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Sex Influences the Safety and Therapeutic Efficacy of Cardiac Nanomedicine Technologies

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Nanomedicine technologies are being developed for the prevention, diagnosis, and treatment of cardiovascular disease (CVD), which is the leading cause of death worldwide. Before delving into the nuances of cardiac nanomedicine, it is essential to comprehend the fundamental sex-specific differences in cardiovascular health. Traditionally, CVDs have been more prevalent in males, but it is increasingly evident that females also face significant risks, albeit with distinct characteristics. Females tend to develop CVDs at a later age, exhibit different clinical symptoms, and often experience worse outcomes compared to males. These differences indicate the need for sex-specific approaches in cardiac nanomedicine. This Perspective discusses the importance of considering sex in the safety and therapeutic efficacy of nanomedicine approaches for the prevention, diagnosis, and treatment of CVD.

1. Introduction

Cardiovascular diseases (CVDs) remain a significant cause of mortality worldwide, affecting both men and women.[1] However, it is well established that sex differences exist in the prevalence, presentation, and outcomes of cardiovascular conditions.[2] In the last decade, the field of nanomedicine has shown tremendous potential in improving current clinical approaches to diagnose and treat CVDs.[3] As sex is an important factor in the safety and therapeutic efficacy of nanomedicine,[4] it is crucial to consider the influence of sex differences when developing and implementing cardiac nanomedicine strategies to ensure optimal therapeutic outcomes. Understanding these differences will enable clinicians and the entire nanomedicine community to tailor treatments and optimize outcomes for both male and female patients.

Sex disparities are increasingly reported in various aspects of nanomedicine, including nanoparticle (NP) distribution, pharmacokinetics, immune responses, and cellular uptake.[5,6] Two major aspects of biological sex can substantially affect the safety and efficacy of nanotechnology-based therapies, including the circulating biomolecules and biosystem (e.g., cell) characteristics and function (**Table 1**).[4]

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Table 1. Representative examples of differences in hormonal and biosystem functions/characteristics between males and females that can affect the safety and therapeutic efficacy of identical nanoparticles.

		Biological difference		Functional difference	
		Male	Female	Male	Female
Estrogen Effect	Plasma lipid level	• Higher LDL • Lower HDL, leptin adiponectin • Higher LP-PLA2	• Lower LDL • Higher HDL, leptin, adiponectin • Lower LP-PLA2	• Lower mitochondria efficacy • More oxidative stress and cardiac damage • More likely to swell during ischemia injury • More inflammation with vascular wall	• Higher mitochondria efficacy • Less oxidative stress and cardiac damage • Less likely to swell during ischemia injury • Less inflammation with vascular wall • Myocardium mitochondria are less likely to swell • Upregulated oxidative metabolism
	PI3K pathway	• Lower Activation	• Higher activation		
	Calcium handling	• Lower expression of Calcium • Lesser Calcium uptake • Channels sodium-calcium exchanges	• Higher expression of calcium channels and sodium-calcium exchanges • Reduction in Calcium loading		
Energy Metabolism	Mitochondrial permeability transition pore (MPTP)	• Greater sensitivity for MPTP opening	• Less sensitivity for MPTP opening		
Vasculature Function	Nitrate oxide (NO) release	• NO synthase is lower	• NO synthase is higher	• Higher chances of endothelial dysfunction	• Better response to myocardium stretching • Functional endothelium regulation
	Renin–angiotensin–aldosterone system (RAAS)	• Higher RAAS activity • Higher ACE activity • Less likely to have similar beneficial response to ACE inhibitor	• Lower RAAS activity • Lower ACE activity • Better response to ACE inhibitor		

(*Continued*)

Table 1. (Continued).

		Biological difference		Functional difference	
		Male	Female	Male	Female
	Endothelial function	• Higher levels of sESAM and SDMA, and lower levels of homoarginine • Lower levels of natriuretic peptides levels	• Lower levels of natriuretic peptides levels		Higher levels of natriuretic peptides levels
Post Injury Response	Peri-infarct apoptosis	Post Injury Response	• Activation of Akt to prevent apoptosis • Lower expression of apoptosis genes	• Higher rate of apoptosis • More adapted and severe structural remodeling • No enhancement in heart contractibility	• Lower rate of apoptosis • Greater left ventricle (LV) systolic function • Minimal dynamic changes in LV geometry
	ECM remodeling	• Less angiogenesis and ECM remodeling	• Upregulated angiogenesis and ECM remodeling		
	LV chamber remodeling	• Lower density of cardiomyocytes	• Higher density of cardiomyocytes • Lower number of inflammatory cytokines and		
Contractability	Stronger cellular contraction	• Larger cardiomyocytes	• Higher percentage of ventricular cardiomyocytes • Higher density of smaller cardiomyocytes	• Higher density of smaller cardiomyocytes	• Higher ejection fraction • Higher contraction frequency
Plaque Change		• More inflammatory and migratory macrophages	• Less inflammatory and migratory macrophages due to higher anti-inflammatory signaling	• More likely to have plaque rupture	• More likely to have plaque erosion

2. The Effects of Sex on Biomolecular Corona in Cardiac Nanomedicine Technologies

Circulating biomolecules are important in the formation of the biomolecular corona at the surface of NPs.[7] More specifically, when NPs enter a biological fluid, various types of biomolecules interact with their surface and create a biomolecular shell (i.e., biomolecular corona). The abundance, conformation, and exposure sites of these biomolecules on the NP surface dictate their interactions with biosystems.[8] Various circulating biomolecules are present during the occurrence and progress of CVD in males versus females; for example, significant differences were found in 30 biomarkers between male versus female CVD patients, which were mostly related to the endothelial dysfunction, recruitment of inflammatory cells, and cardiac injuries.[9] Another study that probed sex differences in circulating biomarkers of CVD patients revealed that out of 61 biomarkers examined, 37 were present at higher levels in females compared to males.[10] The most prominent sex-specific biomarkers in females included leptin, ceruloplasmin, and hemopexin, which are associated with inflammation, immune response, and adiposity, respectively. The other 24 biomarkers were found at higher levels in males compared to females, with the most significant differences observed in the concentrations of platelet and fibrosis markers (e.g., GMP140, sGP130, BCHE, CD56, and myoglobin), which are related to fibrosis and platelet homeostasis pathways.[10]

Other studies demonstrate that females have higher levels of fibrinogen than males,[11,12] and elevated levels are associated with greater risk of ischemic injury, hypercoagulation, vascular damage, and thrombosis.[13] Plasminogen activator inhibitor type-1 (PAI-1) plays an important role in modulating inflammation, hemostasis, and tissue remodeling. Higher levels of PAI-1 are associated with significantly increased risk of ischemic infarct in both males and females.[14]

Levels of several biomolecules that show variations between the sexes can alter due to CVD occurrence and progression. For example, endothelial dysfunction, which is a type of coronary artery disease, perturbs the secretion levels of endothelial cell-selective adhesion molecule (sESAM), symmetric dimethylarginine (SDMA), and homoarginine (sESAM and SDMA levels are inversely associated with endothelial health).[15] Females have lower levels of sESAM and SDMA, and higher levels of homoarginine.[16] As another example, B-type natriuretic peptide and atrial natriuretic peptide are released by the myocardium when cardiomyocytes stretch, and levels of natriuretic peptides levels were found to be consistently higher in females compared to males.[17] Besides circulating biomolecules, sex-specific secretion of paracrine factors and other types of small molecules by local cells can alter the composition of the biomolecular corona.[18,19] In addition, molecules that can change the biological fluid balance and pressure can also affect the composition of the biomolecular corona. For example, nitrous oxide (NO) release relaxes vessel walls and the expression of NO synthase (eNOS) is significantly higher in the female heart.[20] The renin–angiotensin–aldosterone system (RAAS) regulates multiple organ vasculature by controlling the fluid balance and pressure. There is evidence that sexual dimorphism affects plasma levels of RAAS proteins and receptors. Estrogen might help protect against hypertension and induce hypertrophic cardiac remodeling by regulating the RAAS proteins in various organ systems.[21] Specifically, males have higher RAAS activity than females, likely caused by androgen stimulation.

Based on the above discussions, one can expect that the biomolecular corona formed on the surface of identical NPs would be substantially different between males and females which, in turn, could cause different effects on the safety and therapeutic efficacy of NPs in males versus females. Studies probing the biomolecular coronas of various NPs in biological fluids (e.g., plasma) have indeed revealed substantial sex-specific differences in coronas in several biomolecular categories, including the hormones and lipids.[22,23]

2.1. Hormones

Sex hormones, primarily estrogen and testosterone, have important and well-documented effects on cardiac response to CVDs. These hormonal disparities are responsible for divergent cellular responses to cardiac stressors and may also contribute to the sex differences observed in CVDs. For example, heart failure is lethal for 37% of males and 33% of females within two years of diagnosis. Six years after diagnosis, this difference in mortality rate increases to 82% and 67% in males versus females, respectively[24]; however, females exhibit a worse prognosis and a higher risk of disease after menopause, indicating the protective role of estrogen (E2) against CVD.[25] Early menopause, from either natural or surgical causes, increases the risk of ischemic heart disease in females.[26] The endocrine hormone E2 is found at higher levels in females (predominantly synthesized by the ovaries until menopause). Although extragonadal E2 has mainly paracrine effects, it becomes the main source of endocrine E2 in post-menopausal females.[27] The cardioprotective role of gonadal E2 was confirmed by the finding that female rats exhibited smaller infarcts than ovariectomized female rats.[28]

E2 incorporation within the biomolecular corona of NPs and the exposure of its active site to biosystems could result in substantial variation in cellular response to NPs in males versus females. For example, E2 binding to intracellular receptors induces dimerization of the complex, which is then able to bind to the promoter sites on DNA called estrogen response elements (ERE).[29] In this way, E2 regulates protein expression and exerts its other effects. Females express E2 receptors (ER) in their vessels but this expression decreases with age.[30] This further explains sex differences in the prevalence of ischemic cardiomyopathy and the variations between pre- and post-menopausal females.

2.2. Lipids

It has recently been demonstrated that lipids (e.g., cholesterol) have substantial effects on the composition and thickness of the biomolecular corona.[31] Elevated cholesterol levels can increase the participation of apolipoproteins and decrease the involvement of complement proteins in the biomolecular corona.[31] In addition, higher cholesterol can substantially reduce the thickness of the biomolecular corona, which can reduce its shielding effect on the NP.[31,32] Therefore, sex-specific variation in lipid

levels can substantially change the biomolecular corona formed on identical NPs, altering their safety and therapeutic efficacy.

Clinical studies have revealed lower levels of low-density lipoproteins (LDL) and higher levels of high-density lipoproteins (HDL) and leptin in females versus males, between the ages of 20 and 55 years.[33] However, after age 55, and mainly due to menopause, HDL cholesterol levels in females decrease rapidly and LDL levels rise.[34] Recent clinical studies have also demonstrated that the same degree of lipid profile improvement produces a greater reduction in CVD risk in females than in males.[35,36] Healthy females also have significantly higher adiponectin levels,[37] and these higher levels are inversely associated with CVD risk in general.[38] Lipoprotein-associated phospholipase-A2 (LP-PLA2) interacts with LDL and contributes to inflammation in vascular walls.[39,40] LP-PLA2 levels have been shown to be lower in women, which may be associated with the sex-specific risk of developing CVD.

Overall, careful consideration of alterations in the biomolecular corona profiles of NPs, caused by sex-specific biomolecules such as hormones and lipids, is essential in the development and administration of nanomedicine technologies. Factors such as menopause and pregnancy can greatly alter hormone and lipid profiles in females and should therefore be taken into consideration in the design, development, and administration of future nanomedicine therapies.

3. Sex and Biosystems' Characteristics/Functions – Influence on Cardiac Nanomedicine Technologies

The second important factor that affects the fate of identical NPs in males versus females is the biosystem characteristics and functions. There are significant differences between male and female hearts, from size and structure to gene expression, cellular composition, and function.[41] Left ventricular (LV) mass is similar for infants of both sexes, but after puberty, men can have up to ≈40% higher LV mass.[42] This is unsurprising since male hearts typically have larger cardiomyocytes, while female hearts possess a higher density of smaller cardiomyocytes.[43] An opposite trend is observed for cardiac function and LV ejection fraction (LVEF), where women have higher LVEF than men of the same age.[44] At the molecular level, for example, RNA sequencing and microarray datasets of human heart tissue reveal 300–400 sexually differentiated genes, expressed in all cell populations within the heart.[45,46] These genes include MYL4 (important for contractile function via increasing force production),[47] SCN10A (associated with hypertrophic cardiomyopathy),[48] and NPPB (associated with cardiomyopathy).[49] At the cellular level, on average, healthy female hearts contain a higher percentage of ventricular cardiomyocytes (56% ± 9%) compared to the male hearts (47% ± 11%).[50] These differences contribute to variations in heart size, contractility, and electrical properties, potentially affecting overall cardiac function.[43] Molecular, cellular, and structural variations in CVD-related biosystems between males and females can alter the effects of biological pathways (e.g., intracellular trafficking) on identical NPs in males versus females.

3.1. Functional Differences

Functional differences of biosystems between sexes can substantially affect the safety and therapeutic efficacy of NPs in male versus female patients. However, the role of sex in biosystems has rarely been the subject of the cardiac nanomedicine literature, for example, in detection and removal of atherosclerotic plaques.[51] In the initial stages of plaque formation, endothelial cells are first activated via several stimuli, including genetics, environmental, biochemical, and inflammatory cues. Activated endothelial cells recruit monocytes into the intima, where they take up lipids and mature into foam cells. In the later stages of plaque formation, more smooth muscle cells divide and increase the formation of extracellular matrix (ECM). The accumulated ECM forms a fibrous cap, attaching the plaque to the endothelial wall and allowing the accumulation of lipids and cholesterol to form a necrotic core.[52] An unstable or vulnerable plaque constitutes high risk due to its tendency to embolize. Macrophages can not only attach to the fibrous cap and release proteolytic enzymes to dissolve the cap but may also engulf lipids from the necrotic core and form foam cells. This ultimately activates other inflammatory cells that release further proteolytic enzymes and promote plaque formation.[53,54] Consequently, macrophages are the main determinants of the detachment and mobilization of plaques.[55] The sexual dimorphism in macrophage activity indicates sexual differences in vulnerable plaque formation. Compared to males, female macrophages are less inflammatory and migratory due to their higher levels of anti-inflammatory markers.[56] NPs such as superparamagnetic iron oxide NPs[57] can substantially affect the functionality and inflammatory cytokine release of macrophages; thus, the impact of the macrophage sex on their interactions with NPs would be essential in formulating applications involving plaque imaging and/or treatment. This is mainly because NPs may exacerbate inflammation, further damaging vessels and increasing the risk of plaque disruption[58] in a sex-specific manner. Such considerations enable clinicians and the nanomedical community to design safer nanomedicine products and adjust dosage in a sex-specific manner.

Both clinical and experimental investigations have demonstrated that females undergo a stronger beneficial effect of remodeling and the adaptive response to myocardial infarction compared to males. Females exhibit better functional recuperation, which has been associated with a reduction in the infarct size, fewer neutrophils, and diminished production of inflammatory cytokines following ischemic injury. More specifically, females exhibit relatively subtle changes in the left ventricle (LV) geometry after ischemic injury, while males undergo more dramatic changes in myocardial structure. However, these more pronounced changes in geometry post-infarction do not enhance the contractility in male hearts.[59] On the other hand, females have fewer apoptotic cardiomyocytes. Clinical analysis of apoptosis-related gene expression revealed that males exhibited 10-fold higher rate of apoptosis than females in the peri-infarct region.[60] Post-mortem analyses of myocardium also suggest that males and females experience different regulation of the apoptotic pathways.[61] Following infarction, females present with better LV function and cardiac performance compared to males. Females also have higher density of cardiomyocytes following remodeling, which is partially explained by lower

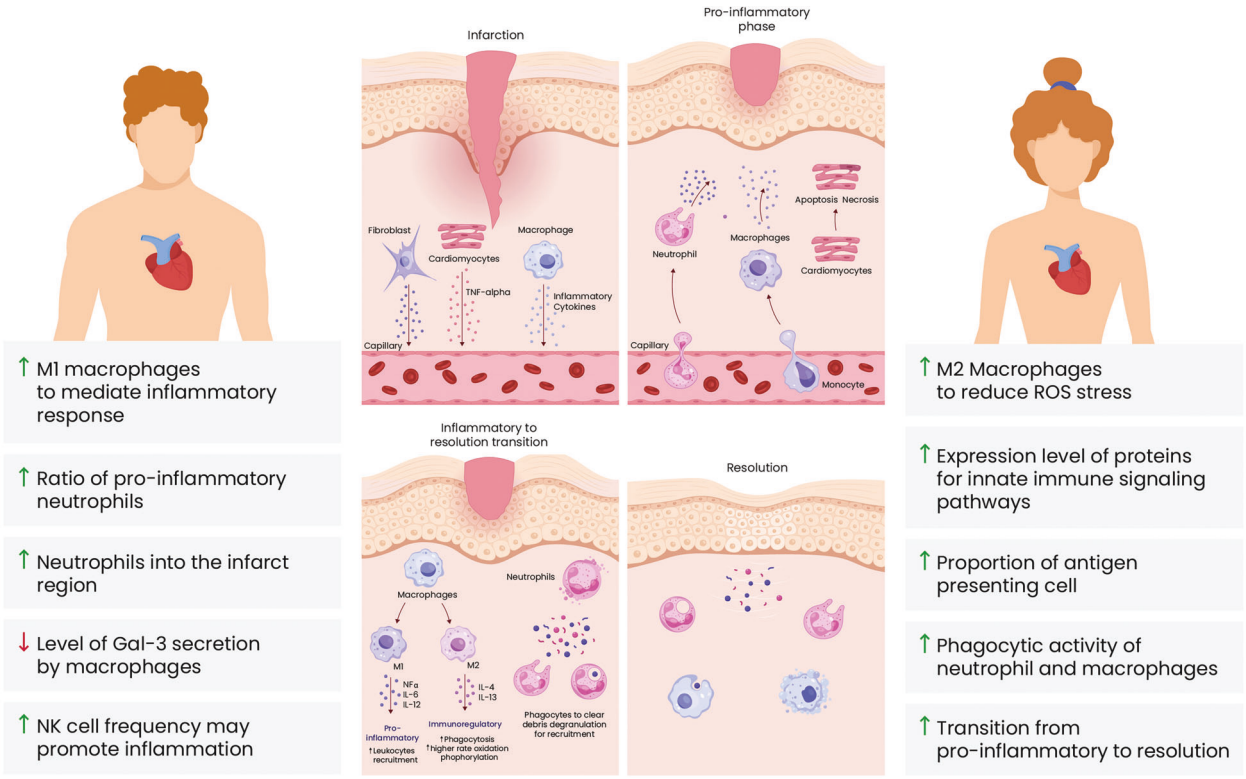

Figure 1. Schematic illustration of major differences between male and female immune system response to myocardial infarction.

apoptosis.[62] Such sex-specific differences in post-infarction responses reflect the superiority of female remodeling, i.e., avoiding more severe changes and maintaining higher numbers of functional cardiomyocytes.

3.2. Structural Differences

At the cellular level, cardiac cells exhibit sexual dimorphism in mitochondrial activity. Mitochondria in males have lower Ca^{2+} uptake than those in females. Moreover, females have lesser mitochondria but with higher efficiency, thus creating fewer reactive oxygen species (ROS) and thus less oxidative stress and cardiac damage.[63] Additionally, the E2 receptors on mitochondria in cardiomyocytes further reflect sexual dimorphism at the cellular level. Mitochondrial function plays an important role in ischemic injury by regulating mitochondrial permeability transition pore (MPTP) opening. Myocardial mitochondria are less likely to swell during ischemic injury and high calcium loading in females, and males have a lower capacity for calcium retention and greater sensitivity for MPTP opening. Some types of diagnostic and therapeutic NPs (e.g., metallic-based NPs) are known to produce ROS inside cells.[64] The use of these NPs may more severely aggravate overall ROS concentration in male compared to female cardiomyocytes. ROS may also alter membrane permeability and gene expression, hindering the NP uptake.

In addition, cellular characteristics (e.g., size) also play a crucial role in NP uptake and intracellular trafficking. Chromosomes have repeated ends called telomeres, a small portion of which is lost at each cell division. Telomerase is an enzyme that replenishes the lost telomeres. One of the molecular-level sex-specific differences in cardiomyocyte is the discrepancy in telomerase functionality with age. As males age, their telomerase activity decreases, while it increases in females. As a result, the number and size of cardiomyocytes decrease in males but are maintained in females.[65,66] Higher numbers of cardiomyocytes would mean more surface area and more cell-cell communication via gap junctions, potentially providing more sites for NP adhesion and uptake in females as compared to males. More cell-cell communication can also aid in NPs mobilization between cardiac cells.

3.3. Sex-Specific Immune Response

CVD involves endothelial dysfunction and vascular inflammation, which activate the innate immune responses (**Figure 1**). After myocardial infarction, for instance, macrophages, neutrophils, and mast cells rapidly respond by activating innate immune mechanisms, including the Toll-Like Receptor-mediated pathways, the complement cascade, and secreting signals such as chemokines and cytokines to recruit immune cells, contain inflammation, and promote cardiac tissue repair. Careful consideration of sex differences in the initiation of inflammatory and reparative response in the infarcted area is essential for understanding the sex-specific differences in the infarct healing process in males and females.[67]

Neutrophils are recruited to the newly infarcted myocardium during the first few hours via cytokine and chemokine signaling, leading to local inflammation, necrotic tissue removal, and tissue

degradation. Macrophages are resident immune cells that play major roles in engulfment of the dead cells and timely resolution of inflammation after infarction. The switch from the inflammatory response to resolution via the dual functionality of innate immune cells is a requirement for a good prognosis.[68] Higher mortality rates in males after infarction may be explained by a higher number of neutrophils infiltrating the infarct and pre-infarct regions, with a higher percentage of pro-inflammatory neutrophils in males than in females and a slower transition from proinflammatory stage to resolution.[69] Females also benefit from stronger phagocytic activity of neutrophils and macrophages and more efficient antigen presentation than males, resulting in a more efficient inflammatory response.[70,71] Males exhibit more macrophage-mediated inflammation with higher levels of classically activated M1, whereas females present with higher levels of M2. A higher rate of oxidation phosphorylation of M2 could also explain the superior female resistance to oxidative damage.[72] Gal-3, secreted by macrophages, is thought to manifest in cardiac fibrosis and heart failure via initiating adverse ventricular modeling following infarction.[73,74] Upregulation of Gal-3 contributes to cardiac dysfunction and adverse remodeling, while downregulation of Gal-3 reduces fibrosis.[75–78] Multiple studies have shown that Gal-3 concentrations are consistently higher in females.[79,80]

The adaptive immune response also differs between sex. Lower numbers of circulating lymphocytes count are predictive of poor outcome in ischemic myopathy.[81] The primary protective effect of lymphocytes is mediated through T-reg cells, which suppress inflammatory processes and activate fibrosis a few days following infarction. CD4+ T-cells are attracted to the injured myocardium and may participate in cytokine release and infiltration, which may worsen myocardial inflammation.[68] However, CD4+ T-cells also promote macrophage recruitment and arteriogenesis.[82] An animal study showed that a deficiency of CD8+ T cells is associated with better recovery of heart functionality but impaired scar formation; CD8+ T lymphocytes release Granzyme B, leading to cardiomyocyte apoptosis and hindering remodeling.[83] However, such lymphocytes promote removal of necrotic tissue via accelerated recruitment of neutrophils and may suppress the inflammatory response.[84] This dual effect is likely caused by dysregulated fibrosis and inflammation.[68] Females have higher CD4+ T cell counts and higher CD4/CD8 ratios, but males have higher CD8+ T cell frequencies.[85] CD8+ T cells in females also have upregulated proinflammatory genes compared with males.

4. Challenges and Recommendations in Considering Sex in Cardiac Nanomedicine Studies

The significance of sex differences in nanomedicine is becoming increasingly recognized.[4] For example, it was demonstrated that cell sex can significantly affect cellular uptake and intracellular trafficking of various types of identical NPs.[19,86] As nanomedicine continues to advance, embracing sex-specific precision medicine will pave the way for a more personalized and effective approach to cardiac care, benefiting all patients. Structural, hormonal, electrophysiological, immunological, pharmacokinetic, and regenerative disparities between the sexes play a pivotal role in cardiovascular health and must be considered in the development and implementation of cardiac nanomedicine. By acknowledging sex disparities in cardiovascular diseases, clinicians and the nanomedicine community, together with regulatory bodies and policymakers,[87] can address these challenges to promote sex-specific precision cardiac nanomedicine.

Some of the barriers in the field of cardiac nanomedicine that need to be carefully addressed include the rarity of using both cell sexes in the in vitro analyses, limited representation of female subjects in preclinical and clinical studies, lack of standardized methodologies for assessing sex-specific responses, and inadequate awareness among healthcare professionals. In order to gain a better understanding of the role of sex in cardiac nanomedicine, one of the primary recommendations is that, in preclinical studies utilizing animal models of cardiac nanomedicine, researchers should include both male and female subjects to accurately evaluate sex-specific responses. It is noteworthy that the current progress in the field of 3D bioprinting[88] technologies may enable researchers in the near future to bypass the use of animal models. Additionally, large-scale clinical trials should strive to enroll sufficient numbers of both male and female participants to assess sex-related differences in treatment outcomes. The outcomes of such preclinical and clinical trials enable the nanomedicine community to better understand how sex, as a crucial biological factor, affects the safety and biological identity of nanomedicine technologies. Such sex-specific information can then be used in the design and development of safe and efficient sex-specific nanomedicine technologies. For example, the identification of sex-specific biomolecular corona profiles of identical NPs [23] provides distinct opportunities to the nanomedicine community to finetune the physicochemical properties of NPs in a way to recruit more of the important proteins in corona layer that can be enhance safety and therapeutic efficacies of NPs for each sex.

Another crucial, yet, overlooked factor in the design and development of cardiac nanomedicine technologies is the sex-specific disparities in cardiac function. For instance, on average, female hearts beat faster and have larger ejection fraction and contractile strain compared to males, but also they exhibit smaller cardiac outputs and lower blood pressure.[43] These disparities can affect the decoration of the biomolecular corona on the NP surface and their interactions and availability to male versus female cardiac cells.

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Conflict of Interest

Z. L. Z. J. contributed equally as first authors. M.M. discloses that (1) he is a co-founder and director of the Academic Parity Movement (www.paritymovement.org), a non-profit organization dedicated to addressing academic discrimination, violence and incivility; (2) he is a co-founder of and shareholder in NanoServ Corp. and Targets' Tip Corp.; and (3) he receives royalties/honoraria for his published books, plenary lectures and licensed patents.

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