Biological Effects of Nanoparticles on Macrophage Polarization in the Tumor Microenvironment

Derek Reichel¹, Manisha Tripathi¹,4 and J. Manuel Perez¹,2,3,5

¹. Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA 90048
². Biomedical Imaging Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048
³. Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA 90048
⁴. Current Address: Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, TX 79430, USA

Corresponding author: J Manuel Perez, PhD. Department of Biomedical Sciences & Department of Neurosurgery, Biomedical Imaging Research Institute & Samuel Oschin Comprehensive Cancer Institute, Cedar Sinai Medical Center, 127 S. San Vicente Blvd, Suite A8113, Los Angeles CA, 90048. Email: jmanuel.perez@cshs.org

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Received: 2018.09.18; Accepted: 2018.12.02; Published: 2019.01.01

Abstract

Biological interactions between tumor-associated macrophages (TAMs), cancer cells and other cells within the tumor microenvironment contribute to tumorigenesis, tumor growth, metastasis and therapeutic resistance. TAMs can remodel the tumor microenvironment to reduce growth barriers such as the dense extracellular matrix and shift tumors towards an immunosuppressive microenvironment that protects cancer cells from targeted immune responses. Nanoparticles can interrupt these biological interactions within tumors by altering TAM phenotypes through a process called polarization. Macrophage polarization within tumors can shift TAMs from a growth-promoting phenotype towards a cancer cell-killing phenotype that predicts treatment efficacy. Because many types of nanoparticles have been shown to preferentially accumulate within macrophages following systemic administration, there is considerable interest in identifying nanoparticle effects on TAM polarization, evaluating nanoparticle-induced TAM polarization effects on cancer treatment using drug-loaded nanoparticles and identifying beneficial types of nanoparticles for effective cancer treatment. In this review, the macrophage polarization effects of nanoparticles will be described based on their primary chemical composition. Because of their strong macrophage-polarizing and antitumor effects compared to other types of nanoparticles, the effects of iron oxide nanoparticles on macrophages will be discussed in detail. By comparing the macrophage polarization effects of various nanoparticle treatments reported in the literature, this review aims to both elucidate nanoparticle material effects on macrophage polarization and to provide insight into engineering nanoparticles with more beneficial immunological responses for cancer treatment.

Key words: Nanoparticles, Macrophage polarization, Tumor microenvironment, Cancer, Iron oxide

Introduction

The tumor microenvironment is a unique biological environment that promotes tumorigenesis, tumor metastasis and therapeutic resistance [1-4]. Interactions between different cell populations within tumors contribute to the formation and proliferation of the tumor microenvironment [5], and tumor-associated macrophages (TAMs) are a major constituent within tumors [6]. Although the biological origins of TAMs are not fully understood, experimental evidence suggests that TAMs are a heterogeneous group of cells derived from both tissue-resident macrophages and bone marrow-derived monocytes [7, 8]. While tissue-resident macrophages support cell growth, destroy foreign invaders and protect healthy tissues from aberrant remodeling, TAMs aid tumor
development, demonstrate suppressed immune activity and assist in tissue remodeling. [9]. Figure 1 describes some of the interactions between macrophages and other types of cells within tumors. TAMs secrete growth factors such as vascular endothelial growth factor A (VEGF-A) or placental growth factor (PGF) that can increase blood vessel formation by tumor endothelial cells [10]. These new blood vessels increase nutrient delivery to cancer cells and enhance cancer cell growth within dense tumors. In addition to secreting growth factors, TAMs also secrete enzymes such as matrix metalloproteinase-9 (MMP-9), serine proteases and cathepsins that can degrade the extracellular matrix (ECM), facilitate tumor invasion to adjacent organs, and promote metastasis [11, 12]. Both adipose cells and fibroblasts increase macrophage recruitment into tumors, which amplifies their microenvironment-remodeling capabilities and further promotes tumor invasion and metastasis [13, 14]. TAMs also enhance the survival, migration and chemoresistance of cancer-associated stem cells, which may allow tumors to regrow following otherwise successful treatments [15]. Recent reports suggest that TAMs can suppress Natural Killer (NK) and T cell activity within tumors by preventing immune cells from properly identifying cancer cells [16, 17], which can hinder targeted immune responses to damaged cancer cells and reduce chemotherapy efficacy. Because TAMs contribute to many interactions within tumors that form the tumor microenvironment, TAMs are considered to be a promising therapeutic target for cancer treatment [18].

Figure 1. Biological effects of macrophages on cellular components of the tumor microenvironment. Tumor-associated M2-like macrophages contribute to tumor microenvironment remodeling by removing growth barriers such as the extracellular matrix and promoting vascular growth. In addition, tumor-associated M2-like macrophages promote an immunosuppressive tumor microenvironment that protects cancer cells from targeted immune responses. Following polarization towards an M1-like state, these macrophages can initiate both non-specific and targeted immune responses towards cancer cells.
Macrophages themselves can undergo significant phenotypic changes in response to extracellular signals or foreign entities, such as macromolecules or bacteria, through a process called polarization [19]. Initially, many researchers hypothesized that macrophage polarization divided macrophages into two distinct subtypes: type 1 macrophages (Mφ1) and type 2 macrophages (Mφ2) [20]. Mφ1 were described as pro-inflammatory, antibacterial macrophages because they are often found at sites of inflammation or bacterial infection. Both obesity-induced inflammation in adipose tissue and insulin resistance have been attributed to Mφ1 [21, 22]. In addition, increased Mφ1 prevalence in cancer patients could also contribute to immune-related adverse events from immune checkpoint inhibitor therapies, which can be difficult to manage clinically [23]. In contrast, Mφ2 were classified as anti-inflammatory macrophages because of their presence at wound sites following injury [24]. However, recent studies have shown that macrophage polarization is more accurately described as shifts toward an idealized M1 or M2 polarization state that results in a spectrum of various macrophage phenotypes [25, 26]. For simplicity, this review will refer to any macrophages with M1-like phenotypes as M1-like macrophages and any macrophages with M2-like phenotypes as M2-like macrophages. This paradigm has also been used to describe the behavior of TAMs [27, 28]. M1-like macrophages within tumors can identify cancer cells and initiate targeted immune responses, while M2-like macrophages within tumors can promote tumor growth and proliferation [29]. Cancer researchers typically consider TAMs within patients to consist of predominantly M2-like macrophages [30], and treatments targeting M2-like macrophages within tumors have shown promise to improve cancer treatment. Specifically, Georgoudaki et al. demonstrated that the treatment of tumors with M2-like macrophage-targeting antibodies increased the prevalence of M1-like macrophages within tumors and promoted a T-cell dependent immune response that decreased tumor size, prevented cancer progression and inhibited metastasis in mouse models [31].

Recent evidence suggests that nanoparticle uptake can contribute to macrophage polarization within tumors [32, 33]. Nanoparticles are defined as small (<200 nm diameter) particles comprised of metals, polymers or other materials that often contain hydrophobic regions enveloped by a hydrophilic shell. Because these hydrophobic regions can entrap hydrophobic drugs, nanoparticle delivery of hydrophobic anticancer drugs to tumors has gathered significant interest as a cancer treatment approach [34, 35]. Nanoparticles have been recognized to localize within both primary and metastatic tumors following systemic administration, and the biological factors leading to this accumulation profile have been thoroughly studied [36-38]. More specifically, nanoparticles have been shown to accumulate within both TAMs and healthy macrophages following systemic administration [39, 40]. Previous investigations have suggested that the sustained release of drugs from nanoparticle-containing TAMs into the surrounding cancer cells can alter the therapeutic efficacy of drug-loaded nanoparticles [41, 42]. Specifically, Miller et al. demonstrated that the accumulation of platinum prodrug-loaded polymeric nanoparticles within tumor-associated macrophages alters the spatial distribution of drug within tumors and controls nanoparticle treatment efficacy, while the depletion of macrophages from tumors reduces nanoparticle treatment efficacy [42]. However, this review will focus more on the nanoparticle effects on macrophage polarization, rather than macrophage effects on nanoparticle drug delivery to tumors. By comparing the macrophage polarization effects of various nanoparticle treatments to each other, the effect of nanoparticle material composition on macrophage polarization can be elucidated, which may provide design considerations for drug-loaded nanoparticle formulations.

Measurement of Macrophage Polarization

Because the presence of M2-like macrophages within tumors is correlated with poor patient prognosis in several types of cancer [43-45], there is interest in both quantifying macrophage polarization states in tumors and determining the polarization effects of nanoparticles within tumors. Macrophage polarization states are often measured using three main cell properties: cell surface protein expression, enzymatic activity and cytokine secretion. Table 1 summarizes some of the most common markers for macrophage polarization that increase in response to the indicated polarization state, and indicates some characterization methods for these markers that are described in the literature [46-50]. Because these markers can be affected by multiple biological factors and are time-dependent, researchers often measure multiple M1 and M2 polarization markers states at several timepoints. It should be noted that there is no standardized protocol to quantify macrophage polarization, and research groups often select various markers and detection timepoints based on their experience. In addition, researchers often use various nanoparticle treatment conditions to observe polarization effects. As a result, research groups often report inconsistent or contradictory results, and
additional experiments are needed to quantitatively compare the biological effects of nanoparticles on macrophage polarization.

**Macrophage Responses to Nanoparticles**

Macrophage responses to nanoparticles can depend on factors such as dose, route of administration, size, nanoparticle composition and nanoparticle surface properties. Macrophages in patients are typically exposed to nanoparticles in three main ways: 1) oral or parenteral administration of nanoparticle-based pharmaceutical formulations, 2) inhalation of airborne nanoparticles from pollution or occupational exposure, or 3) generation of nanoparticles in the body due to the degradation of metallic implants. Once nanoparticles enter the body, macrophages can identify nanoparticles as foreign bodies due to surface opsonization and take them up by endocytosis or phagocytosis [51]. Because these materials are not recognized as inert, macrophages will often respond to their uptake by undergoing polarization. The exact contributions of specific nanoparticle properties, such as material type, core and shell composition, therapeutic payload, or shape, to their overall macrophage polarization properties are not fully understood. Because nanoparticles are often described in the literature based on their core composition, nanoparticles in this review are categorized based on their core material rather than size or surface charge. Based on this classification system, eight main types of nanoparticles were identified: silica, gold, polymeric, cationic polymer, liposome, carbon, metallic and iron oxide. These nanoparticle types represent both nanoparticles in clinical trials or in the environment due to pollution. **Table 2** summarizes the effects of nanoparticle treatment on macrophage polarization markers that will be described in detail in the following sections. Some of the nanoparticle effects on specific polarization markers could not be identified from the literature, indicating the need for additional macrophage polarization characterization experiments.

In order to evaluate the significance of nanoparticle-induced macrophage polarization effects, they must be considered in comparison to small molecule-induced macrophage polarization effects. The immunological effects of anticancer drugs such as doxorubicin or paclitaxel within tumors have been investigated for many years [52-54]. Although some anticancer drugs have been shown to suppress immune activity within tumors and increase tumor growth [53], many of these drugs remain in clinical use for cancer treatment. Macrophages are often polarized *in vitro* by treatment with biomolecules such as interferon-γ (IFN-γ), IL-4, IL-10 or lipopolysaccharide (LPS). By comparing the polarization of nanoparticle-treated macrophages to either drug- or biomolecule-treated macrophages, researchers can gain a more detailed understanding of nanoparticle-induced macrophage polarization. Towards this goal, significant efforts are needed to identify reference treatment conditions for accurate comparisons between small molecule- and nanoparticle-induced effects.

| Marker                  | Polarization State | Detection Methods        | Type             | Reference |
|-------------------------|--------------------|--------------------------|------------------|-----------|
| IL-1β                   | M1-Like            | ELISA/PCR                | Cytokine         | [48, 170, 171] |
| IL-6                    | M1-Like            |                          | Cytokine         | [48, 171, 172] |
| IL-10                   | M2-Like            |                          | Cytokine         | [48, 50, 170-172] |
| IL-12                   | M1-Like            |                          | Cytokine         | [48, 50, 170, 172] |
| IL-23                   | M1-Like            |                          | Cytokine         | [50] |
| TGF-β                   | M2-Like            |                          | Cytokine         | [48, 171-173] |
| TNF-α                   | M1-Like            |                          | Cytokine         | [48, 170-172] |
| CD68                    | M1-Like            | FACS/IHC                 | Cell Surface Protein | [170, 172, 173] |
| CD80                    | M1-Like            |                          | Cell Surface Protein | [170, 173] |
| CD86                    | M1-Like            |                          | Cell Surface Protein | [170, 172] |
| CD163                   | M2-Like            |                          | Cell Surface Protein | [48, 49, 170, 172] |
| CD206                   | M2-Like            |                          | Cell Surface Protein | [170, 172] |
| Ferroportin             | M2-Like            |                          | Cell Surface Protein | [149, 153] |
| Reactive Oxygen Species (ROS) | M1-Like            | Fluorescence-Based Assays | Enzymatic Product | [171] |
| Nitric Oxide            | M1-Like            |                          | Enzymatic Product | [49, 50, 170-173] |
| Arginase                | M2-Like            | IHC/Western Blot         | Enzyme           | [49, 50, 172, 173] |
| Ferritin                | M1-Like            |                          | Cell Protein     | [149, 153] |
| Inducible Nitric Oxide Synthase (iNOS) | M1-Like            |                          | Enzyme/Enzymatic Product | [49, 50, 170-173] |
| Transferrin Receptor 1  | M2-Like            |                          | Cell Surface Protein | [149] |
Table 2. Effects of nanoparticle treatment on macrophage cell surface protein expression. Size range refers to the size of the nanoparticles as reported in the references. Because of their inconsistent effects, biological-based nanoparticles were excluded from the table.

| Nanoparticle Type | Overall Size Range (nm) | CD68/CD86/IL-23/IL-10 | ROS Generation | IL-1β/IL-6/IL-12/IL-23/TNF-α | CD63/CD206 | IL-10 | TGF-β | Arginase-I | Reference |
|-------------------|-------------------------|------------------------|----------------|-------------------------------|-------------|-------|-------|-----------|----------|
| Silica M1-Like    | 10-1000                 | No Change | Increase | Increase | - | No Change | Increase | - | [59-64] |
| Gold M1-Like      | 10-300                  | No Change | Increase | Increase | - | Decrease | - | - | [60,70-73] |
| Polymeric M2-Like | 30-600                  | Decrease | Decrease | Decrease | Increase | Decrease | Decrease | - | [77-80] |
| Cationic Polymer  | 110-22000               | Increase | Increase | Decrease | Increase | Decrease | Decrease | - | [85-93] |
| Liposome M2-Like  | 70-400                  | Decrease | Decrease | Increase | No Change | No Change | Increase | - | [96, 98, 99] |
| Carbon M1-Like    | 70-70000                | Increase | Increase | Increase | Decrease | Increase | No Change | Increase | [104-111] |
| Metallic M1-Like  | 20-200                  | Increase | Increase | Increase | Decrease | Increase | Decrease | - | [126-129, 136, 137, 139, 140] |
| Iron Oxide M1-Like| 30-280                  | Increase | Increase | Increase | Decrease | Decrease | Decrease | - | [150, 151, 154, 155, 161, 162, 165, 174] |

Silica Nanoparticles

Partially porous silica-based nanoparticles called mesoporous silica nanoparticles (MSNs) have been investigated as PET imaging devices entrapping radioisotopes such as copper-64 or zirconium-89 [55, 56]. Clinical trials have been performed to assess the lymph node mapping and photothermal therapy capabilities of these nanoparticles [57, 58]. Because of their widespread use in medical fields, MSNs with a variety of sizes have been prepared, and the size effects of MSNs on macrophage polarization have been investigated. For example, Kwon et al. measured the treatment effects of 100-200 nm diameter MSNs on macrophage polarization [59]. Smaller diameter MSN treatments increased ROS generation in macrophages compared to larger diameter MSN treatments. This observation suggests that the surface area-to-volume ratios of MSNs control their ROS generation potential. Furthermore, none of the MSNs in this study affected IL-6 or TNF-α secretion from macrophages. In contrast, Bancos et al. found that treatment with 10 nm but not 300 nm diameter MSNs increased TNF-α secretion from macrophages after 24 h [60]. Smaller diameter MSNs in this study demonstrated greater intracellular accumulation than larger diameter MSNs following macrophage treatment, suggesting that the intracellular accumulation of MSNs causes TNF-α secretion. However, the MSN treatments in this study did not affect CD80, CD86 or CD40 expression on macrophages. Other research groups observed that the treatment of macrophages with silica-based microparticles (1-100 µm diameter) had no effect on either IL-6 or IL-10 secretion [61]. In a different study, Park et al. found that MSN treatments increased IL-1, IL-6, TNF-α and iNOS secretion from peritoneal macrophages isolated from mice [62]. Together, these results suggest that smaller diameter MSNs promote greater M1 marker cytokine secretion from macrophages than larger diameter MSNs or microparticles.

In order to elucidate the contributions of surface area and diameter to the silica nanoparticle size effects on macrophage polarization, Kusaka et al. prepared a silica particle library with diameters between 30 and 10000 nm, and then measured IL-1β secretion from silica particle-treated macrophages as a polarization marker [63]. The group further investigated the mechanism of size-dependent IL-1β secretion, and a summary of their findings is in Figure 2. The smaller diameter MSNs had stronger effects on IL-1β secretion than larger diameter MSNs, even after accounting for differences in their surface area-to-volume ratios (Figure 2A). Macrophage treatment with the microfilament inhibitor cytochalasin D (Cyto D) prevented intracellular uptake of the silica particles, as indicated by the cell surface marker CD11b (Figure 2B). Because Cyto D pretreatment of macrophages mitigated IL-1β secretion increases, they concluded that silica particle uptake via a cytoskeleton-dependent pathway is necessary for IL-1β secretion (Figure 2C). The pretreatment of macrophages with the lysosomal acidification inhibitor bafilomycin A also mitigated potential increases in IL-1β secretion, indicating that silica particle-induced lysosomal damage may also contribute to IL-1β secretion increases (Figure 2D). Taken together, these experiments suggest that larger diameter MSNs and silica microparticles have minimal macrophage polarization effects due to decreased intracellular accumulation and minimal lysosomal disruption.

Surface modifications to MSNs have also been investigated as a method to modulate their
macrophage polarization properties. Wan et al. prepared MSNs that were coated with human plasma proteins [64]. By treating these MSNs with deglycosylases, the surface glycosylation of these MSNs could be modulated without altering their protein coating. Regardless of their surface glycosylation state, these MSNs increased IL-1β secretion, decreased TGF-β secretion, and had no effect on TNF-α secretion from M1-like macrophages. Both MSN treatments also increased TNF-α secretion from M2-like macrophages but had no effect on IL-1β or TGF-β secretion. However, surface glycosylation of MSNs reduced the magnitude of the observed M1 polarization effects. In another study, macrophage treatment with TAT peptide-functionalized or bovine serum albumin-functionalized MSNs was found to increase ROS generation in macrophages compared to unmodified MSN treatments due to increased cellular uptake [65]. Taken together, these reports indicate that increasing the size of silica particles can reduce their cellular uptake and minimize their M1 polarizing capabilities, and surface modifications to MSNs can further control their cellular uptake and modulate their polarization effects.

**Figure 2.** IL-1β secretion from macrophages treated with silica particles of various diameters. IL-1β secretion is graphed as a function of mass-based (left), mole-based (middle) or surface area-based (right) concentration (A). Macrophage uptake of FITC-labelled silica particles (green) with and without Cyto D pre-treatment. Red indicates CD11b, while green indicated silica particles (B). Quantification of IL-1β secretion from macrophages following silica particle treatment with and without Cyto D pre-treatment. (C). Quantification of IL-1β secretion from macrophages following silica particle treatment with and without bafilomycin A1 pre-treatment (D). ATP treatment was used as a control for potential cytotoxicity-induced effects on IL-1β secretion in (C) and (D). Adapted from [63] with permission.
Gold Nanoparticles

Gold nanoparticles have been investigated extensively in pre-clinical and clinical studies as tools for drug delivery, diagnostics and biomedical imaging due to their optical, acoustic and Raman properties [66-69]. In one study, gold nanoparticles were developed as a delivery system for the antiretroviral drug stavudine (STA) [70]. Figure 3 summarizes the findings of this study. Stavudine-loaded gold nanoparticles (AuNP-STA) were prepared by mixing gold nanoparticles with stavudine in water. These AuNP-STA nanoparticles were expected to accumulate within macrophages and release STA into cells, which would induce proinflammatory macrophage activation and improve drug efficacy (Figure 3A). Indeed, nanoparticle complexation of STA increased its uptake into macrophages compared to free STA treatments (Figure 3B). In addition, stavudine complexation did not affect the uptake of gold nanoparticles. The treatment of macrophages with empty gold nanoparticles increased both IL-1β and IL-10 secretion, while free stavudine treatment had no effect on these markers (Figure 3C). Surprisingly, treatment with stavudine-complexed gold nanoparticles drastically increased IL-1β secretion from...
macrophages compared to either individual treatment. In addition, stavudine-complexed gold nanoparticle treatments increased IL-10 secretion from macrophages, suggesting that nanoparticle complexation of stavudine enhances its action in macrophages. These findings highlight a potential strategy for drug-loaded nanoparticles to improve the treatment efficacy of antiretroviral drugs by targeting macrophages, increasing macrophage uptake of drug-loaded nanoparticles compared to free drug, and modulating macrophage activity to promote an effective immune response towards viruses.

Several researchers have observed changes in cytokine secretion profiles from macrophages following treatment with gold nanoparticles. Bastus et al. demonstrated that the treatment of macrophages with amyloid growth inhibitory peptide- or sweet arrow peptide-conjugated gold nanoparticles increased TNF-α, IL-1β and IL-6 secretion due to interactions between peptide-conjugated nanoparticles and toll-like receptor 4 [71, 72]. Nanoparticle-treated macrophages in this study also demonstrated increased iNOS expression as part of their immune response. In another study, Pal et al. measured the effects of citrate-coated gold nanoparticle treatment on tumor-associated macrophages from tumor-bearing mice, splenic macrophages from tumor-bearing mice and macrophages from healthy mice [73]. ROS generation increased more in tumor-associated macrophages than in splenic macrophages from tumor-bearing mice or macrophages from healthy mice. In addition, gold nanoparticle treatments decreased both TNF-α and IL-10 secretion from tumor-associated macrophages while also increasing IL-12 secretion. The contradictory cytokine secretion observations between the two previously discussed reports may be attributed to differences between citrate and peptide surface coatings of gold nanoparticles; however, elucidating the effects of nanoparticle surface coating on macrophage polarization while keeping other factors such as surface charge constant remains difficult. These results indicate the significance of nanoparticle surface coatings to macrophage polarization, and further emphasize the need to measure multiple polarization markers to accurately characterize macrophage polarization.

Polymeric Nanoparticles

Polymeric nanoparticles with a core-shell structure that are prepared from biocompatible polymers such as poly(lactic-co-glycolic acid) (PLGA) or polylactic acid have been used as biocompatible drug delivery systems for the controlled release of a variety of drugs [74-76]. Because the chemical properties of polymers can be finely tuned during their synthesis, the physical-chemical properties of polymeric nanoparticles can be finely tuned in comparison to other types of nanoparticles by using libraries of selected polymers with desired properties during nanoparticle preparation. By developing and testing several polymeric nanoparticles with varying chemical properties, the contributions of specific physical-chemical nanoparticle properties to macrophage polarization can be elucidated. Towards this goal, Ye et al. prepared polymers consisting of starch and octanoic acid, and then further modified these polymers with sulfobetaine groups in order to prepare either uncharged or zwitterionic nanoparticles [77]. By treating macrophages with these nanoparticles, the group could investigate the effects of protein binding to the nanoparticle surface, also called opsonization, on macrophage polarization. Although the uncharged surface nanoparticle increased in size and gained a negative surface charge during storage in 10% serum due to protein binding, zwitterionic nanoparticles remained stable in 10% serum. As a result of protein binding, the uncharged nanoparticles demonstrated greater accumulation in macrophages than the zwitterionic nanoparticles. The uncharged nanoparticles increased IL-6 and TNF-α secretion from macrophages in comparison to zwitterionic nanoparticles, presumably due to the presence of serum proteins on the nanoparticle surface. Based on these results, the group hypothesized that the opsonization of polymeric nanoparticles contributes greatly to their macrophage uptake and polarization effects. In addition to opsonization-induced macrophage interactions, sugar groups can also induce macrophage interactions. Su et al. developed polymeric nanoparticles with various sugar groups in the corona, in order to target macrophage surface receptors such as CD206 (mannose receptor) [78]. Macrophage treatment with these nanoparticles increased CD86 expression, while also decreasing CD23 and CD206 expression. These nanoparticles also increased IL-12, MCP-1 and TNF-α secretion from macrophages, while also decreasing IL-10 secretion. It should be noted that the polyethylene glycol (PEG)-modified nanoparticles used in this study had no effect on macrophage polarization, presumably because they were not taken up by macrophages. Together, these results indicate that increasing the surface ionization or adding inert polymers to the surface of nanoparticles to reduce their opsonization and prevent specific nanoparticle-macrophage interactions can reduce their macrophage polarization capabilities.

To elucidate the effects of specific polymeric nanoparticle surface properties on macrophage
polarization, Wang et al. prepared a library of 13 polymeric nanoparticles with different functional groups on the surface and characterized their macrophage polarization properties [79]. These nanoparticles had minimal differences in surface charge or melting temperature. The surfaces of these nanoparticles were characterized using a 22-parameter polymer property model, and both IL-10 and TNF-α secretion was measured from nanoparticle-treated macrophages. To quantify the specific effects of nanoparticle chemical properties on macrophages, the group developed mathematical models relating IL-10 and TNF-α secretion to nanoparticle surface properties. These mathematical models, also called quantitative structure-activity relationships (QSARs), indicated that hydrogen bond donors and acceptors on the nanoparticle surface predicted cytokine secretion from macrophages. The group speculated that hydrogen bond donors and acceptors on the nanoparticle surface facilitated opsonization, which caused macrophage polarization following uptake. In additional work by this group, QSARs were developed to predict in vivo macrophage polarization [80]. Figure 4 highlights some of the key results from this study. First, the group treated mice with various nanoparticles and isolated macrophages from these mice. Next, the group characterized TNF-α secretion from these macrophages (Figure 4A). Then, IL-10 secretion was measured from these macrophages (Figure 4B). After that, the ratio of arginase to iNOS activity was measured (Figure 4C). Using these measured data, QSARs were developed to predict cytokine secretion or enzymatic activity in macrophages. These QSARs identified key physical and chemical properties of polymeric nanoparticles that contributed to in vivo macrophage polarization. In addition, the QSARs demonstrated that specific polymer properties affect each macrophage polarization marker differently, and that the surface properties of a polymeric nanoparticle must be considered carefully when controlling the macrophage polarization properties of a polymeric nanoparticle. Overall, these findings indicate that polymeric nanoparticle surface properties affect each macrophage polarization marker differently, and polymeric nanoparticles must be carefully designed to control their polarization properties based on several markers.

Cationic Polymers

Cationic polymers have been used to prepare nanoparticle delivery systems for both nucleic acids and anionic drugs by charge neutralization [81-83]. Depending on the chemical structures of the polymer and payload, the cationic groups in these nanoparticles can either be free, exposed on the nanoparticle surface or concealed in the nanoparticle core. Unlike other polymeric nanoparticles that are typically developed with inert polymers, cationic polymers have been shown to cause toxicity by interacting with cellular components such as the mitochondria [84]. As a result, the biological effects of cationic polymer-containing nanoparticles are often considered to be distinct from neutral or anionic polymeric nanoparticles. Treatment of macrophages with cationic polymers such as polyethylenimine (PEI) or cationic dextran has been demonstrated to induce M1-like polarization through toll-like receptor 4 (TLR4) signaling and promote immune cell infiltration into tumors [85]. In another study, Lunov et al. demonstrated that the treatment of macrophages with cationic polystyrene nanoparticles increased IL-1β secretion and promoted inflammasome formation [86]. In contrast, macrophage treatment with non-ionic or anionic polystyrene nanoparticles in this study had no effect on these markers. Regardless of their surface functionalization, polystyrene nanoparticle treatments did not affect TNF-α secretion from macrophages. To assess the role of ROS generation in macrophage polarization, the group loaded the nanoparticles with ROS scavengers and then treated macrophages. Because these nanoparticles had minimal effects on macrophage polarization, the polarization effects of cationic polystyrene nanoparticles were concluded to be ROS-mediated. However, other reports found that both cationic and anionic polystyrene nanoparticle treatments of M2-like macrophages decreased CD163 and CD200R expression without affecting CD86 expression [87]. The treatment of M2-like macrophages with either nanoparticle also decreased IL-10 secretion without affecting TNF-α secretion, IL-1β secretion, or NO2 expression. Together, these results suggest that cationic groups on the surface of nanoparticles can induce M1-like polarization in macrophages.

In addition to the charged groups on the surface, polymeric nanoparticles can also conceal charged groups in the core. Castro et al. used the cationic carbohydrate chitosan and the anionic peptide poly-γ-glutamic acid to prepare nanoparticles and measured their effects on macrophage polarization [88]. Macrophages that were treated with these nanoparticles demonstrated increased CD40, CD86 and HLA-DR expression. In addition, these nanoparticles increased IL-12, IL-23, TNF-α, IL-6 and IL-10 secretion from macrophages, while also preventing IL-10-induced M2-like polarization. Goncalves et al. confirmed the effects of chitosan/poly-γ-glutamic acid nanoparticle...
treatments on macrophages [89]. In further experiments, Goncalves et al. demonstrated that loading the anti-inflammatory drug diclofenac into these nanoparticles mitigated their effects on IL-12, IL-23 and TNF-α secretion from macrophages. In a different study, Ma et al. also demonstrated that chitosan nanoparticle treatments increased IL-1, IL-6 and TNF-α secretion from macrophages [90]. However, the treatment of macrophages with arginine-based polyester amide/hyaluronic acid nanoparticles increased IL-10 secretion, TNF-α secretion and arginase expression [91]. It should be noted that the nanoparticles in this study contained a molar excess of hyaluronic acid, and charge neutralization due to ionic bonding could mitigate the M1-polarizing effects of the cationic arginine-based polyester amide polymer in these nanoparticles. Taken together, these results suggest that concealing or neutralizing charged groups on cationic polymers in the core of a nanoparticle does not mitigate their M1 polarizing capabilities, probably due to nanoparticle dissociation following cellular uptake.

Figure 4. Development of qualitative structure-activity relationships to describe macrophage polarization following treatment with various polymeric nanoparticles. Mice were treated with various nanoparticles, and their macrophages were isolated and characterized. TNF-α secretion was measured from each group of isolated macrophages (A). IL-10 secretion was measured (B). The ratio of arginase to iNOS activity was measured (C). Mathematical relationships were developed using these observed values to predict these values based on nanoparticles properties. To verify the accuracy of these predictions, the observed values were compared to the predicted values for each nanoparticle treatment. Figure was adapted with permission from [80].
In contrast to the previously described nanoparticles that are formed by charge neutralization of cationic polymers, unimolecular polymeric nanoparticles containing cationic groups in the core have also been developed. These nanoparticles are expected to remain stable upon dilution and shield charged groups from the nanoparticle surface. Bernal et al. prepared unimolecular nanoparticles by conjugating mannose to the surface of PEI [92]. Mannose-conjugated PEI (Man-PEI) treatments slightly decreased IL-6 mRNA expression increases in response to LPS pre-treatment, but cytokine secretion from macrophages in response to LPS treatment was mostly unaffected by Man-PEI [93]. Man-PEI treatments mitigated LPS-induced increases in IL-6 and IL-10 secretion from macrophages during a second LPS stimulation. These results suggest that while cationic polymer treatments typically cause M1-like polarization, chemical modifications to these polymers to prevent nanoparticle dissociation and charge-based interactions can mitigate their macrophage polarization capabilities.

**Liposomes**

Liposomal formulations of anticancer drugs such as FDA-approved Doxil (doxorubicin) or Onivyde (irinotecan) have reduced chemotherapy drug toxicity compared to traditional formulations [94, 95], but the effects of liposome treatments on macrophage polarization have only recently been considered. Towards this goal, Rajan et al. treated tumor-bearing mice with PEG-coated liposome nanoparticles (PLNs) and measured their effect on tumor growth [96]. Figure 5 summarizes these findings and presents a mechanism for PLN effects on tumor growth. PLN treatment increased tumor growth in normal mice compared to saline treatment; however, PLN treatment did not affect tumor growth in macrophage-depleted mice (Figure 5A). To further investigate the effects of PLN treatments on TAMs, the group prepared PLNs that were loaded with alendronate, a bisphosphonate that has been implicated to interfere with TAM activity [97]. The treatment of mice with alendronate-loaded PLN (PLN-ALEN) reduced tumor growth compared to either empty liposome treatments or a physical mixture of empty liposomes and alendronate (Figure 5B). In addition, tumor-bearing mice treated with PLN-ALEN had longer overall survival than PLN-treated mice (Figure 5C). Based on these findings and supplemental biological characterization of cells isolated from liposome-treated tumors, the group proposed a mechanism of macrophage polarization by liposome treatment involving an autocrine/paracrine feedback loop of TGF-β that increases CCL-2 secretion, which recruits other myeloid-derived cells to tumors that further enhance the immunosuppressive properties of the tumor microenvironment (Figure 5D). Together, these cells are believed to suppress the activity of cytotoxic T lymphocytes within tumors and increase tumor survival and progression. The results of this group highlight the complex relationship between nanoparticle treatments, immune cells and macrophage polarization within tumors.

The macrophage polarization effects of nanoparticles such as liposomes can be difficult to measure in vitro, and contradictory conclusions about nanoparticle properties are often reported in the literature. Although differences in liposome chemical composition between studies may contribute to these contradictory conclusions, experimental design differences are also likely to contribute. To assure that the observed macrophage polarization properties of a nanoparticle are accurate and representative of macrophage behavior in the tumor microenvironment, great care must be taken when designing experiments to observe nanoparticle-induced macrophage polarization. In agreement with Rajan et al., other groups observed M2-like polarization in macrophages isolated from dipalmitoyl phosphatidylcholine (DPPC)/cholesterol liposome-treated mice [98]. However, Bartneck et al. reported that PEG-coated liposome treatments did not affect protein expression or cytokine secretion from macrophages in vitro [99]. This paradoxical observation suggests that TAM polarization requires interactions between cancer cells and tumor-associated macrophages to occur, and that in vitro experiments must be carefully designed to mimic these in vivo conditions and observe relevant macrophage behavior to the tumor microenvironment.

**Carbon-Based Nanoparticles**

Carbon-based nanoparticles are composed primarily of carbon nanotubes (CNTs) and carbon nanospheres (CNSs), which are also called buckyballs. These nanostructures have unique mechanical properties, high conductivity and the capability to encapsulate both drugs and imaging agents [100-102]. Because of these properties, these materials have been extensively studied and used as reinforcements for metallic implants and to deliver drugs. For these reasons, understanding the harmful effects of carbon-based nanoparticles on macrophages is important. In contrast to other types of nanoparticles, Smith et al. demonstrated that following intravenous injection of CNTs, a significant portion of CNTs were
localized within circulating Ly-6Chi monocytes [103]. The number of CNT-loaded monocytes entering tumors increased up to 7 days post-treatment, and labelling the CNTs with RGD peptide increased the fraction of monocyte-entrapped CNTs within tumors. However, it should be noted that more than 70 percent of CNTs remained outside of these monocytes. Nonetheless, these results suggest that the unique cellular distribution of CNTs may reduce their localization within macrophages following intravenous administration and lessen their macrophage polarization potential.

In addition to intravenous administration of CNTs, these nanoparticles have also been injected intracranially. VanHandel et al. compared the effects of CNT treatment on macrophages in both healthy and brain tumor-bearing mice [104]. Following intracranial CNT injections, brain tumors in mice had reduced macrophage recruitment compared to injection sites of healthy mice brains. The macrophages isolated from brain tumors had increased TNF-α, IL-6, IL-12 and IL-10 secretion compared to macrophages isolated from healthy mice brain injection sites. Despite these observed cytokine secretion effects in brain tumors, intracranial CNT injections did not affect brain tumor growth compared to untreated mice, presumably because the macrophage polarization effects were only transient. In different studies, other groups have also observed that CNT treatments induce M1-like polarization of macrophages in the lungs and liver [105, 106]. Meng et al. also reported that CNT treatment slightly increased cytokine secretion, TLR4 expression and CD206 expression on macrophages [107]. However, it should be noted that many of the effects of CNT treatments on macrophage polarization are time-dependent. Long-term studies of macrophage polarization following CNT treatment indicated that iNOS expression and TNF-α secretion increases for 24 to 72 h post-treatment and returns to normal levels after 7 days, but arginase expression increases for up to 14 days post-treatment [108]. Other studies reported that CNT-treated macrophages returned to an M2-like phenotype 7 days post-treatment [109]. The time-dependency of nanoparticle-induced macrophage polarization effects has not been thoroughly investigated with other types of nanoparticles. Together, these results emphasize the need to measure the long-term effects of nanoparticles on macrophage polarization.

Figure 5. PLN-induced tumor growth is mediated by macrophages. In vivo depletion of systemic macrophages abolished PLN-induced tumor growth (A). PLN-induced tumor progression is mitigated by encapsulated alendronate but not free alendronate (B). Survival of PLN- and PLN-ALEN-treated mice (C). Proposed mechanisms of PLN-induced tumor growth and immunosuppression in tumors based on interactions between liposomes, macrophages and other immune cells (D). Adapted with permission from [96].
The macrophage polarization effects of carbon nanospheres (CNS) have also been investigated. Tang et al. treated macrophages with polyhydroxylated CNS and measured treatment effects on macrophage polarization [110]. CNS-treated macrophages demonstrated a 1.2- to 1.5-fold increase in TNF-α, IL-1β and IL-6 secretion compared to untreated macrophages, indicating a mild M1-like polarization state. In a macrophage-cancer cell co-culture system, CNS-pretreated macrophages decreased the viability of cancer cells compared to non-pretreated macrophages, despite small changes to macrophage cytokine secretion profiles. However, CNS treatments did not affect cancer cells in the absence of macrophages. Based on these results, the group hypothesized that CNS treatments activate macrophages to selectively kill cancer cells. To further investigate this hypothesis, CNS-pretreated macrophages were infused into the peritoneal cavity of mice in a lung metastasis model. The infusion of CNS-pretreated macrophages decreased metastatic nodule formation in mice following an intravenous injection of lung cancer cells compared to mice infused with saline. In contrast, there was no difference in metastatic nodule formation between mice infused with non-pretreated macrophages and mice infused with saline, indicating that CNS-treated macrophages can kill cancer cells in vivo despite moderate changes to their in vitro cytokine secretion profiles. Pacor et al. also found that CNS with a positive surface charge reduced their intracellular accumulation, reduced their mitochondrial localization, and decreased ROS generation in macrophages compared to CNS with an uncharged surface [111]. Taken together, these findings suggest that carbon-based nanoparticles induce short-term M1 macrophage polarization following treatment regardless of their shape, and this polarization can cause cancer cell death in metastatic tumors but may not significantly affect primary tumors.

**Biological-Based Nanoparticles**

Nanoparticles consisting of biomolecules, such as proteins, nucleic acids, sugars, lipids and lipoproteins, or cellular components, such as exosomes and microvesicles, have gathered recent interest as biocompatible drug delivery systems. Because some of these nanoparticles have only been developed recently, their macrophage polarization properties have not been thoroughly characterized, and additional studies are needed to elucidate their biological effects. Due to the chemical diversity found within this nanoparticle group, these nanoparticles often have disparate polarization effects. As a result, these nanoparticles were not included in Table 2. Some examples of biological-based nanoparticles are Abraxane (nanoparticle albumin-bound paclitaxel, or nab-paclitaxel) [112] and the spherical nucleic acid-based nanoparticle NU-0129 is undergoing clinical trials. Cullis et al. investigated the macrophage polarization properties of Abraxane nanoparticles in pancreatic cancer in detail [113]. Abraxane treatment of macrophages increased the in vitro secretion of IL-1α, IL-1β, IL-6, IL-12 and TNF-α compared to free paclitaxel treatment, indicating a shift to an M1-like polarization state. Macrophage treatment with a TLR4 inhibitor abrogated the M1-like polarizing effects of Abraxane. These results concur with other reports describing the TLR4-associated effects of paclitaxel on cancer cells and macrophages [114, 115]. To further investigate the in vivo effects of Abraxane in pancreatic cancer treatment, Cullis et al. treated mice bearing orthotopic pancreatic tumors with Abraxane nanoparticles and gemcitabine, which is a clinically used drug combination for pancreatic cancer treatment [116]. Figure 6 describes some of the findings in this experiment. Immunohistochemical staining of tumors demonstrated that the tumors contained F4/80-labelled macrophages (Figure 6A). Fluorescence imaging of tumors that were treated with Oregon green-labelled Abraxane showed that the F4/80-labelled macrophages took up Abraxane (Figure 6B). Abraxane treatment increased the presence of M1-like polarized macrophages, as indicated by MHCII/CD80/CD86+ cells (Figure 6C). The increased amount of MHCII/CD80/IL-1α+ cells confirmed this M1 polarization observation (Figure 6D). However, gemcitabine treatment alone had no effect on macrophage polarization. This study highlights the potential for drug-loaded albumin-based nanoparticles to amplify the M1-polarizing effects of a drug payload to improve tumor treatment.

Sugar- and lipid-based nanoparticles have been used as biocompatible polymers for drug delivery. Oxidized dextran treatment of macrophages was shown to increase CD206 expression but had no effect on CD86 expression, suggesting that polysaccharide treatments can cause M2-like polarization shifts in macrophages [117]. In addition, oxidized dextran treatments reduced LPS-induced increases in CD86 expression. The treatment of macrophages with lipid-based nanoparticles consisting of phosphatidylserine decreased MCP-1, TNF-α, IL-6 mRNA levels and increased TGF-β and IL-10 mRNA levels [118]. These results suggest that sugar- and lipid-based nanoparticles can induce weak M2-like polarization in the absence of a drug payload.

Researchers have also investigated the effects of nucleic acid-based nanoparticles on macrophages.
Macrophage treatment with tetrahedral DNA nanostructures (TDNs) increased TNF-α, IL-1β and IL-6 secretion from macrophages [119]. TDNs slightly increased iNOS expression and decreased arginase expression in macrophages. Zhang et al. also demonstrated that DNA-based nanoflowers increased TNF-α, IL-6 and IL-10 secretion from macrophages [120]. In contrast to DNA-based nanoparticles, RNA-based nanoparticle treatments did not affect TNF-α or IL-6 secretion from macrophages [121]. To directly compare the effects of various types of nucleic acid-based nanoparticles on cytokine activation, Afonin et al. prepared DNA-based and RNA-based nanoparticles with similar structures and treated peripheral blood mononuclear cells (PBMCs) [122]. In these studies, RNA nanoparticles significantly increased IL-1β secretion from PBMCs while DNA nanoparticles had no effect. Differences between this study and other studies could be attributed to biological differences between PBMCs and macrophages. Overall, these findings suggest that the structure, conformation and chemical composition of nucleic acid-based nanoparticles can significantly affect their macrophage polarization potential.

Metallic Nanoparticles

Metallic nanoparticles can enter the body due to either environmental exposure or the breakdown of metallic implants [123-125]. Although the cytotoxicity of these nanoparticles in various cell types has been studied, the macrophage polarization properties of these nanoparticles are less thoroughly described in the literature. Armstead et al. demonstrated that tungsten carbide-cobalt nanoparticle (WC-Co-NP) treatment decreased CD206 expression on macrophages in a co-culture system of macrophages and healthy endothelial cells [126]. WC-Co-NP-treated macrophages also demonstrated increased TNF-α and IL-1β secretion. Scherbart et al. investigated the size-dependent effects of titanium nanoparticles (Ti-NPs) on macrophage polarization [127]. Smaller diameter Ti-NP treatments increased HO-1 expression, iNOS expression and TNF-α secretion from macrophages, while larger diameter Ti-NP treatments had no effect on these three
polarization markers. However, macrophage treatment with either diameter nanoparticle increased ROS generation in macrophages. Kumar et al. also found that Ti-NP treatments increased IL-1β, IL-6 and TNF-α secretion in macrophages that were isolated from mice [128]. Furthermore, Sarkar et al. demonstrated that macrophages that were treated using silver nanoparticles with various diameters and surface coatings increased IL-1β and IL-8 mRNA expression in cells [129]. Together, these results suggest that various types of ROS-generating metallic nanoparticles can induce M1-like polarization in macrophages.

In contrast to many metallic nanoparticles, cerium oxide nanoparticles (nanoceria) have demonstrated antioxidant properties under physiological conditions. The antioxidant properties of nanoceria have been employed to develop biosensors and to inhibit bacterial growth [130-134]. Selvaraj et al. found that nanoceria treatment of macrophages decreased TNF-α, IL-1β, IL-1α, MIP-3β, MCP-1 and MCP-3 secretion following LPS pretreatment [135]. Nanoceria treatment also reduced iNOS expression in macrophages, which decreased ROS generation and ROS-induced liver damage in nanoceria-treated rats. Both nanoceria and copper nanoparticle treatments have been shown to increase arginase expression in macrophages and shift their activity towards M2-like polarization [136, 137]. Similarly, the treatment of macrophages with chromium oxide nanoparticles did not affect TNF-α, MCP-1 or MIP-1α mRNA expression [138]. Taken together, these results indicate that antioxidative nanoparticles can induce M2-like polarization in macrophages.

To better understand the mechanisms behind macrophage polarization following drug-loaded metallic nanoparticle treatment, the potential synergistic effects between drugs and metallic nanoparticles on macrophage polarization must be considered. One example of a drug-loaded metallic nanoparticle with synergistic effects on macrophage polarization is doxorubicin (DOX)-loaded zinc oxide nanoparticles (ZnO-DOX). Zinc oxide nanoparticle treatments were shown to increase M1 marker cytokine secretion from macrophages [139, 140]; however, some anticancer drugs such as DOX have been reported to induce M2-like macrophage polarization [141-143]. Wang et al. investigated the effects of ZnO-DOX on tumors, and Figure 7 describes their approach and some of the key findings in their study related to macrophages. The group proposed that zinc oxide nanoparticles affect macrophages, cancer cells and cancer stem cells within tumors, and a combination of these effects inhibits cancer progression (Figure 7A). While doxorubicin treatment alone decreased TNF-α secretion from macrophages, zinc oxide nanoparticle treatment slightly increased TNF-α secretion (Figure 7B). In contrast to both treatments, ZnO-DOX significantly increased TNF-α secretion from macrophages. ZnO-DOX treatments also increased IL-6 secretion in comparison to individual treatments (Figure 7C). Macrophages treated with ZnO-DOX also demonstrated increased CD80, CD86 and MHCII expression in comparison to either DOX or zinc oxide nanoparticles (Figure 7D). These findings demonstrated that nanoparticle encapsulation of anticancer drugs altered its macrophage polarization properties synergistically, and that investigators should consider potential synergistic effects between drugs and nanoparticles when designing drug-loaded nanoparticles for effective cancer treatment.

Iron Oxide Nanoparticles

Iron oxide nanoparticles are currently used clinically as iron replacement therapies [144]. Several groups have also developed iron oxide-based nanoparticles as theranostic drug delivery systems [145-147]. Because iron exposure regulates the expression of iron transport-related proteins that are correlated to macrophage polarization states [148, 149], researchers have hypothesized that iron oxide nanoparticles would have strong effects on macrophage polarization within the tumor microenvironment. As a result, the effects of iron oxide nanoparticle treatments on macrophage polarization within tumors and the therapeutic consequences of iron oxide nanoparticle-induced macrophage polarization have been investigated extensively.

Super-Paramagnetic Iron Oxide Nanoparticles (SPIONs) are iron oxide nanoparticles consisting of an iron oxide core coated with a biocompatible polymer coating such as dextran or polyethylene glycol. Kodali et al. compared the effects of silica nanoparticle and SPION treatments on lung macrophages by measuring changes in gene expressions following either treatment [150]. Their results showed that SPION treatments changed the expression of 1029 genes, while silica nanoparticle treatments changed the expression of only 67 genes. SPION treatments reduced IL-10 secretion from macrophages more than silica nanoparticle treatments. In addition, SPION treatments increased TNF-α secretion from macrophages in comparison to silica nanoparticle treatments. As a result, researchers often consider iron oxide nanoparticles to have stronger M1-like polarization capabilities than other types of M1-polarizing nanoparticles.
Figure 7. Zinc oxide nanoparticle effects on tumor cells. Proposed mechanism for zinc oxide nanoparticle effects on macrophages, cancer cells and cancer stem cells (A). TNF-α and IL-6 secretion of macrophages treated with either doxorubicin (DOX), zinc oxide nanoparticles (ZnO) or doxorubicin-loaded ZnO (ZnO-DOX) (B). DOX treatment enhances TNF-α and IL-6 secretion increases following ZnO treatment. DOX also enhances CD80 and CD86 increases following ZnO treatment but has a minimal effect on MHCII expression (C). Adapted with permission from [140]. Copyright 2017 American Chemical Society.

The potential effects of both the surface coating and the iron oxide core on macrophage polarization must be considered. In order to investigate potential surface coating effects on macrophage polarization, Rojas et al. prepared dimercaptosuccinic acid-, aminopropyl silane- and aminodextran-coated SPIONs for macrophage treatment [151]. Macrophage treatment with these SPIONs increased ROS generation and ferroportin expression and shifted macrophages towards an M1-like phenotype regardless of surface coating. The observation of SPION treatment-induced ferroportin expression agrees with previous reports [152, 153]. Other groups demonstrated that macrophage treatment with PEI-coated SPIONs increased CD80, CD86 and ferritin expression on macrophages, while also increasing IL-12 and IL-10 secretion from macrophages [154]. In patients, intravenous injections of amine-functionalized polyvinyl alcohol-coated SPIONs increased IL-1β secretion from isolated macrophages and increased cytokine concentrations in the blood for up to 72 h [155]. However, Manuelli et al. showed that PEG-coated SPION treatments did not affect TNF-α secretion from macrophages, despite
observed TNF-α secretion increases from macrophages following citrate-coated and α-Tn mucin antigen-coated SPION treatment [156]. In this study, the macrophage polarization differences between PEG-coated SPION treatments and other SPION treatments were attributed to decreased cellular uptake, which agrees with previous experimental observations of PEG-coated SPION treatments [157, 158]. Taken together, these results suggest that the metallic core of iron oxide nanoparticles, rather than the surface coating, control their macrophage polarization properties.

Feraheme (also called ferumoxytol) is an FDA-approved carboxymethyl dextran-coated iron oxide nanoparticle that is used to treat iron deficient anemia. This nanoparticle has been used as a drug delivery system for anticancer drugs and for contrast agent delivery [35, 159, 160]. Zanganeh et al. investigated the effects of empty ferumoxytol on macrophage polarization in detail [161]. Figure 8 summarizes the key findings of their study. After ferumoxytol treatment, macrophages in co-culture with cancer cells demonstrated increased M1 marker and decreased M2 marker gene expression (Figure 8A). Ferumoxytol treatment increased TNF-α secretion (Figure 8B) and decreased IL-10 secretion (Figure 8C) from macrophages in co-culture with cancer cells. To further investigate these effects, mice were injected with a mixture of cancer cells and physiologically relevant amounts of ferumoxytol. Ferumoxytol reduced tumor growth in a dose-dependent manner (Figure 8D). Because

![Figure 8](http://www.ntno.org)

**Figure 8. Effects of ferumoxytol treatment on macrophages and tumor growth.** Ferumoxytol treatment increased M1-associated gene expression while also decreasing M2-associated gene expression (A). TNF-α secretion was decreased from ferumoxytol-treated macrophages in the presence of cancer cells (B). Ferumoxytol inhibited IL-10 secretion from macrophages in the presence of cancer cells (C) and also reduced tumor growth in a dose-dependent manner (D). In contrast, dextran nanoparticles had no effect on tumor growth (E). Tissue sections from tumors after 7 or 21 days were stained for CD11B, CD80 or CD206 (F). Ferumoxytol decreased CD206 expression and increased CD80 expression on tumor-associated macrophages for up to 21 days (F). Adapted with permission from [161].
injections of dextran did not affect tumor growth, tumor growth effects of ferumoxytol were attributed to the iron oxide core (Figure 8E). In addition, tissue sections from ferumoxytol-containing tumors were taken at 7 and 21 days post-injection and analyzed for CD80 and CD206 expression (Figure 8F). Ferumoxytol decreased CD206 expression and increased CD80 expression at 7 days post-injection (Figure 8G). These effects were maintained at 21 days post-injection. These findings suggest that ferumoxytol nanoparticles hold promise to improve cancer treatment by inducing tumor-suppressive macrophage polarization within tumors.

In addition to Feraheme, several other iron oxide-based nanoparticle have been used clinically as MRI contrast agents. The treatment of M2-polarized macrophages with the carboxy-dextran-coated SPION Resovist (ferucarbotran) increased ferritin, CD86 and cathepsin L expression while also increasing TNF-α secretion, indicating an M1-like polarization shift [162]. However, other reports suggest that macrophage labeling with either the dextran-coated iron oxide nanoparticle Feridex IV (ferumoxide) or the ultra-small SPION Combidex (ferumoxytran-10) had no significant effect on macrophage behavior [163, 164]. The reported differences in macrophage polarization effects between these experiments could be attributed to lower iron doses in macrophage labeling experiments (0.1-0.5 mg iron/ml media) compared to cancer treatment experiments (3.9 mg iron/ml media).

Clinical evidence also indicates that iron accumulation near tumors due to hemolysis induces M1-like polarization. Costa da Silva et al. examined iron accumulations in tissue samples from non-small cell lung cancer patients [165]. In these tissue samples, hemolysis-induced iron accumulation near the periphery of lung tumors was positively correlated to CD68 expression on TAMs and inversely correlated to tumor size in patients. Iron-containing macrophages in tissue samples exhibited increased iNOS and CD86 expression, along with decreased CD206 expression. In addition, these macrophages also demonstrated increased IL-6 secretion and decreased IL-10 secretion, further verifying an M1 polarization shift. This group hypothesized that the observed hemolysis-induced tumor size decreases could also be caused by iron oxide nanoparticle accumulation near tumors. To test this hypothesis, the group treated lung tumor-bearing mice with iron oxide nanoparticles. In these mice, iron accumulation near tumors decreased CD206 expression on TAMs and decreased tumor size. Overall, this research suggests that iron-induced M1-like polarization of TAMs has clinical applications to decrease tumor size and improve cancer therapy.

Alternating magnetic field (AMF) therapy has been proposed as a method for controlling drug release from SPIONs and inducing hyperthermia in tumors [166-168], but AMF effects on macrophage polarization have been less thoroughly studied. Kang et al. investigated the effects of AMF therapy on macrophage polarization following treatment with an RGD peptide-labelled amino-silica-coated SPION [169]. Figure 9 describes their experimental protocol and findings. These SPIONs were attached to a matrix and implanted into mice, which were then exposed to alternating magnetic fields with or without injection of M2-polarizing cytokines (Figure 9A). Following nanoparticle implantation, macrophages in mice that were exposed to a low frequency AMF exhibited decreased iNOS and increased arginase-1 expression in macrophages, indicating an M2-like polarization state (Figure 9B). In contrast, macrophages in mice that were exposed to a high frequency AMF demonstrated an M1-like polarization state. The AMF-induced macrophage polarization effects could also overcome any effects from M1- or M2-polarizing cell media treatment in vitro. Overall, these results suggest that AMF therapy holds promise to control macrophage polarization following nanoparticle treatment.

Conclusions and Future Perspectives

Nanoparticle-based drug delivery systems offer a unique opportunity to target tumor-associated macrophages, which contribute to tumorigenesis, metastasis and therapeutic resistance in patients. The contributions of nanoparticles to macrophage polarization within the tumor microenvironment must be considered when developing effective nanoparticle-based therapies for cancer treatment. In general, M2-like macrophages increase tumor growth and suppress immune responses to cancer cells, while M1-like macrophages can reduce tumor growth by selectively killing cancer cells within the tumor microenvironment. Because there is no standardized method to measure the effects of a nanoparticle treatment on macrophage polarization, researchers often use inconsistent markers, timepoints and measurement conditions to quantify nanoparticle effects on macrophage polarization. Because of this inconsistency, researchers can report inconsistent findings and conclusions. To overcome this issue, standardized conditions are needed for researchers to measure and report the macrophage polarization effects of nanoparticles. In general, reducing the cellular uptake of a nanoparticle by decreasing its diameter, increasing its surface charge or adding an inert surface coating can reduce its macrophage polarization effects. In contrast, increasing the
Macrophage uptake of a nanoparticle by adding a macrophage-targeting moiety to the surface can enhance its macrophage polarization properties. Macrophage-polarizing drug-loaded nanoparticles may also demonstrate synergistic effects with their therapeutic payload. The literature suggests that polymeric nanoparticles and liposomes can cause M2-like polarization following macrophage treatment, while other types of nanoparticles can cause M1-like polarization. However, a variety of clinically-approved iron oxide nanoparticle treatments have also been shown to induce strong M1-like polarization in TAMs as compared to other nanoparticles and reduce tumor growth in animal models. In the future, additional work is needed to elucidate both the dose- and time-dependency of nanoparticle treatments on macrophage polarization, particularly for iron oxide nanoparticles. Overall, nanoparticles are a promising treatment approach to modulate macrophage polarization in the tumor microenvironment, and researchers developing nanoparticle-based cancer treatments should consider the macrophage-polarizing potential of nanoparticles to maximize their therapeutic efficacy.

Figure 9. Alternating Magnetic Field (AMF) therapy as an approach to control macrophage polarization. Schematic of AMF-induced macrophage polarization measurement in vivo (A). Low frequency AMF decreased iNOS expression in isolated macrophages, while high frequency AMF increased iNOS expression (B). In contrast, low frequency AMF increased arginase-1 expression in isolated macrophages in M2 polarizing conditions, while high frequency AMF decreased arginase-1 expression. Reproduced with permission from [169]. Copyright 2017 American Chemical Society.
Abbreviations

BSA: bovine serum albumin; CD: cluster of differentiation; CNS: carbon nanosphere; CNT: carbon nanotube; Cyto D: cytochalasin D; DOX: doxorubicin; DPPC: dipalmitoyl phosphatidylcholine; ECM: extracellular matrix; FITC: fluorescein isothiocyanate; h: hours; IL: interleukin; LPS: lipopolysaccharide; MHCII: major histocompatibility complex type II; MMP-9: matrix metalloproteinase-9; Mφ1: type 1 macrophage; Mφ2: type 2 macrophage; MSN: mesoporous silica nanoparticle; μm: micron; NK cell: natural killer cell; nm: nanometer; PEG: polyethylene glycol; PEI: polyethyleneimine; PFG: placental growth factor; PLGA: poly(lactic-co-glycolic acid); PLN: PEG-coated liposome nanoparticles; PLN-ALEN: Alendronate-loaded PEG-coated liposome nanoparticles; QSAR: quantitative structure-activity relationship; ROS: reactive oxygen species; SPION: superparamagnetic iron oxide nanoparticle; TAM: tumor-associated macrophage; TAT: trans-activating transcriptional activator; TLR: toll-like receptor; VEGF: vascular endothelial growth factor; ZnO: zinc oxide nanoparticle; ZnO-DOX: doxorubicin-loaded zinc oxide nanoparticle.

Acknowledgements

The authors thank the National Institute of Health (NIH) for financial support through grant EB 019288 and Cedars Sinai Medical Center for providing additional financial support.

Competing Interests

The authors have declared that no competing interest exists.

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