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The Possible Uses and Challenges of Nanomaterials in Mast Cell Research

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Mast cells are tissue-resident immune cells that are involved in inflammation and fibrosis but also serve beneficial roles, including tissue maintenance, angiogenesis, pathogen clearance, and immunoregulation. Their multifaceted response and the ability of their mediators to target multiple organs and tissues means that mast cells play important roles in numerous conditions, including asthma, atopic dermatitis, drug sensitivities, ischemic heart disease, Alzheimer disease, arthritis, irritable bowel syndrome, infections (parasites, bacteria and viruses), and cancer. As a result, mast cells have become an important target for drug discovery and diagnostic research. Recent work has focused on applying novel nanotechnologies to explore cell biology. In this brief review, we will highlight the use of nanomaterials to modify mast cell functions and will discuss the potential of these technologies as research tools for understanding mast cell biology. *The Journal of Immunology*, 2020, 204: 2021–2032.

Nanobiology, the use of nanomaterials to influence biological processes, is a rapidly growing field, in which molecular-level design can directly affect biological outcomes. Nanomaterials range in shape, size, and chemical composition but will have one or more dimensions smaller or equal to 100 nm. Normally, synthetic nanomaterials used for medical applications are synthesized using specific polymers, lipids, and/or proteins that may bear surface modifications that enhance their targeting to specific cells or tissues.

The field of nanomedicine has garnered significant interest recently, as it employs custom-designed, nanoscale pharmaceuticals to target specific biological targets. Nanomedicine is particularly well suited for conditions in which an easily identifiable cellular target is available, such as allergic inflammation, in which mast cells play an essential role in the production of

proinflammatory mediators. The biological applications of nanotechnologies have increased over the past decade with studies examining the effect of nanomaterials on cell biology. In this review, we will focus how these technologies have been applied to the study of specific immune regulatory cells called mast cells. We will highlight the importance of mast cells in mediating allergic inflammation, how numerous nanomaterials have been developed to modify mast cell functions, and how these nanoscale materials can serve as novel mast cell–targeted research tools.

Mast cells are important to tissue maintenance, angiogenesis, pathogen clearance, and immunoregulation, making them significant contributors to allergy and inflammation (1). Mast cells are tissue-resident cells that originate from bone marrow progenitor cells (2–6). Following migration through the circulation as partially differentiated cells, they reach a fully granulated and differentiated state when they reach the tissue microenvironment (7). In general, mast cells are large granular leukocytes that contain many densely packed granules (8) (Fig. 1A). Mast cells are a heterogeneous group of cells that “acquire” different phenotypes and functions, depending upon the tissue in which they reside.

Although mast cells are ubiquitously distributed in tissues throughout the body, they predominate in tissues that interface with the external environment, such as the skin, gut, and lungs. Because of this close proximity to the environment and their unique receptor repertoire, mast cells are among the first cells to respond to Ags and invading pathogens. Mast cells are activated by many different receptors, including Ig receptors (IgE, FcεRI) and IgG (FcγR) (9–11), TLRs that bind pathogen-derived molecules (12), complement receptors [CD11b/CR3 (13), CD11c/CR4 (14) and CD88/C5aR (15)], histamine receptors H2 and H4 (16–18), Mas-related G protein–coupled receptors (Mas-related G protein–coupled receptor X2 [MrgprX2] or MrgprB2) (19), and various cytokine and chemokine receptors (20). Ligation of these cell

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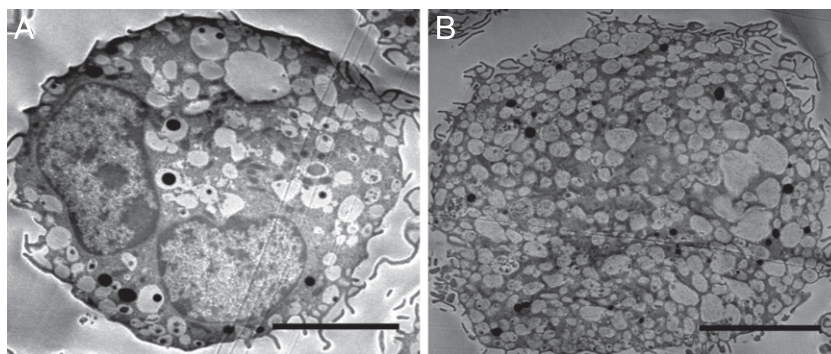
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Abbreviations used in this article: BMMC, bone marrow–derived mast cell; DLinkKC2-DMA, 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane; DODAP, 1,2-dioleoyloxy-3-dimethylaminopropane; DODMA, 1,2-dioleoyloxy-3-dimethylaminopropane; EET, *cis*-epoxyeicosatrienoic acid; LAD2, Laboratory of Allergic Diseases 2; LNP, lipid NP; MrgprX2, Mas-related G protein–coupled receptor X2; NP, nanoparticle; PLGA, poly(lactic-co-glycolic) acid; RBL-2H3, rat basophilic leukemia 2H3 cell; ROS, reactive oxygen species; siRNA, small interfering RNA.

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FIGURE 1. (A) Transmission electron microscopy (TEM) image of a human mast cell (LAD2). **(B)** TEM image of an FcεRI-activated LAD2 mast cell with numerous empty (non-electron-dense) cytoplasmic granules following degranulation. Scale bar, 5 μm.



surface receptors results in a variety of mast cell responses. Initially, mast cells rapidly release preformed mediators from cytoplasmic granules (termed degranulation, Fig. 1B), which may also be followed by a delayed release of additional de novo synthesized lipid and protein mediators. The initiation and strength of each of these responses are dictated by the specific stimuli encountered by the mast cell (21).

Mature mast cells offer a unique avenue of therapeutic intervention because they reside in every tissue (apart from blood) and have been implicated in a wide variety of pathologies, including but not limited to asthma (22), autoimmune diseases (23), neuroinflammation (24), and gastrointestinal diseases (25). This therapeutic potential has made mast cells a target for vaccine adjuvant development (26) in the treatment of solid tumors (27–30) and bacterial infections (31). The significant clinical impact of mast cells has spurred the development of mast cell-specific targeting strategies that not only target specific mast cell phenotypes but also overcome some of the complexities of working with mature mast cells. For these potential strategies to be efficacious, it may be necessary to inhibit specific mast cell functions while maintaining others. For example, it may be beneficial to enhance mast cell chemotaxis to a site of infection while inhibiting the release of lipid mediators via FcεRI activation. During inflammatory reactions, it would be beneficial to ligate an inhibitory mast cell receptor and block proinflammatory mediator release for a prolonged period of time, even within the dynamic environment of an inflamed tissue. Yet, during a parasitic infection, it may be necessary to rapidly but transiently activate the release of histamine without triggering cytokine production. These “customizable” requirements and targeted modification strategies require precise molecular approaches that are tailored to the study of biological processes in the “nano” scale.

The use of new and improved nanoscale materials, such as drugs or molecular nanocarriers, have become prevalent in medical research over the years. This has led to diverse formulations that have been extensively studied for their biochemical effects on cell toxicity, metabolism, cell-to-cell communication, and cell morphology (Fig. 2). In this regard, the biochemical and physicochemical features, such as chemistry, size, shape, and charge, have a direct impact on a nanomaterial's ability to elicit specific biological responses. Synthetic nanoparticles (NPs) and nanomaterials can be tailored physicochemically to alter their size, polydispersity, shape, zeta potential, surface chemistry, and surface hydrophobicity/hydrophilicity as well as their temperature sensitivity, ionic strength, osmolality, loading capacity, encapsulation efficiency, and bioavailability. NPs with high zeta potentials (negative or positive) are less

prone to aggregation and are more electrically stable (32). In lipid NPs (LNPs), higher lipid concentrations promote the viscosity of the organic phase, resulting in larger particles (33). In contrast, higher surfactant concentrations promote smaller-sized particles because of reduced tension between organic and aqueous phases, forming smaller-sized droplets, while the addition of emulsifiers improves the uniformity of the nanomaterials (34–36). The surface charge and size of nanomaterials influence their toxicity as large cationic NPs are more cytotoxic and proinflammatory than smaller anionic or neutral NPs (33). Nanomaterials smaller than 100 nm have higher surface area to volume ratios, allowing for the release of encapsulated drugs by diffusion into cells and/or by phagocytosis. Overall, these properties enhance the entry of encapsulated drugs into cells and tissues. In addition, studies by Mahmoudi et al. (37–39) have reported that physicochemical properties have a profound effect on nanomaterial cellular uptake and functionality. However, the use of some NPs as drug delivery systems has had limited success. This is due to multiple factors such as their inherent size, toxicity, and immunomodulatory activity, which can alter their capacity to deliver their cargo into specific tissues and intracellular compartments.

The application of nanotechnologies to the mast cell field has increased over the past decade because of the desire to discover novel modulators of mast cell biology and diseases. Several recent studies by Johnson et al. (40, 41), Duan et al. (42), and Feng et al. (43) suggest that nanomaterials can target specific mast cell behaviors by circumventing classically defined FcεRI activation pathways. Even more promising is the application of nanomaterials to mast cell-targeted gene delivery. We will dissect some of this literature and examine the complex and sometimes counterintuitive effects of nanomaterials on mast cell functions. The interaction of nanomaterials with mast cells is still a new area of research and it is largely unknown how specific physicochemical nanomaterial properties influence mast cell responses and phenotypes. By examining studies using multiple cell types, we may better understand the mechanistic effects of these materials on mast cells.

The impact of inorganic NPs on mast cell function

Nanomaterials of various compositions can affect mediator release by mast cells, which may not be a specific response to the nanomaterial itself but a “danger response” to the presence of a foreign particle. This is similar to non-IgE hypersensitivity or pseudoallergic responses by mast cells during adverse drug reactions. Experiments using inorganic NPs have observed stimulatory and inhibitory effects on mast cell

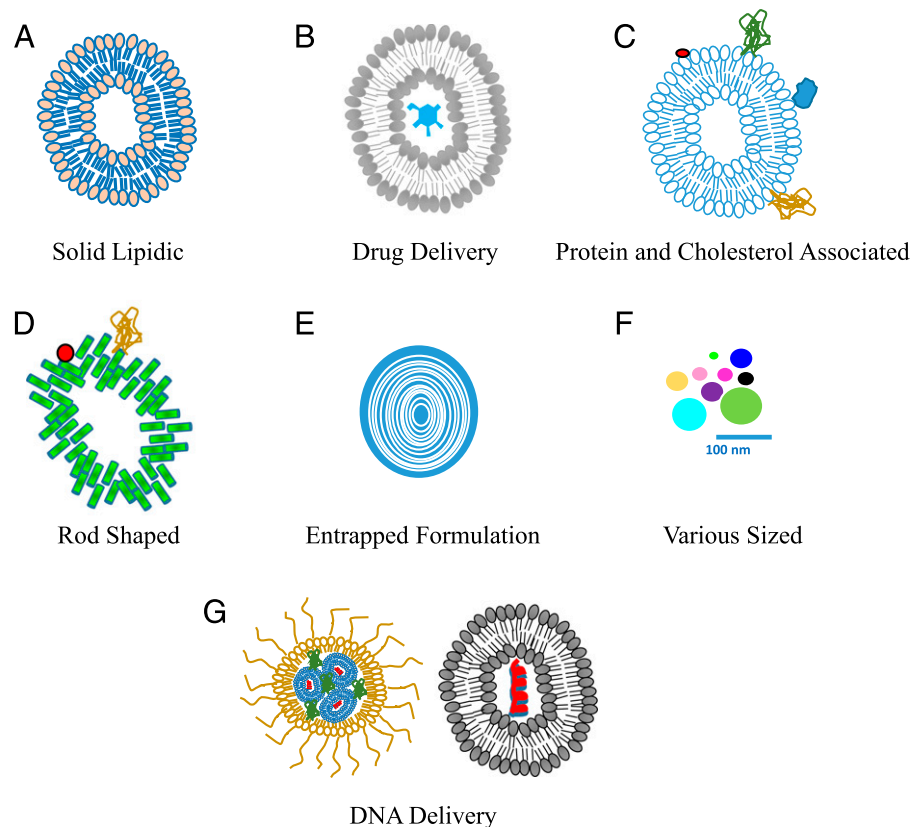


FIGURE 2. Examples of various nano-materials. (A) Solid lipid nanoparticle (LNP), (B) solid LNP containing an encapsulated hydrophobic drug, (C) protein- and cholesterol-associated NP, (D) aggregation of rod-shaped nanomaterials, (E) entrapped formulation, (F) a polydisperse mixture of NPs, and (G) encapsulated nucleic acid in LNP delivery systems.

mediator release (Tables I, II). Silver NPs, which are prevalent in numerous consumer products, stimulate degranulation of mouse bone marrow–derived mast cells (BMMCs) and mast cell–like rat basophilic leukemia 2H3 cells (RBL-2H3) (40, 44–46). This occurs through associations with cell surface scavenger receptors (44) and activates intracellular signaling pathways, ultimately leading to changes in gene expression (41). In those studies, NP size was inversely proportional to the stimulatory activity of the particle, suggesting that NP uptake into intracellular compartments (often dependent upon particle size) may be a factor in the extent of the activation observed (47). Some studies have also examined synthetic NP localization to mast cell intracellular compartments (44, 45, 48, 49). However, in other cell types, NP localization depends upon the engineered geometry, outer core chemistry, and functionalization of the particle. All of these characteristics influence the interaction of the NP with the biological systems they encounter, such as the lipid bilayer, vesicle membranes, protein receptors, and signaling molecules. The ability to bind and interact with specific receptors inside or on the surface of the cell is particularly important (previously reviewed in Ref. 47).

The detailed process of NP internalization by mast cells has not been explored. Mathematical modeling suggests that optimal endocytosis of NPs occurs when there are nonsaturating levels of ligand and receptor interactions between the NP and a cell surface (50). An NP's dimensions within a certain geometric shape will also greatly influence its cellular uptake, such that most cell types prefer to internalize rod-shaped NPs, followed by spheres, cylinders, and cubes of the same size (51). However, size and surface chemistry are also important factors in cellular uptake. For example, gold nanorods coated

with poly(diallyldimethyl ammonium chloride) are readily absorbed by human breast adenocarcinoma cells, followed by nanorods coated with poly(sodium-p-styrenesulfate) or cetyltrimethylammonium bromide (52). Surface chemistry often changes the surface charge of the NP and affects the way in which proteins in the extracellular liquid (i.e., media) bind to the NP, which forms a coating called a corona (53). The degree of protein adsorption by the NPs and the composition of the protein corona correlate with cellular uptake such that gold NPs coated with BSA are three times more likely to be internalized by human breast adenocarcinoma cells compared with gold NPs that had been coated with FBS (54). Even the sex of certain cells appears to influence NP uptake (55). These observations emphasize that NP design is not a “one-size-fits-all” approach and reiterates that NPs must be customized for specific functions and cell types. Currently, it is unclear how any of these parameters influence NP internalization by mast cells. It is possible that a protein corona is necessary for the internalization of many metal NPs because this initiates endocytotic pathways. Because mast cells are not a typically phagocytic cell, it is more likely that they employ endocytotic or pinocytotic mechanisms. In cases in which the NPs are coated with specific ligands, receptor-mediated endocytotic mechanisms likely internalize the NPs and shuttle them to endosomal compartments. The intracellular localization of NPs, their compartmentalization, and the resulting intracellular signaling response in cells is still poorly understood. However, AshaRani et al. (56) showed that intracellular starch-coated silver NPs increased the production of reactive oxygen species (ROS) and disrupt mitochondrial function in human lung fibroblast and glioblastoma cells, presumably by localization to mitochondria. In our own unpublished

Table I. Inorganic NPs that inhibit mast cell function

Type	Particle Size ^a	Particle Properties or Modifications	Particle Concentration	Model System	Functional Effect	Effect on Mast Cell Viability	Proposed Mechanism	Reference
Au	8–50 nm	Citrate-capped, thiolated DNP	0.01–3 nM	RBL-2H3, murine peritoneal mast cell	Inhibitory (IgE- and non-IgE-mediated degranulation; 72-h exposure)	Decreased (75–85%)	Interference with vesicle transport, steric hindrance	66, 68
BN	50–117 nm (373–400 nm)	UF, MF	25–100 µg/ml	BMMC	Inhibitory (IgE-mediated degranulation)	Unchanged	Decreased IgE binding	40
CeO ₂	3–9 nm (31 nm)	Polyacrylic acid	50–200 µg/ml	RBL-2H3	Inhibitory (IgE-mediated degranulation; phospho-ERK1/2 protein detection)	Unchanged	Decreased IgE binding and/or phospho-ERK1/2	63
Fe ₂ O ₃	4–28 nm (147–251 nm)	Unmodified, polyacrylic acid	25–200 µg/ml	BMMC, RBL-2H3	Inhibitory (IgE-mediated degranulation)	Unchanged	Decreased IgE binding	40, 63
MgO	95 nm (793 nm)		25–100 µg/ml	BMMC	No effect (spontaneous degranulation; IgE-mediated degranulation)	Unchanged		40
SiO ₂	24–25 nm (555–797 nm)	Nonporous, porous	0–400 µg/ml	Murine peritoneal mast cells	Inhibitory (number/frequency of released granules; non-IgE-mediated degranulation)	Decreased (~75%, nonporous only)	Interference with vesicle transport	61
TiO ₂	11 nm (1055 nm)	Nonporous	0–400 µg/ml	Murine peritoneal mast cells	Inhibitory (non-IgE-mediated degranulation)	Unchanged	Interference with vesicle transport	61
	3–9 nm (31 nm)	Polyacrylic acid	50–200 µg/ml	RBL-2H3	Inhibitory (IgE-mediated degranulation; phospho-ERK1/2 protein detection)	Decreased (67–88%)	Decreased IgE binding and/or decreased phospho-ERK1/2	63
ZnO	49 nm (696 nm)	Unmodified	25–100 µg/ml	BMMC	Inhibitory (IgE-mediated degranulation)	Unchanged	Inhibition of intracellular calcium flux	40
	21 nm	UF	2.5–10 µg/ml	RBL-2H3	Inhibitory (IgE-mediated degranulation; phospho-protein detection)	Unchanged		60
	3–9 nm (64 nm)	Polyacrylic acid	50–200 µg/ml	RBL-2H3	Inhibitory (IgE-mediated degranulation)	Unchanged	Decreased IgE binding	63
	(36–250 nm)	Pristine, surfactant treated	10–30 µg/ml	RBL-2H3, BMMC	Inhibitory (IgE-mediated degranulation and calcium flux; spontaneous degranulation)	Decreased for doses >40 µg/ml		62
	25 nm (294 nm)		25–100 µg/ml	BMMC	Inhibitory (IgE-mediated degranulation)	Unchanged		40

^aParticle sizes are reported as diameters from electron microscopy measurements or dynamic light scattering measurements (in brackets). DNP, 2,4-dinitrophenyl; MF, microfine; UF, ultrafine.

Table II. Inorganic NPs that stimulate mast cell function

Type	Particle Size (nm) ^a	Particle Properties or Modifications	Particle Concentration	Model System	Functional Effect	Effect on Mast Cell Viability	Proposed Mechanism	Reference(s)
Ag	5–112 (7–133)	Citrate or polyvinylpyrrolidone	0.15–100 µg/ml	BMMC, RBL-2H3,	Stimulatory (spontaneous degranulation, smaller NPs only; IgE-mediated degranulation, smaller NPs only; and protein phosphorylation; calcium flux and ROS levels, RBL-2H3 only)	Decreased (at high doses with smaller NPs only)	Scavenger receptor B1, calcium, and PI3K dependent	40, 44–46
	6 and 83 (10 and 106)	Polyvinylpyrrolidone	40 µg/12.5 cm ² (topical, mice)	NC/Nga mice (female)	Stimulatory (mast cell migration, smaller NPs only)	Not reported		45
Ag (nanoplates)	42–75 (38–71)	Polyvinylpyrrolidone	6.25–50 µg/ml	BMMC	Stimulatory (spontaneous degranulation, p550 > p850)	Unchanged		44
Ag (nanowires)	105 (w) × 1250 (l) (316)	Polyvinylpyrrolidone	6.25–50 µg/ml	BMMC	Stimulatory (spontaneous degranulation)	Unchanged		44
Au	28	Citrate-capped	0.01–1 nM	Murine peritoneal mast cell, RBL-2H3	Stimulatory (non-IgE-mediated degranulation, NPs ≥19.8 nm only)	Decreased in RBL-2H3 (75–85%)	Interference with vesicle transport	67, 68
CeO ₂	8–50 (70 (90))	Thiolated DNP	0.01–3 nM (in vitro)	RBL-2H3 BMMC	Stimulatory (IgE-mediated degranulation) Stimulatory (IgE-mediated degranulation and cytokine production, BMMC and C57BL/6J mice)	Not reported Unchanged	Steric hindrance	66 57
			10–100 µg (in vivo)	C57BL/6J and B6.Cg-Kit ^{W-sh} mice (male)	Stimulatory (IgE-mediated PGD ₂ release, BMMC)			
CuO	92 (73)		25–100 µg/ml	BMMC	Stimulatory (non-IgE-mediated degranulation)	Unchanged		40
Fe ₃ O ₄	10 (45–89)	Uncoated, dextran-coated, and polyethylene glycol-coated	5 mg Fe/kg (i.v.)	Wistar rats (female)	Stimulatory (mast cell infiltration, variable depending on NP modification)	Not reported		59
SiO ₂	13 (190)		7 mg/kg (i.v.)	Wistar rats (male)	Stimulatory (mast cell infiltration; liver, lung, and heart)	Liver tissue remodeling		58
	22 and 64 (530 and 816)		25–100 µg/ml	BMMC	Stimulatory (non-IgE-mediated degranulation)	Unchanged		40
TiO ₂	60 (83)	Mixture (rutile and anatase)	0.1–1 mg/ml	RBL-2H3	Stimulatory (non-IgE-mediated degranulation, calcium flux, and ROS production)	Not reported	Disruption of calcium channels	64
	75 (474)	Anatase	10–200 mg/kg	Sprague Dawley rats	Stimulatory (mast cell infiltration, to stomach of young rats only)			65
	(25–34)	Rutile and anatase	10–30 µg/ml	RBL-2H3, BMMC	Slightly stimulatory (non-IgE- and IgE-mediated degranulation, rutile only)			62

^aParticle sizes are reported as diameters from electron microscopy measurements or dynamic light scattering measurements (in parentheses).

observations, we have detected similar disruptions of mitochondrial function, although it is unclear whether the NPs are localizing to these compartments specifically or whether they are causing overall cell toxicity and loss of viability.

Enhanced production of proinflammatory cytokines by mast cells occurs in mice treated with cerium oxide NPs (57). Particular types of modified superparamagnetic iron oxide or silica NPs also stimulate mast cell infiltration into tissues, such as the lungs, heart, and liver, through NP-induced tissue damage (58, 59). In this case, the observed effects were not a direct effect on mast cells but an inflammatory response to tissue damage caused by the NPs. NP formulations, such as silicon dioxide, titanium dioxide, zinc oxide, and polyacrylic acid functionalized metal-oxide NPs, suppress mast cell degranulation (40, 60–63) when the mast cells are activated by IgE-dependent (40, 60, 62, 63) or IgE-independent mechanisms (61) (Tables I, II). It is important to note that, in other studies, titanium dioxide NPs had stimulatory effects on mast cell functions that may be a result of differences in NP dose, NP properties, or the cell models used in these studies (Table II) (64, 65).

Interestingly, titrating gold NP concentration and the density of IgE ligands bound to the NP surface was found to modulate IgE-mediated receptor cross-linking on the surface of RBL-2H3 cells, resulting in either stimulatory or inhibitory signals (66). Others have shown that citrate-capped gold NPs could stimulate degranulation from murine peritoneal mast cells following exposure for up to 48 h, after which prolonged exposure (72 h) to gold NPs inhibited granule exocytosis (67, 68), suggesting that exposure time may also directly affect mast cell function regardless of surface functionalization. The potential medical applications for inorganic NPs include diagnostics and therapeutics (69) and fine-tuning the size and functionalization of NPs (66) may lead to the discovery of novel, mast cell–targeted therapeutics. However, more research into mitigating the undesired effects of both engineered and environmental inorganic NP exposure (70) and their effects on immune cells must be explored.

The impact of carbon-containing and organic NPs on mast cell function

Carbon-containing and organic NPs are thought to be more biodegradable and biocompatible, which has led to significant interest in their use by nanobiologists. Whereas these types of NPs are not devoid of off-target effects, carbon-containing and organic NPs have proven to be a very effective means of targeting specific biochemical processes. Although there are few studies in mast cells, investigators have examined the effects of fullerenes, spherical or cylindrical carbon-based nanotubes, and polymeric NPs on IgE-dependent (71–77) and IgE-independent mast cell degranulation (49, 71, 76) (Table III). These studies identified these NPs as mast cell inhibitors. C₇₀ fullerenes derivatized with tetraglycolate stimulated the production of *cis*-epoxyeicosatrienoic acids (EETs) in the lungs of C₇₀-tetraglycolate–treated mice, and because EETs can directly inhibit human lung mast cell mediator release, it was observed that this indirect strategy could be used to treat allergic asthma (74). C₇₀ fullerene derivatives can also ameliorate other mast cell related diseases, such as in a mouse model of rheumatoid arthritis (77); however, the observation of mast cell-independent effects in vivo suggests

that fullerene-based NPs may inhibit inflammation in multiple ways.

Conversely, there may be circumstances in which activation of mast cells in a specific tissue may be advantageous. Mast cells release antimicrobial and antiviral mediators that are an important facet of the innate immune response. Ligands bound to NPs lead to IgE–FcεRI cross-linking and degranulation of RBL-2H3 mast cells in vitro, suggesting that NPs can be used as surrogate stimulatory Ags. This same strategy can be used to generate FcεRI antagonists by changing the size of the NP and the Ag density of NPs, both of which influence the IgE–FcεRI cross-linking such that some of these NP could bind but not activate mast cells (66). These strategies merit further examination because engineering NP with different ligands may lead to precision therapies tailored to a patient's own allergic profile.

The potential for nanocarrier-mediated gene delivery into mast cells

Structured nanocarriers and functionalized solid LNPs have been used very successfully as gene delivery tools to modify gene expression in multiple cell types, with the goal of modifying cell function and/or growth (78). Many of these nanocarriers are composed of organic molecules or biocompatible polymers that facilitate cellular uptake into intracellular organelles. Chitin, and its derivative chitosan, have generated considerable interest for their biocompatibility and numerous applications, which include but are not limited to drug delivery, antimicrobial substances, and immunomodulatory agents (79, 80). Chitosan/cyclodextrin nanocarriers are extremely versatile because they can encapsulate various macromolecules, including DNA (81, 82). In rat mast cells, chitosan/cyclodextrin nanocarriers could deliver heparin intracellularly, which led to a suppression of IgE-independent histamine release (83) (Table IV). Moreover, a recent in vivo study in mice has shown that cedrol, a naturally occurring, marginally water-soluble sesquiterpene and mast cell stabilizer, could be encapsulated and delivered into mouse peritoneal mast cells (48) (Table IV). Lipid-based carriers are useful tools to facilitate the emulsification of water-insoluble substances or to protect bioactive compounds for systemic delivery. Cedrol-containing lipid nanocarriers had a suppressive effect on IgE-independent histamine or β-hexosaminidase release from ex vivo mouse mast cells at doses two to three times lower than cedrol alone; in addition, cedrol-loaded nanocarriers were efficient in suppressing compound 48/80–induced anaphylactic reactions when used prophylactically in mice (48).

Purely lipid-based carriers that use cationic lipids or polymers can encapsulate nucleic acids (such as antisense oligonucleotides, small interfering RNA [siRNA], microRNA, mRNA, or plasmid DNA) with high efficiencies; however, toxicity is often cited as the largest obstacle to widespread application of these types of NPs (84). The advent of ionizable, cationic lipids for nucleic acid packaging has greatly improved toxicity profiles (85–87), and the application of microfluidic technologies has fostered the efficient and reproducible generation of LNPs for nucleic acid delivery (88, 89). Specifically, LNPs have been shown to modulate the function of various immune cells in both in vitro and in vivo models. Multiple studies have elegantly demonstrated that LNPs can deliver siRNA into B cells (90), siRNA into T cells (91–93), or siRNA or mRNA into monocytes, macrophages, or dendritic cells (94–98). More recently, LNPs

Table III. Carbon-containing and organic NPs used to inhibit mast cell functions

Type	Particle Size (nm) ^a	Particle Properties or Modifications	Particle Concentration	Model System	Functional Effect	Effect on Mast Cell Viability	Proposed Mechanism	Reference(s)
C ₆₀ Fullerenes	Not reported	Polyhydroxy, <i>N</i> -ethyl-polyamino	0.01–1000 ng/ml	Human skin mast cells	Inhibitory (IgE-mediated degranulation, GM-CSF release, and ROS production; phospho-Syk detection) Inhibitory (anti-FcεRI-mediated degranulation, PGD ₂ release, and TNF-α release) Inhibitory (non-IgE-mediated degranulation) Inhibitory (IgE-mediated degranulation) Inhibitory (IgE-mediated serum histamine levels)	Unchanged	Reduction of intracellular ROS	71
C ₇₀ Fullerenes	1–50	Pristine or hydroxylated	10–100 μg/ml	RBL-2H3	Inhibitory (IgE-mediated degranulation)		FcεRI-mediated signaling inhibition	76
		Polyhydroxy, <i>N</i> -ethyl-polyamino	50–250 ng, 2–10 μg/kg (i.p. injection)	C57BL/6 mice	Inhibitory (anti-FcεRI-mediated degranulation, all except tetrainositol)			71
		Biotin, C ₃ , C ₇₀ -OH, CCC, dimethylaminoethanol, ethanalamine, niacin, NSAID, (OH) ₁₂ , (PC) ₄ , tetraglutamate, tetraglycolate, tetrainositol, tetraphosphate, tetrapyrindine, tetrasulfonate, TTA	10–100 μg/ml or 0.01–10 μM	Human skin mast cells	Inhibitory (anti-FcεRI-mediated degranulation, all except GM-CSF release, all except tetrapyrindine) Inhibitory [non-IgE-mediated degranulation, all except for biotin, CCC, (PC) ₄ , tetrainositol, and TTA] Inhibitory (non-IgE-mediated TNF-α release, all except CCC, NSAID) Inhibitory (IC-mediated degranulation, all except for C ₃ and niacin)	Unchanged	FcεRI-mediated signaling inhibition; changes in intracellular signaling	72, 75, 77
		ALM (liposome-encapsulated C ₇₀), amine, biotin, C ₃ , C ₇₀ -OH, niacin, (PC) ₄ , tetraglycolate	10 μg/ml	Human skin mast cells, BMMC (FcγRII-null)	Inhibitory [IC-mediated NF-κB expression, all except amine, biotin, and (PC) ₄]	Not reported	FcεRI-mediated signaling inhibition	77
Fullerenes (carbon nanotubes)	(10–21 × 150–2000)	Tetraglycolate, tetrainositol	100 ng (i.p. injection), 20 μg (intranasally, multiple doses)	C57BL/6 and BALB/c mice (female)	Inhibitory (decreases in IgE-mediated inflammation, histamine, and cytokine levels; tetraglycolate only)	Unchanged (liver toxicity or kidney toxicity)	Upregulation of <i>cis</i> -EETs	72, 74
		Single-walled, hydroxylated	10–100 μg/ml	RBL-2H3	Inhibitory (non-IgE- and IgE-mediated degranulation) Inhibitory (calcium flux and phospho-protein detection)	Not reported	Inhibition of intracellular calcium flux, FcεRI-mediated signaling inhibition	76
Poly(DL-lactide-coglycolide)	(240–338)	^{+/–} Chitosan	0–2.5 mg/ml	RBL-2H3 ^{+/–} IgE	Inhibitory (IgE-mediated degranulation, unmodified > chitosan-modified) Stimulatory (non-IgE-mediated degranulation, IgE-sensitized cells only)	Unchanged	Disruption of SNARE-dependent exocytosis	73
	(240–338)	Coumarin-labeled ^{+/–} chitosan	20 mg in PBS (i.p. injection)	C57BL/6 mice (male)	Inhibitory (IgE-mediated anaphylaxis, +chitosan only)			

^aParticle sizes are reported as diameters from dynamic light scattering measurements or electron microscopy measurements (in brackets). IC, immune complex; NSAID, nonsteroidal anti-inflammatory drug; SNARE, soluble *N*-ethylmaleimide attachment protein receptor.

Table IV. Nanocarriers for drug delivery into mast cells

Nanocarrier Type	Particle Size (nm) ^a	Particle Properties and Cargo	Particle Concentration	Model System	Functional Effect	Effect on Mast Cell Viability	Proposed Mechanism	Reference
Chitosan-based	221–729	Na-carboxymethyl- β -cyclodextrin, heparin-loaded	1.6–200 μ g/ml (heparin)	Rat pleural and peritoneal mast cells	Inhibitory (non-IgE-mediated degranulation)	Slight decrease	Heparin-dependent inhibition	83
Lipid-based	56–92	Cedrol-loaded	IC ₅₀ : 10.5–17.0 μ M (in vitro), 12.5–200 mg/kg (in vivo, oral)	Mice peritoneal mast cells, BALB/c mice	Inhibitory (non-IgE-mediated degranulation, calcium flux, and anaphylaxis [in vivo])	CC ₅₀ >1 mM	Cedrol-dependent inhibition	48
Lipid-based	80–145	Lipids, cholesterol, polyethylene glycol, ⁺ /– plasmid DNA	LNP containing 2 μ g DNA/5 $\times$ 10 ⁷ cells	HMC-1.1, HMC1.2, and LAD2	DNA delivery into mast cells (expression of EGFP reporter)	5–25% decrease (dependent on formulation)		100

^aParticle sizes are reported as diameters from dynamic light scattering measurements.

CC₅₀, half maximal cytotoxic concentration; EGFP, enhanced GFP; HMC-1.1/1.2, human mast cell line 1.1/1.2; LAD2, Laboratory of Allergic Diseases 2.

have been used to efficiently package and deliver plasmid DNA into both human cancer cells and chicken embryos (99). Thus far, very little research has focused on applying nucleic acid-containing LNPs to mast cell research for the modification of gene expression. Our recent work demonstrates that LNPs containing the ionizable cationic lipids 1,2-dioleoyloxy-3-dimethylaminopropane (DODMA) or 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLinkC2-DMA) are more effective than traditional lipofection reagents for the delivery of plasmid DNA into HMC and Laboratory of Allergic Diseases 2 (LAD2) cell lines (100). Our initial LNP experiments tested various LNP formulations containing cationic lipids (1,2-dioleoyloxy-3-dimethylaminopropane [DODAP], DODMA, or DLinkC2-DMA) for their ability to transfect HMC-1.2 cells. We observed that DODAP, DODMA, and DLinkC2-DMA LNPs were well tolerated in mast cells and that DODMA and DLinkC2-DMA LNP-mediated plasmid delivery resulted in robust transgene expression. Notably, we observed distinct lipofection efficiencies between two different human mast cell lines, HMC-1 and LAD2. Supplementing LNPs with apolipoprotein E4 enhanced the lipofection of HMC-1 cells but not LAD2 cells, suggesting that LNPs are not as highly dependent on uptake through the apolipoprotein pathway in LAD2 mast cells. This highlights the benefit of customizing and optimizing NP properties for each cell type.

Some siRNA or drug-encapsulating LNP formulations have been investigated in clinical trials (101) and similar strategies may be applicable in inflammatory diseases in which mast cells play a major role. Moreover, strategies, such as covalently coupling carbohydrates, folate, peptides, or Abs to the surface of LNPs, are currently being investigated to facilitate cell- or tissue-specific targeting of nucleic acid-containing LNPs (90, 93, 102–107). These strategies may be useful in treating diseases, such as allergen-specific inflammation, in which specific subsets of mast cells are implicated.

Nanostructures as scaffolds to regulate mast cell function and growth

Nanostructures are another means of manipulating cell behavior. Cell biologists have been using nanostructures, such as three-dimensional growth matrices, to activate adhesion molecules and initiate differentiation signals in cells for decades. However, the recent increase in our understanding of these scaffolds as structures with nanostructural features has prompted us to re-evaluate their use in manipulating specific cell behaviors. These features have applications in immunotherapy where there is a great need to inhibit or enhance specific cell responses in a localized manner. Tissue-resident cells, such as mast cells, rely heavily on environmental signals obtained through their adhesion molecules bound to the surrounding matrix. In these circumstances, mast cell responses can be activated and finely tuned to address their extracellular milieu, such as during inflammation. It is logical to begin to design nanoscaffolds that can modulate these microenvironment-mediated signals.

Structures assembled from nanoscale intermediates can be functionalized such that they modulate the functions of embedded mast cell populations or provide matrices in which cells can differentiate and proliferate, which simulates the natural tissue environment. Poly(lactic-coglycolic) acid (PLGA) microfibers (108) functionalized with chemokine stromal-derived factor-1 α and administered s.c. significantly reduce local mast cell numbers and degranulation, promoting angiogenesis and

tissue healing in the vicinity of PLGA scaffolds (109) (Table V). Oh and Lee (110) demonstrated that the immune-suppressive effect of a cardiovascular stent coated with a nanofiber hydrogel, containing a mixture of NO donors and ROS scavengers, stabilized mast cells and protected mast cells from the ROS-mediated immune response. In addition to having a role in often detrimental inflammatory processes, mast cells also have beneficial roles in tissue homeostasis through the promotion of angiogenesis and wound healing. Bioresorbable grafts assembled from electrospun nanoscaffolds coated with fibronectin under the appropriate conditions can promote BMMC adhesion, survival, and cytokine secretion (111) (Table V). Moreover, our group demonstrated that self-assembling, peptide-based hydrogels can serve as nanoscaffolds for unstimulated BMNCs and may suppress the egress of mast cell mediators into the surrounding environment (112) (Table V). Future efforts to modify nanostructures could develop novel substrates that favor mast cell proliferation. This could occur through the addition of growth factors or mimetics, that act as scaffolds to selectively suppress deleterious inflammatory responses or that promote wound healing mediated by the release of mast cell mediators. Based on our research, self-assembling peptide matrices were able to alter the activation of human mast cells in cell and primary tissue culture. This hydrogel matrix may provide a new platform to modulate localized mast cell functions, thereby facilitating their protective role in the skin (113).

Future perspectives for nanotechnology-enabled mast cell research

The continued exploration of nanotechnology applications for biological research will provide not just potential therapeutics but also novel means to study molecular and cell biology. As key immunomodulatory cells, mast cells and their released mediators have a vast array of functions within the body and form intricate signaling networks with both immune and nonimmune cells. We believe that the position and function of mast cells throughout the body make them good models for studying the impact of NPs in biological systems and are appropriate targets for NP-mediated therapies. Additionally, research investigating NP effects on mast cell function have employed the highly versatile mouse and rat model systems in the form of primary cell, cell line, and in vivo studies (Tables I–V). We believe that additional important insights on how NPs impact mast cell biology can be obtained by using ex vivo human mast cell cultures, such as those employed by Ryan et al. (71), Norton et al. (72), and Dellinger et al. (49, 75), or human mast cell lines, such as LAD2, LUVA, and ROSA (114–116).

Cell surface receptors, such as MRGPRX2 or subunits of high-affinity IgE receptors (FcεR1s) that are enriched on human connective tissue mast cells (19, 117–121), can be exploited to target NPs to specific subsets of mast cells in inflamed tissues. However, as MRGPRX2 is also found on cells in the CNS (122) and FcεR1s are also found on other immune cells, including basophils and APC (123–128), it will also be important to define any impact of targeted NPs on these additional cell types. Similarly, strategies that exploit peanut- or ragweed-specific IgE on mast cell surfaces can be used to target only those mast cells and basophils that are predisposed to activation by these allergens. By recognizing the receptors on these cell types in their tissue environments, NPs could be tailored to

Table V. Nanoscaffolds used to support mast cell growth and function in vitro

Nanoscaffold Synthesis Method	Pore Size (nm)	Nanoscaffold Composition	Model System	Effect of Nanoscaffold on Mast Cells	Reference
Electrospinning	>1 (inferred from SEM images)	Polycaprolactone, polydioxanone, or silk fibroin with fibronectin	BMMC	Promotion of mast cell adhesion (IL-3/SCF/IgE treated), proliferation (IL-3/SCF/IgE/Ag treated), and cytokine secretion (IL-3/SCF/IgE/Ag treated)	111
Peptide self-assembly	5–200	(RADA) ₄	BMMC	Promotion of mast cell adhesion (IL-3/SCF/IgE treated) Inhibition of IgE-mediated degranulation	112

RADA, arginine–alanine–aspartic acid–alanine; SCF, stem cell factor; SEM, scanning electron microscopy.

alleviate specific allergies in individual patients, thereby opening a new frontier in precision therapeutics. With the increasing interest in genetic modification through clustered regularly interspaced short palindromic repeat-mediated editing, nucleic acid-containing NPs could be engineered to modify the gene(s) necessary for regulating degranulation. This approach may interfere with detrimental mast cell inflammatory responses while leaving protective innate immune or tissue homeostatic functions intact.

The tissue microenvironment strongly influences mast cell differentiation. However, most *in vitro* mast cell studies are performed in a suspension culture in the absence of a scaffold or adhesion matrix. Thus, developing an extracellular matrix-mimicking scaffold that can be programmed with biochemicals (e.g., bioactive peptides, lipids, etc.) for three-dimensional mast cell culture is essential for fundamental studies. Mast cells are widely distributed in tissues that interface with the external environment and are filled with large amounts of preformed protective compounds; this makes mast cells ideal targets for immunotherapies and treatments for conditions, such as wound healing, angiogenesis, and infection. Engineered nanoscaffolds, especially *in situ*-forming nanoscaffolds that can be easily combined with bioactive peptides, are ideal platforms for modulating inflammatory responses. The biochemical nature of nanoscaffolds makes them easy to manipulate and the injectability of *in situ*-forming nanoscaffolds will enhance their clinical application. The large surface area and porous structure of nanofibers facilitate their interaction with immune cells (129). Moreover, designing nanoscaffolds to be sensitive to mast cell inflammatory mediators, such as histamine, proteases, cytokines, and chemokines, could enhance inflammation responsiveness and self-regulated drug release from these scaffolds. Moreover, the use of nanomaterials as vehicles for biomolecule delivery holds great promise as an efficient means to modify mast cell biological functions.

Conclusions

With the continued need for novel technologies to accelerate cell biology research and therapeutic development, the use of novel nanomaterials, coupled with a growing repository of genetic tools, highlights the potential for NPs, LNPs, and nanoscaffolds to drive the advancement of mast cell research.

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Disclosures

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