermore, a higher accumulation of silver nanoparticles was recorded in the lungs and kidneys of female mice compared to the male mice.[22] Another study demonstrated that albumin-coated silver nanoparticles accumulated in higher amounts in male rats' livers than female rats.[23] A different approach exploits nanoparticle–protein corona interactions to serve as a diagnostic tool.[65] Liu and co-workers leveraged these unique interactions combining mass spectrometry and deep proteome profiling to distinguish between healthy and diseased blood samples.[66] As sex alters specific protein coronas such as sex hormone regulators, detection should consider sex differences when proposing a disease-associated protein corona profile. For example, distinguish interactions will apparently be detected in males and females when diagnosing sex-dependent disease expression.[67] These studies demonstrate that sex-specific and nanoparticle-specific protein coronas affect nanoparticles' PK in the body and should be accounted for in medicinal nanoparticles' engineering.

2.3. Anti-PEG Antibodies

PEG is used in many nanoformulations to extend circulation time and improve nanoparticle stability. Anti-PEG antibodies can be found in healthy individuals after exposure to PEG in beauty products, medicines, and personal care. Anti-PEG antibodies will bind to PEGylated-moieties introduced into the bloodstream and alter their biodistribution and PK, leading to accelerated blood clearance.[68] Many modern nanocarriers are

Table 4. Main considerations of sex differences in nanotechnology.

Consideration	Evidence	Possible outcome	Ref.
Females have slower overall clearance of nanoparticles	General slower clearance	Higher chance of toxicity for females, greater efficacy for females	[17,18]
	Lower MPS function		[18a,41,54]
	Female-specific protein corona		[22]
Females have higher nanoparticles' distribution to the kidneys compared to males	Higher accumulation at the kidneys	Nanoparticles' metabolism and elimination in females are mainly by the renal route	[20–22]
	Greater kidneys toxicity and damage		[25]
	Dominant renal excretion		[44a]
	Higher levels of metal-binding proteins (metallothionein)		[20,51]
Males have higher nanoparticles' distribution to the liver compared to females	Higher accumulation at the liver	Nanoparticles' metabolism and elimination in males are mainly by the hepatic route. In addition, the lower accumulation at the kidneys is due to higher activity of renal function.	[23,25]
	Greater liver toxicity and damage		[23,25,40]
	Testosterone stimulates renal function		[20]
	Higher glomerular filtration		[55]
	Higher levels of ApoE		[52a,56]

functionalized with PEG to increase circulation time and hence increase therapeutic efficacy.[13,69] Recently, it was discovered that anti-PEG antibodies are generated after the administration of PEGylated nanoparticles.[70] Repeated exposure to PEGylated drugs resulted in faster clearance through the MPS and premature drug release, therefore suppressing the benefits of nanotherapeutics.[71] Anti-PEG antibodies may also be the reason behind adverse infusion reactions seen in a small fraction of patients after administering PEGylated nanotherapeutics.[70a] In one study that looked at 20 human serum donors, more than 50% of samples were positive to anti-PEG2000 IgM and 20% to IgG. The overall prevalence of anti-PEG antibodies was higher in females as serum antibodies reacted against PEG alone and PEGylated liposomes. Surprisingly, when serum samples were incubated with only PEGylated liposomes, antibody reaction was obtained only in female samples.[70a] A separate study that evaluated preexisting IgG and IgM antibodies against PEG in 1504 patients corroborated these results.[72] Sex differences in anti-PEG antibodies should be further evaluated to better understand how they affect nanomedicine's efficacy and fate in the body.[73]

2.4. Cell XX, XY Chromosomes Affect Nanoparticle Uptake

The influence of cell-origin (XX or XY) on nanoparticle uptake is considered one of the predominant overlooked features in the bio–nano interface.[74] Cell-origin has an impact on the molecular function and cytoskeletal organization of the cell.[75] A recent study showed that quantum dot (Qdot, 2–3 nm) uptake is cell-origin (XX or XY) and type dependent.[37] Female human amniotic stem cells (hAMSC) displayed higher Qdot uptake compared to male cells.[37] The opposite was recorded in primary fibroblasts derived from the salivary gland, favoring male cells. Variations in the secretion of cytokines, cytoskeleton, and mitochondrial changes have all been suggested to affect sex differences in nanoparticle uptake.[37] An additional theory for these differences may be related to autophagy, the natural process of protein and organelle turnover inside the cell, which is impacted by sex.[76] Under similar stress conditions, female cells tend to survive longer due to increased activation of autophagic response, compared to male cells, where an increase in apoptotic signals results in cell death.[76] Nanoparticles' internalization by cells can lead to altered trafficking pathways, which manifest differently in female and male cells.[77] Therefore, the development of sex-specific formulations to address differences at the cellular level may improve the therapeutic activity.

2.5. Physiological Sex Differences

Physiological differences between sexes also affect the distribution and PK of drugs. The blood volume, cardiac output (the blood volume pumped by the heart to the organs), and average blood flow to organs are higher in males, affecting both drug distribution and clearance.[11b] For example, silver nanoparticles had higher accumulation in female rat kidneys when compared to male rat kidneys.[20] Renal clearance is dependent on the glomerular filtration rate, which is higher in males than in non-pregnant females.[11b] However, Sparreboom et al.[44a] observed that Abraxane elimination via renal excretion was significantly higher in female rats than in male rats. Interestingly, when implementing the same treatment in humans, no differences were recorded.[44] Liposomal irinotecan administered intravenously to patients with solid ovarian, breast, and lung tumors showed 1.5-fold higher V_d in males compared to females due to greater blood volume in males.[41] On average, females have a higher body fat content (16.5 kg) relative to males (13.5 kg), which increases the volume of distribution (V_d) of lipid-soluble drugs.[11b] Contrarily, males have higher average body-water content that results in increased V_d of water-soluble drugs or nanocarriers. For example, liposomes, lipid-based vesicles, were shown to favor fat tissue uptake, relative to muscle.[78] Another key difference is the reproductive system in males and females. One of the main examples is the mucosal layer in the female reproductive system which is often targeted as a promising delivery route.[79] There are currently several nanotechnology applications that deal specifically with reproductive health and are designed by targeting either male or female reproductive tract.[80]

3. Sex-Specific Metabolism and Excretion of Nanoparticles

Hepatic metabolism depends on enzyme activity, which differs between females and males.[11c,81] Cytochrome CYP3A4, a liver enzyme involved in the metabolism of 30% of clinically used drugs, is more active in females.[82] In contrast, CYP2D6, which is involved in the metabolism of 20% of drugs, is more active in males.[11b,81,83] Injections of PEGylated liposomes induced the expression of CYP450 enzymes, resulting in faster nanoparticle clearance from the system circulation.[84] This, along with sex-specific CYP450 enzyme activity, may be a factor in a sex-related variance of nanoparticle PK. Moreover, it was shown that Apolipoprotein E-dependent hepatic lipase activity is increased by 30% in males compared to females.[85] As lipid carriers are often trafficked by ApoE and metabolized by lipases;[52b] this may cause metabolic variations in lipid nanoparticle clearance between males and females.[56] Another example of proteins affecting nanoparticles' traffic in the body is specific metal-binding proteins, such as metallothionein. These proteins are found in higher concentrations in females than males[20,51] and were suggested to cause higher accumulation of silver nanoparticles in female rat kidneys when compared to male rat kidneys.[20]

The metabolism of nanoparticles depends on their composition and properties. Inorganic nanoparticles, such as metallic nanoparticles, quantum dots, and silica, may remain in the body for prolonged periods of time.[86] Organic nanoparticles such as liposomes and polymeric nanoparticles are biodegradable and subject to additional sex-dependent excretion pathways.[11b,c] For example, carbon nanotubes are degraded by the enzyme myeloperoxidase inside neutrophils.[53a] Females present a higher intracellular concentration of myeloperoxidase, thereby possibly favoring the degradation of carbon nanotubes in a sex-specific manner.[87]

4. Sex Differences in Oral and Inhaled Administration of Nanoparticles

Females have a longer mean transit time in the gastrointestinal tract (91.7 h) than males (44.8 h),[88] which can lead to increased nanoparticle absorption and subsequent exposure to higher drug concentrations.[89] Orally delivered nanotherapeutics are subject to gastric degradation and clearance through the feces.[86] Nanoparticles that evade secretion are absorbed into the systemic circulation and lymphatic systems through Peyer's patches and other regions in the intestine wall (size range of 50–200 μm).[90] Intestine motility and transit times change during the female menstrual cycle, being longer after the ovulatory phase.[91] This also differs between fertile and postreproductive (menopausal) females, where longer transit times were recorded in the former.[91] Furthermore, gastric fluid in the stomach is more acidic in males (pH = 1.92) than in females (pH = 2.59).[91] This may be critical when pH-responsive nanoparticles are used for gastric delivery,[92] or when nanocarrier stability has to be maintained within the harsh gastric environment.[93]

The gut microbiome also plays a role in differences in nanoparticle absorption. The levels of fecal bifidobacteria, a gut microbiota, are higher in females. At the same time, Bacteroides and Prevotella are higher in males.[94] Another essential consideration is sex-related differences in gastric enzymes such as alcohol dehydrogenase, whose activity is lower in females than in males, or glutathione-*S*-transferase, which is more active in females than in males.[11b] Gastric enzymes and the gut microbiome are responsible for gut-protein corona formation on the nanoparticles' surface, affecting their colloidal stability and absorption.[95] For example, in zebrafish orally administered silver nanoparticles showed that the male gut microbiome was affected while the females' remained unchanged. The interaction of nanoparticles with the gut microbiome is currently the subject of multiple studies, promising to shed light on these interactions.[95]

Generally, metabolomics analysis implied sex-dependent gene expression after respiratory inhalation of aluminum oxide nanoparticles.[31] Similarly, a different publication showed sex-dependent gene expression patterns in rat kidney cells after prolonged exposure to silver nanoparticles.[21] A total of 163 genes showed sex-related differences, where male-predominant gene expression was related to metabolic enzymes, while female-predominant genes were related to extracellular signaling.[21]

5. Sex Hormones and Fertility

Sex hormones have distinct expression patterns in males and females.[96] The hypothalamic–pituitary–gonadal axis regulates sex-hormone production (Figure 2A), impacting multiple pathways, such as fertility, the immune system, and drug metabolism.[97]

Nanoparticle administration may affect the levels of sex hormones and alter hormonal activity[98] (Figure 2B, Table 3). For example, exposure to nickel nanoparticles resulted in decreased levels of follicle-stimulating hormone (FSH) and testosterone in male rats. In contrast, female rats exposed to nickel nanoparticles exhibited lowered estrogen levels and higher FSH levels and luteinizing hormone (LH).[33] On the other hand, male rats injected cerium dioxide nanoparticles had an increase in testosterone and LH levels, resulting in the renewal of sperm production.[34] Elevation in testosterone levels in male rats was also observed after administration of titanium dioxide nanoparticles.[28] In this case, the opposite effect was observed in female rats. Changes in testosterone levels are explained by a targeted accumulation of titanium oxide nanoparticles in the reproductive organs.[28] In several studies on adult female mice, it was found that prolonged exposure to TiO_2 resulted in an imbalance in progesterone and estrogen production, resulting in ovarian damage due to alterations in gene expression.[99] Similarly, polymeric PEG-*b*-PLA (polylactic acid) nanoparticles increased progesterone levels in female rats and disrupted their ovulation cycle due to changes in insulin signaling.[42]

Nanoparticle properties, such as size and charge, play an essential role in affecting sex-related hormonal activity. For example, testosterone levels increased in male mice only after injecting carbon black nanoparticles smaller than 95 nm.[35] On the contrary, carbon nanotubes and nanoscale graphene oxide were not reported to affect hormone levels in male mice, suggesting that nanoparticles' effect on sex hormones involves

both size and composition of nanoparticles. Moreover, modifications on the surface of nanoparticles are also an important parameter. PEGylated amine-modified gold nanoparticles led to increased testosterone levels in male mice without harming fertility, while nanoparticles without the amine modification did not affect hormone levels.

Hormones can also influence the biodistribution of nanoparticles (Figure 2B). It was suggested that female hormones prevent aggregation of PEGylated gold nanoparticles in blood plasma, thus resulting in renal clearance, instead of the hepatic clearance seen in males.[25] Furthermore, female rats had a higher accumulation of silver nanoparticles in the kidneys compared to male rats. The suggested explanation for this difference is that testosterone is known to stimulate renal function; thus, elimination is faster in males.[20] Another example is a significantly higher nanoparticle accumulation in female brains following traumatic brain injury (TBI).[100] The blood–brain barrier (BBB) permeability is suggested to be related to sex-hormone levels, thus resulting in sex-dependent accumulation of nanoparticles.[100]

Hormonal profiles change during the menstrual cycle, thereby affecting the PK of nanoparticles (Figure 2C). During the female menstrual cycle, estrogen and progesterone levels alter hepatic enzyme activity, thereby affecting drug accumulation.[11b] As CYP3A4 metabolizes estrogen, the competition between estrogen and other CYP3A4 substrates during the luteal phase of the menstrual cycle or hormonal replacement therapy has been studied.[101] In later adulthood, females enter menopause, which spans over more than one-third of their lifetime. During this period, females may be prescribed hormone replacement therapy. It has been shown that premenopausal females had higher activity of CYP3A4 than postmenopausal females, but the effect of menopause on CYP3A4 was not reversed with hormone replacement.[102] On the other hand, other studies showed no significant differences in small molecule drug clearance between young females or menopausal females treated with hormone replacement therapy, arguing that menopause or hormone replacement therapy does not affect CYP3A4 activity.[103] As female sex hormones affect nanoparticle distribution and vice versa, it is possible that hormonal profile changes during the female lifetime will also influence nanoparticles' fate in the body. Hormonal contraceptives[104] and cross-sex hormonal therapy,[105] which affect the female hormonal cycle, can alter hepatic enzyme activity, affecting PK parameters; however, their effect on nanoparticles' biodistribution and activity needs further investigation.[106]

During pregnancy, the body undergoes multiple changes accompanied by an increased plasma volume that result in different drug concentrations in pregnant females compared to nonpregnant females.[11c] One study has shown that gold nanoparticles (1.4, 18, and 80 nm) accumulated preferentially in pregnant rats' reproductive systems compared to nonpregnant rats following i.v. administration.[27] Furthermore, during ovulation there is an increase in blood vessel density in the female reproductive system. This resulted in a twofold increase in nanoparticles' accumulation in mice ovaries and a 2.5-fold increase in the uterus.[16]

For males, changes in the hormonal profile such as lowered testosterone levels during later life may lead to erectile dysfunction and infertility.[107] Males that suffer from this condition are treated with hormonal therapies or medications, such as sildenafil,[108] which may affect nanoparticle distribution;[109] however, this aspect has not been studied yet.

6. Sex-Related Nanotoxicity

Sex may also affect nanoparticle toxicity and adverse effects. Doxorubicin-loaded polylactic-*co*-glycolic (PLGA) nanoparticles, administered intravenously, caused increased gastrointestinal toxicity and weight loss in female rabbits compared to males.[43] On the other hand, oral exposure to copper nanoparticles resulted in more significant hepatic, renal, splenic, and gastric toxicity in male mice compared to female mice.[32] One of the adverse reactions to copper nanoparticles was acute ulcers among males, attributed to excessive gastric acid secretion.[110] Another study found that the porosity level of silica nanoparticles affects their sex-related toxicity.[38] Dose-dependent toxicity of mesoporous spherical silica nanoparticles was found in both sexes; however, the maximum tolerated dose (MTD) was approximately twofold lower in male mice (95 mg kg^{-1}) compared to female mice (40 mg kg^{-1}).[38] This difference was explained by the sex-related immunologic response of T-helper cells (Th1 and Th2 cells) that play a major role in immune activation.[111] Chen et al. showed increased levels of hepatic enzymes aspartate transaminase (AST) and alanine transaminase (ALT) in male mice versus female mice following an injection of PEGylated gold nanoparticles, suggesting greater liver toxicity.[25] Contrarily, a sharp decrease in serum creatinine was found in female mice, which may indicate renal damage.[25] In the reproductive system, TiO_2 nanoparticles disrupted female follicles causing reproductive dysfunction and reduced sperm motility in males.[30] Gold nanoparticles were found to decrease sperm motility in male rats, while in the female reproductive system, they tended to accumulate in the uterus without any significant toxic effects.[112] Several studies conducted on male mice suggest that the accumulation of 20–30 nm carbon nanotubes in the testes results in reversible damage by reducing the seminiferous epithelium's thickness. However, there is no indication as to whether they affect sperm health or fertility.[36] Alternatively, in female mice, 10 nm carbon nanotubes affect pregnancy by delaying litter delivery.[113]

7. Conclusion and Outlook

Here, we review how sex differences affect the fate and activity of nanotechnologies in the body. While we sourced the literature, it was evident that the field of "sex nanotechnology" is underresearched. For example, the term "sex" or "gender" appeared in less than 0.1% of publications containing the term "nanoparticle" in the PubMed search engine. This comes with no surprise because females were previously excluded from clinical trials to preserve their fertility. While this logic is reasonable, it was already proven wrong for small molecule drugs as females were reincluded in clinical trials to reveal crucial sex-related drug differences.[4]

As nanotechnologies' use is relatively new, it is possible that only at this point there is enough evidence to state that we should

be aware of sex difference in this field as well. Nonetheless, custom-designed personalized nanotechnology systems are being implemented to overcome complex physiological parameters such as local tissue microenvironment[114] and personalized protein corona.[115] Thus, the nature of precision nanomedicine can also answer the needs raised by sex-dependent differences in nanotechnology. As we show herein, sex differences may have a great importance to the outcome of preclinical and clinical studies of nanotechnologies. The sex differences in small molecule drug disposition that were uncovered until now are also true for nanotechnologies; however, there are several factors we discuss here that are specific for nanoparticles such as protein corona, anti-PEG antibodies, and immune cell activation. These factors, affecting sex-specific nanoparticle biodistribution, metabolism, and excretion, should be accounted for in nanoparticle design and implementation. Although considering these factors may not always minimize sex differences, the awareness for nanotechnology-derived differences in response between sexes can improve patient care on its own. In accordance, the global effort to fight COVID-19 pandemic using nanotechnology[116] assured the importance of sex differences when comparing males and females throughout the vaccine development.[69] While no significant differences are shown in clinical trials, pooled meta-analysis reports increased efficacy and adverse events in male and female, respectively.[117] Cumulative data are incomplete, thus making it difficult to draw a comprehensive conclusion, yet this correlation should be referred to when designing a national vaccination strategy.

From the data gathered in this review, three main sex-specific considerations arise regarding nanoparticles' PK (Figure 3, Table 4). Females present an overall slower clearance rate of nanoparticles; this can possibly lead to higher toxicities albeit greater efficacy. As for clearance route, females have higher nanoparticles' distribution to the kidneys, hence dominant renal clearance. On the other hand, males have higher nanoparticles' distribution to the liver, leading to dominant hepatic clearance. These points can help improve dose adjustment according to sex in order to maximize drug efficacy and minimize nanotoxicity.

The consideration of sex as a nanotechnological biological parameter should take place at the early stages of nanomedicines development—at the cellular level where nanoparticles' uptake can be evaluated and in animal studies that provide a platform for PK and biodistribution assessment of sex differences. Unraveling the governing mechanisms that affect sex-related nanotechnology differences will encourage discoveries leading to improved medical nanotechnologies and more effective clinical implementation.

Acknowledgements

M.P. wishes to thank the Ministry of Science and Technology for the Shulamit Aloni Doctoral Fellowship. This work was supported by ERC-STG-2015-680242. The authors also acknowledge the support of the Technion Integrated Cancer Center (TICC), the Russell Berrie Nanotechnology Institute, the Lorry I. Lokey Interdisciplinary Center for Life Sciences & Engineering, and the Israel Ministry of Economy for a Kamin Grant (52752, 69230); the Israel Ministry of Science Technology and Space—Office of the Chief Scientist (3-11878); Israel Innovation Authority for Nofar Grant (67967), the Israel Science Foundation (1778/13, 1421/17, 1881/21); the Israel Ministry of Science & Technology (3-16963); the Israel Cancer Association (2015-0116); the German-Israeli Foundation for Scientific Research and Development for a GIF Young grant (I-2328-1139.10/2012); the European Union FP-7 IRG Program for a Career Integration Grant (908049); the Phospholipid Research Center Grant; the Louis family Cancer Research Fund, a Mallat Family Foundation Grant; the Unger Family Fund; and a Carrie Rosenblatt Foundation for Cancer Research. A.S. acknowledges Alon and Taub Fellowships. A grant number was added to the Israel Science Foundation on December 8, 2022 after initial publication online.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

hormones, nanotechnology, pharmacokinetics, sexes

Received: June 24, 2022
Revised: August 30, 2022
Published online: October 27, 2022

[1] M.-L. Pardue, T. M. Wizemann, *Exploring the Biological Contributions to Human Health: Does Sex Matter?* National Academies Press, Washington, DC **2001**.
[2] B. Pelaz, C. Alexiou, R. A. Alvarez-Puebla, F. Alves, A. M. Andrews, S. Ashraf, L. P. Balogh, L. Ballerini, A. Bestetti, C. Brendel, S. Bosi, M. Carril, W. C. W. Chan, C. Chen, X. Chen, X. Chen, Z. Cheng, D. Cui, J. Du, C. Dullin, A. Escudero, N. Feliu, M. Gao, M. George, Y. Gogotsi, A. Grünweller, Z. Gu, N. J. Halas, N. Hampp, R. K. Hartmann, et al., *ACS Nano* **2017**, *11*, 2313.
[3] C. Tannenbaum, R. P. Ellis, F. Eyssel, J. Zou, L. Schiebinger, *Nature* **2019**, *575*, 137.
[4] K. A. Liu, N. A. Dipietro Mager, *Pharm. Pract.* **2016**, *14*, 708.
[5] T. J. Nicolson, H. R. Mellor, R. R. Roberts, *Trends Pharmacol. Sci.* **2010**, *31*, 108.
[6] S. K. Lee, *BMB Rep.* **2018**, *51*, 167.
[7] J. Heinrich, GAO-01-286R, ed Office USGA, Washington, DC **2001**.
[8] V. Regitz-Zagrosek, *Sex and Gender Differences in Pharmacology*, Vol. *214*, Springer Science & Business Media, Berlin **2012**.
[9] U. F. a. D. A. (FDA), **2013**.
[10] J. Schwartz, *Clin. Pharmacol. Ther.* **2007**, *82*, 87.
[11] a) S. L. Klein, K. L. Flanagan, *Nat. Rev. Immunol.* **2016**, *16*, 626; b) O. P. Soldin, S. H. Chung, D. R. Mattison, *J. Biomed. Biotechnol.* **2011**, *2011*, 187103; c) O. P. Soldin, D. R. Mattison, *Clin. Pharmacokinet.* **2009**, *48*, 143; d) C. Taube, M. Schirner, *Agents Actions Suppl.* **1992**, *37*, 369.
[12] Q. Cheng, T. Wei, L. Farbiak, L. T. Johnson, S. A. Dilliard, D. J. Siegwart, *Nat. Nanotechnol.* **2020**, *15*, 313.
[13] Y. C. Barenholz, *J. Controlled Release* **2012**, *160*, 117.
[14] a) F. Dormont, R. Brusini, C. Cailleau, F. Reynaud, A. Peramo, A. Gendron, J. Mougin, F. Gaudin, M. Varna, P. Couvreur, *Sci. Adv.* **2020**, eaaz5466; b) G. J. Grant, Y. Barenholz, E. M. Bolotin, M. Bansinath, H. Turndorf, B. Piskoun, E. M. Davidson, *Anesthesiology* **2004**, *101*, 133; c) E. M. Davidson, Y. Barenholz, R. Cohen, S. Haroutiunian, L. Kagan, Y. Ginosar, *Anesthesia Analg.* **2010**, *110*, 1018.
[15] a) W. P. Caron, G. Song, P. Kumar, S. Rawal, W. C. Zamboni, *Clin. Pharmacol. Ther.* **2012**, *91*, 802; b) A. Rodallec, S. Benzekry,

B. Lacarelle, J. Ciccolini, R. Fanciullino, *Crit. Rev. Oncol. Hematol.* **2018**, *129*, 1.
[16] M. Poley, P. Mora-Raimundo, Y. Shammai, M. Kaduri, L. Koren, O. Adir, J. Shklover, J. Shainsky-Roitman, S. Ramishetti, F. Man, *ACS Nano* **2022**, *16*, 5246.
[17] G. Song, H. Wu, N. M. La-Beck, B. A. Zamboni, S. Srychor, M. M. Santory, S. R. Deither, W. C. Zamboni, *AACR* **2010**.
[18] a) N. M. La-Beck, B. A. Zamboni, A. Gabizon, H. Schmeeda, M. Amantea, P. A. Gehrig, W. C. Zamboni, *Cancer Chemother. Pharmacol.* **2012**, *69*, 43; b) W. Caron, G. Song, P. Kumar, S. Rawal, W. Zamboni, *Clin. Pharmacol. Ther.* **2012**, *91*, 802.
[19] Y. Ma, L. Song, Y. Lei, P. Jia, C. Lu, J. Wu, C. Xi, P. R. Strauss, D.-S. Pei, *Environ. Sci. Nano* **2018**, *5*, 740.
[20] W.-Y. Kim, J. Kim, J. D. Park, H. Y. Ryu, I. J. Yu, *J. Toxicol. Environ. Health, Part A* **2009**, *72*, 1279.
[21] M. S. Dong, J.-Y. Choi, J. H. Sung, J. S. Kim, K. S. Song, H. R. Ryu, J. H. Lee, I. S. Bang, K. An, H. M. Park, *Toxicol. Mech. Methods* **2013**, *23*, 437.
[22] Y. Xue, S. Zhang, Y. Huang, T. Zhang, X. Liu, Y. Hu, Z. Zhang, M. Tang, *J. Appl. Toxicol.* **2012**, *32*, 890.
[23] R. Barbir, W. Goessler, M. Ćurlin, V. Micek, M. Milić, B. Vuković, M. Milić, M. Ljubojević, D. Domazet Jurašin, I. Vinković Vrček, *Part. Part. Syst. Charact.* **2019**, *36*, 1900174.
[24] J. Gao, L. Lin, A. Wei, M. S. Sepúlveda, *Environ. Sci. Technol. Lett.* **2017**, *4*, 174.
[25] J. Chen, H. Wang, W. Long, X. Shen, D. Wu, S.-S. Song, Y.-M. Sun, P.-X. Liu, S. Fan, F. Fan, *Int. J. Nanomedi.* **2013**, *8*, 2409.
[26] W. Q. Li, F. Wang, Z. M. Liu, Y. C. Wang, J. Wang, F. Sun, *Small* **2013**, *9*, 1708.
[27] M. Semmler-Behnke, J. Lipka, A. Wenk, S. Hirn, M. Schäffler, F. Tian, G. Schmid, G. Oberdörster, W. G. Kreyling, *Part. Fibre Toxicol.* **2014**, *11*, 33.
[28] R. Tassinari, F. Cubadda, G. Moracci, F. Aureli, M. D'Amato, M. Valeri, B. De Berardis, A. Raggi, A. Mantovani, D. Passeri, *Nanotoxicology* **2014**, *8*, 654.
[29] G. Gao, Y. Ze, B. Li, X. Zhao, T. Zhang, L. Sheng, R. Hu, S. Gui, X. Sang, Q. Sun, *J. Hazard. Mater.* **2012**, *243*, 19.
[30] R. Wang, B. Song, J. Wu, Y. Zhang, A. Chen, L. Shao, *Int. J. Nanomed.* **2018**, *13*, 8487.
[31] X. Zhang, Y. Xu, L. Zhou, C. Zhang, Q. Meng, S. Wu, S. Wang, Z. Ding, X. Chen, X. Li, *Int. J. Environ. Res. Public Health* **2015**, *12*, 15692.
[32] Z. Chen, H. Meng, G. Xing, C. Chen, Y. Zhao, G. Jia, T. Wang, H. Yuan, C. Ye, F. Zhao, *Toxicol. Lett.* **2006**, *163*, 109.
[33] L. Kong, M. Tang, T. Zhang, D. Wang, K. Hu, W. Lu, C. Wei, G. Liang, Y. Pu, *Int. J. Mol. Sci.* **2014**, *15*, 21253.
[34] N. M. Kobyliak, T. M. Falalyeyeva, O. G. Kuryk, T. V. Beregova, P. M. Bodnar, N. M. Zholobak, O. B. Shcherbakov, R. V. Bubnov, M. Y. Spivak, *EPMA J.* **2015**, *6*, 12.
[35] S. Yoshida, K. Hiyoshi, T. Ichinose, H. Takano, S. Oshio, I. Sugawara, K. Takeda, T. Shibamoto, *Int. J. Androl.* **2009**, *32*, 337.
[36] Y. Bai, Y. Zhang, J. Zhang, Q. Mu, W. Zhang, E. R. Butch, S. E. Snyder, B. Yan, *Nat. Nanotechnol.* **2010**, *5*, 683.
[37] V. Serpooshan, S. Sheibani, P. Pushparaj, M. Wojcik, A. Y. Jang, M. R. Santoso, J. H. Jang, H. Huang, R. Safavi-Sohi, N. Haghjoo, *ACS Nano* **2018**, *12*, 2253.
[38] R. Mohammadpour, M. Yazdimamaghani, D. L. Cheney, J. Jedrzkiewicz, H. Ghandehari, *J. Controlled Release* **2019**, *304*, 216.
[39] Y. Hayashi, T. Miclaus, S. Murugadoss, M. Takamiya, C. Scavenius, K. Kjaer-Sorensen, J. J. Enghild, U. Strähle, C. Oxvig, C. Weiss, *Environ. Sci.: Nano* **2017**, *4*, 895.
[40] S. A. Barros, J. A. Gollob, *Adv. Drug Delivery Rev.* **2012**, *64*, 1730.
[41] H. Wu, J. R. Infante, V. L. Keedy, S. F. Jones, E. Chan, J. C. Bendell, W. Lee, B. A. Zamboni, S. Ikeda, H. Kodaira, *Eur. J. Clin. Pharmacol.* **2013**, *69*, 2073.
[42] E. Rollerova, J. Jurcovicova, A. Mlynarcikova, I. Sadlonova, D. Bilanicova, L. Wsolova, A. Kiss, J. Kovriznych, J. Kronek, F. Ciampor, *Reprod. Toxicol.* **2015**, *57*, 165.
[43] E. Pereverzeva, I. Treschalin, M. Treschalin, D. Arantseva, Y. Ermolenko, N. Kumskova, O. Maksimenko, V. Balabanyan, J. Kreuter, S. Gelperina, *Int. J. Pharm.* **2019**, *554*, 161.
[44] a) A. Sparreboom, C. D. Scripture, V. Trieu, P. J. Williams, T. De, A. Yang, B. Beals, W. D. Figg, M. Hawkins, N. Desai, *Clin. Cancer Res.* **2005**, *11*, 4136; b) P. K. Paik, L. P. James, G. J. Riely, C. G. Azzoli, V. A. Miller, K. K. Ng, C. S. Sima, R. T. Heelan, M. G. Kris, E. Moore, *Cancer Chemother. Pharmacol.* **2011**, *68*, 1331.
[45] T. Nadulski, F. Pragst, G. Weinberg, P. Roser, M. Schnelle, E.-M. Fronk, A. M. Stadelmann, *Ther. Drug Monit.* **2005**, *27*, 799.
[46] K. Knaub, T. Sartorius, T. Dharsono, R. Wacker, M. Wilhelm, C. Schön, *Molecules* **2019**, *24*, 2967.
[47] Z. Ş. Aksoyalp, D. Nemutlu-Samur, *Eur. J. Pharmacol.* **2021**, *912*, 174548.
[48] E. Vulpis, F. Giulimondi, L. Digiacomo, A. Zingoni, R. Safavi-Sohi, S. Sharifi, G. Caracciolo, M. Mahmoudi, *Mol. Pharm.* **2021**, *18*, 2448.
[49] a) S. Sharifi, G. Caracciolo, D. Pozzi, L. Digiacomo, J. Swann, H. E. Daldrup-Link, M. Mahmoudi, *Adv. Drug Delivery Rev.* **2021**, *174*, 337; b) M. J. Hajipour, H. Aghaverdi, V. Serpooshan, H. Vali, S. Sheibani, M. Mahmoudi, *Nat. Commun.* **2021**, *12*, 1.
[50] J.-L. J. Yang, R. Narayanamurthy, J. Y. Yager, L. D. Unsworth, *Nano Today* **2021**, *41*, 101292.
[51] D. Zhang, T. Jin, Y.-Q. Xu, Y.-F. Lu, Q. Wu, Y.-K. J. Zhang, J. Liu, *J. Circadian Rhythms* **2012**, *10*, 1.
[52] a) R. L. Seip, R. F. Zoeller, T. J. Angelopoulos, J. Salonia, C. Bilbie, N. M. Moyna, M. P. Miles, P. S. Visich, L. S. Pescatello, P. M. Gordon, *J. Appl. Physiol.* **2011**, *110*, 1021; b) A. Akinc, W. Querbes, S. De, J. Qin, M. Frank-Kamenetsky, K. N. Jayaprakash, M. Jayaraman, K. G. Rajeev, W. L. Cantley, J. R. Dorkin, *Mol. Ther.* **2010**, *18*, 1357.
[53] a) V. E. Kagan, N. V. Konduru, W. Feng, B. L. Allen, J. Conroy, Y. Volkov, I. I. Vlasova, N. A. Belikova, N. Yanamala, A. Kapralov, *Nat. Nanotechnol.* **2010**, *5*, 354; b) S. Nikulshin, I. Tolstikova, A. Bartule, D. Kviluna, D. Gravele, D. Gardovska, *Int. J. Labor. Hematol.* **2015**, *37*, 120.
[54] N. La-Beck, H. Wu, J. Infante, S. Jones, H. Burris III, V. Keedy, H. Kodaira, S. Ikeda, R. Ramanathan, W. Zamboni, *J. Clin. Oncol.* **2010**, *28*, 13003.
[55] O. P. Soldin, S. H. Chung, D. R. Mattison, *BioMed Res. Int.* **2011**, *2011*, 187103.
[56] G. Kolovou, D. Damaskos, K. Anagnostopoulou, D. V. Cokkinos, *Ann. Clin. Labor. Sci.* **2009**, *39*, 120.
[57] S. Wilhelm, A. J. Tavares, Q. Dai, S. Ohta, J. Audet, H. F. Dvorak, W. C. W. Chan, *Nat. Rev. Mater.* **2016**, *1*, 16014.
[58] B. R. Starling, P. Kumar, A. T. Lucas, D. Barrow, L. Farnan, L. Hendrix, H. Giovinazzo, G. Song, P. Gehrig, J. T. Bensen, *Cancer Chemother. Pharmacol.* **2019**, *83*, 61.
[59] a) P. Kramer, S. Kramer, G. Guan, *Arthritis Rheumatism* **2004**, *50*, 1967; b) P. Kramer, V. Winger, S. Kramer, *Mol. Cell. Endocrinol.* **2007**, *279*, 16.
[60] G. Aizik, N. Waiskopf, M. Agbaria, M. Ben-David-Naim, Y. Levi-Kalisman, A. Shahar, U. Banin, G. Golomb, *Nano Lett.* **2019**, *19*, 5844.
[61] J. W. Han, J.-K. Jeong, S. Gurunathan, Y.-J. Choi, J. Das, D.-N. Kwon, S.-G. Cho, C. Park, H. G. Seo, J.-K. Park, *Nanotoxicology* **2016**, *10*, 361.

[62] a) S. Tenzer, D. Docter, J. Kuharev, A. Musyanovych, V. Fetz, R. Hecht, F. Schlenk, D. Fischer, K. Kiouptsi, C. Reinhardt, *Nat. Nanotechnol.* **2013**, *8*, 772; b) Q. Wei, T. Becherer, S. Angioletti-Uberti, J. Dzubiella, C. Wischke, A. T. Neffe, A. Lendlein, M. Ballauff, R. Haag, *Angew. Chem. Int. Ed.* **2014**, *53*, 8004.

[63] a) P. E. Geyer, N. A. Kulak, G. Pichler, L. M. Holdt, D. Teupser, M. Mann, *Cell Syst.* **2016**, *2*, 185; b) K. Miike, M. Aoki, R. Yamashita, Y. Takegawa, H. Saya, T. Miike, K. I. Yamamura, *Proteomics* **2010**, *10*, 2678.

[64] W. Xiao, H. Gao, *Int. J. Pharm.* **2018**, *552*, 328.

[65] J. E. Blume, W. C. Manning, G. Troiano, D. Hornburg, M. Figa, L. Hesterberg, T. L. Platt, X. Zhao, R. A. Cuaresma, P. A. Everley, M. Ko, H. Liou, M. Mahoney, S. Ferdosi, E. M. Elgierari, C. Stolarczyk, B. Tangeysh, H. Xia, R. Benz, A. Siddiqui, S. A. Carr, P. Ma, R. Langer, V. Farias, O. C. Farokhzad, *Nat. Commun.* **2020**, *11*, 3662.

[66] Y. Liu, J. Wang, Q. Xiong, D. Hornburg, W. Tao, O. C. Farokhzad, *Acc. Chem. Res.* **2021**, *54*, 291.

[67] G. Baggio, A. Corsini, A. Floreani, S. Giannini, V. Zagonel, *Clin. Chem. Labor. Med.* **2013**, *51*, 713.

[68] a) C.-J. Chang, C.-H. Chen, B.-M. Chen, Y.-C. Su, Y.-T. Chen, M. S. Hershfield, M.-T. M. Lee, T.-L. Cheng, Y.-T. Chen, S. R. Roffler, *Nat. Commun.* **2017**, *8*; b) T. Tagami, K. Nakamura, T. Shimizu, T. Ishida, H. Kiwada, *J. Controlled Release* **2009**, *137*, 234; c) D. Simberg, S. M. Moghimi, in *Interaction of Nanomaterials with the Immune System*, (Eds: J. C. Bonner, J. M. Brown), Springer International Publishing, Cham **2020**; d) V. P. Vu, G. B. Gifford, F. Chen, H. Benasutti, G. Wang, E. V. Groman, R. Scheinman, L. Saba, S. M. Moghimi, D. Simberg, *Nat. Nanotechnol.* **2019**, *14*, 260.

[69] a) L. R. Baden, H. M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S. A. Spector, N. Rouphael, C. B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B. S. Graham, H. Bennett, R. Pajon, C. Knightly, B. Leav, W. Deng, H. Zhou, S. Han, M. Ivarsson, J. Miller, T. Zaks, *N. Engl. J. Med.* **2020**, *384*, 403; b) F. P. Polack, S. J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E. D. Moreira, C. Zerbini, R. Bailey, K. A. Swanson, S. Roychoudhury, K. Koury, P. Li, W. V. Kalina, D. Cooper, R. W. Frenck, L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K. U. Jansen, W. C. Gruber, *N. Engl. J. Med.* **2020**, *383*, 2603.

[70] a) B. Neun, Y. Barenholz, J. Szebeni, M. Dobrovolskaia, *Molecules* **2018**, *23*, 1700; b) T. J. Povsic, M. G. Lawrence, A. M. Lincoff, R. Mehran, C. P. Rusconi, S. L. Zelenkofske, Z. Huang, J. Sailstad, P. W. Armstrong, P. G. Steg, *J. Allergy Clin. Immunol.* **2016**, *138*, 1712.

[71] E. Chen, B.-M. Chen, Y.-C. Su, Y.-C. Chang, T.-L. Cheng, Y. Barenholz, S. R. Roffler, *ACS Nano* **2020**, *14*, 7808.

[72] B.-M. Chen, Y.-C. Su, C.-J. Chang, P.-A. Burnouf, K.-H. Chuang, C.-H. Chen, T.-L. Cheng, Y.-T. Chen, J.-Y. Wu, S. R. Roffler, *Anal. Chem.* **2016**, *88*, 10661.

[73] A. Gabizon, J. Szebeni, *ACS Nano* **2020**, *14*, 7682.

[74] M. Mahmoudi, *Trends Biotechnol.* **2018**, *36*, 755.

[75] B. A. Aguado, C. J. Walker, J. C. Grim, M. E. Schroeder, D. Batan, B. J. Vogt, A. G. Rodriguez, J. A. Schwisow, K. S. Moulton, R. M. Weiss, *Circulation* **2022**, *145*, 513.

[76] P. Lista, E. Straface, S. Brunelleschi, F. Franconi, W. Malorni, *J. Cell. Mol. Med.* **2011**, *15*, 1443.

[77] R. Duncan, S. C. Richardson, *Mol. Pharm.* **2012**, *9*, 2380.

[78] W. C. Zamboni, S. Strychor, E. Joseph, D. R. Walsh, B. A. Zamboni, R. A. Parise, M. E. Tonda, Y. Y. Ning, C. Engbers, J. L. Eiseman, *Clin. Cancer Res.* **2007**, *13*, 7217.

[79] S. Ferber, R. J. Gonzalez, A. M. Cryer, U. H. von Andrian, N. Artzi, *Adv. Mater.* **2019**, 1903847.

[80] R. Shandilya, N. Pathak, N. K. Lohiya, R. S. Sharma, P. K. Mishra, *Clin. Exp. Reprod. Med.* **2020**, *47*, 245.

[81] U. M. Zanger, M. Schwab, *Pharmacol. Ther.* **2013**, *138*, 103.

[82] X. Yang, B. Zhang, C. Molony, E. Chudin, K. Hao, J. Zhu, A. Gaedigk, C. Suver, H. Zhong, J. S. Leeder, *Genome Res.* **2010**, *20*, 1020.

[83] M. Gandhi, F. Aweeka, R. M. Greenblatt, T. F. Blaschke, *Annu. Rev. Pharmacol. Toxicol.* **2004**, *44*, 499.

[84] F. Wang, Y. Wu, J. Zhang, H. Wang, X. Xie, X. Ye, D. Peng, W. Chen, *Drug Metab. Dispos.* **2019**, *47*, 364.

[85] R. L. Seip, R. F. Zoeller, T. J. Angelopoulos, J. Salonia, C. Bilbie, N. M. Moyna, M. P. Miles, P. S. Visich, L. S. Pescatello, P. M. Gordon, G. J. Tsongalis, L. Bausserman, P. D. Thompson, *J. Appl. Physiol.* **2011**, *110*, 1021.

[86] M. Li, K. T. Al-Jamal, K. Kostarelos, J. Reineke, *ACS Nano* **2010**, *4*, 6303.

[87] S. Nikulshin, I. Tolstikova, A. Bartule, D. Kviluna, D. Gravele, D. Gardovska, *Int. J. Labor. Hematol.* **2015**, *37*, 120.

[88] A. M. Stephen, H. Wiggins, H. Englyst, T. Cole, B. Wayman, J. Cummings, *Br. J. Nutr.* **1986**, *56*, 349.

[89] S. H. Bakhru, S. Furtado, A. P. Morello, E. Mathiowitz, *Adv. Drug Delivery Rev.* **2013**, *65*, 811.

[90] A. des Rieux, V. Fievez, M. Garinot, Y.-J. Schneider, V. Préat, *J. Controlled Release* **2006**, *116*, 1.

[91] A. C. Freire, A. W. Basit, R. Choudhary, C. W. Piong, H. A. Merchant, *Int. J. Pharm.* **2011**, *415*, 15.

[92] S. Thamphiwatana, V. Fu, J. Zhu, D. Lu, W. Gao, L. Zhang, *Langmuir* **2013**, *29*, 12228.

[93] a) A. Makhlof, Y. Tozuka, H. Takeuchi, *Eur. J. Pharm. Sci.* **2011**, *42*, 445; b) H. Yuan, C.-Y. Chen, G.-H. Chai, Y.-Z. Du, F.-Q. Hu, *Mol. Pharm.* **2013**, *10*, 1865.

[94] J. G. Markle, D. N. Frank, S. Mortin-Toth, C. E. Robertson, L. M. Feazel, U. Rolle-Kampczyk, M. Von Bergen, K. D. McCoy, A. J. Macpherson, J. S. Danska, *Science* **2013**, *339*, 1084.

[95] A. Berardi, F. B. Bombelli, *Oral Delivery of Nanoparticles–Let's Not Forget About the Protein Corona*, Taylor & Francis, Milton Park, Oxfordshire, UK **2019**.

[96] a) *J. Womens Health Gend. Based Med.* **2001**, *10*, 433; b) B. S. McEwen, T. A. Milner, *J. Neurosci. Res.* **2017**, *95*, 24.

[97] M. J. Legato, *Principles of Gender-Specific Medicine: Gender in the Genomic Era*, Academic Press, Cambridge MA **2017**.

[98] C.-C. Hou, J.-Q. Zhu, *Oncotarget* **2017**, *8*, 109799.

[99] G. Gao, Y. Zea, B. Lia, X. Zhao, T. Zhang, L. Sheng, R. Hu, S. Gui, X. Sang, Q. Sun, J. Cheng, Z. Cheng, L. Wang, M. Tang, F. Hong, *J. Hazard. Mater.* **2012**, *243*:19.

[100] V. N. Bharadwaj, C. Copeland, E. Mathew, J. Newbern, T. R. Anderson, J. Lifshitz, V. D. Kodibagkar, S. E. Stabenfeldt, *Tissue Eng. Part A* **2020**, *26*, 688.

[101] K. L. Bigos, B. G. Pollock, B. A. Stankevich, R. R. Bies, *Gender Med.* **2009**, *6*, 522.

[102] J. C. Fleishaker, L. K. Pearson, P. G. Pearson, L. C. Wienkers, N. K. Hopkins, G. R. Peters, *J. Clin. Pharmacol.* **1999**, *39*, 260.

[103] R. Z. Harris, S. M. Tsunoda, P. Mroczkowski, H. Wong, L. Z. Benet, *Clin. Pharmacol. Ther.* **1996**, *59*, 429.

[104] C. A. Frye, *Neurology* **2006**, *66*, S29.

[105] R. Vita, S. Settineri, M. Liotta, S. Benvenga, F. Trimarchi, *Maturitas* **2018**, *107*, 92.

[106] a) K. Laine, G. Tybring, L. Bertilsson, *Clin. Pharmacol. Ther.* **2000**, *68*, 151; b) N. L. Benowitz, *N. Engl. J. Med.* **2010**, *362*, 2295; c) G. P. Stoehr, P. D. Kroboth, R. P. Juhl, D. B. Wender, J. P. Phillips, R. B. Smith, *Clin. Pharmacol. Ther.* **1984**, *36*, 683; d) K. L. Slayter, E. A. Ludwig, K. H. Lew, E. Middleton Jr, J. J. Ferry, W. J. Jusko, *Clin. Pharmacol. Ther.* **1996**, *59*, 312; e) X.-D. Wang, J.-L. Li,

Q.-B. Su, S. Guan, J. Chen, J. Du, Y.-W. He, J. Zeng, J.-X. Zhang, X. Chen, M. Huang, S.-F. Zhou, *Br. J. Clin. Pharmacol.* **2009**, *67*, 255.
[107] E. A. Jannini, E. Screponi, E. Carosa, M. Pepe, F. Lo Giudice, F. Trimarchi, S. Benvenga, *Int. J. Androl.* **1999**, *22*, 385.
[108] R. Shabsigh, J. Kaufman, C. Steidle, H. Padma-Nathan, *J. Urol.* **2008**, *179*, S97.
[109] a) M. Doumas, A. Lazaridis, N. Katsiki, V. Athyros, *Curr. Drug Targets* **2015**, *16*, 420; b) K. Greish, M. Fateel, S. Abdelghany, N. Rachel, H. Alimoradi, M. Bakhiet, A. Alsaie, *J. Drug Targeting* **2018**, *26*, 610.
[110] C. V. Fletcher, E. P. Acosta, J. M. Strykowski, *J. Adolesc. Health* **1994**, *15*, 619.
[111] S. A. Huber, B. Pfaeffle, *J. Virol.* **1994**, *68*, 5126.
[112] R. Brohi, L. Wang, H. Talpur, D. Wu, F. Khan, D. Bhattarai, Z. Rehman, F. Farmanullah, L. Huo, *Front. Pharmacol.* **2017**, *8*, 606.
[113] K. S. Hougaard, P. Jackson, Z. O. Kyjovska, R. K. Birkedal, P.-J. De Temmerman, A. Brunelli, E. Verleysen, A. M. Madsen, A. T. Saber, G. Pojana, *Reprod. Toxicol.* **2013**, *41*, 86.
[114] N. Oliva, S. Unterman, Y. Zhang, J. Conde, H. S. Song, N. Artzi, *Adv. Healthcare Mater.* **2015**, *4*, 1584.
[115] J. Ren, R. Cai, J. Wang, M. Daniyal, D. Baimanov, Y. Liu, D. Yin, Y. Liu, Q. Miao, Y. Zhao, *Nano Lett.* **2019**, *19*, 4692.
[116] a) Z. Tang, X. Zhang, Y. Shu, M. Guo, H. Zhang, W. Tao, *Nano Today* **2021**, *36*, 101019; b) Z. Tang, N. Kong, X. Zhang, Y. Liu, P. Hu, S. Mou, P. Liljeström, J. Shi, W. Tan, J. S. Kim, Y. Cao, R. Langer, K. W. Leong, O. C. Farokhzad, W. Tao, *Nat. Rev. Mater.* **2020**, *5*, 847.
[117] A. Bignucolo, L. Scarabel, S. Mezzalira, J. Polesel, E. Cecchin, G. Toffoli, *Vaccines* **2021**, *9*, 825.

Maria Poley is a Ph.D. candidate in Prof. Schroeder's Laboratory for Targeted Drug Delivery and Personalized Medicine at the Technion—Israel Institute of Technology. She earned her B.Sc. degree in biochemical engineering from the Technion—Israel Institute of Technology. She has two main research focuses. The first is the effect of nanotechnology on the female reproductive system and fertility, and the second is targeted drug delivery to treat Parkinson's disease.

Avi Schroeder is a professor of chemical engineering at the Technion—Israel Institute of Technology, where he heads the Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies. Dr. Schroeder conducted his postdoctoral studies at the Massachusetts Institute of Technology, and his Ph.D. jointly at the Hebrew and Ben Gurion Universities. His research interests are at the interface of nanotechnology and personalized medicine.