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Identifying Factors of Microparticles Modified with Arginine Derivatives That Induce Phenotypic Shifts in Macrophages

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ABSTRACT: Macrophages are key players in the progression of many diseases, ranging from rheumatoid arthritis to cancer. Drug delivery systems have the potential not only to transport payloads to diseased tissue but also to influence cell behavior. Here, poly(N-isopropylacrylamide-co-acrylic acid) (pNIPAm-co-AAc) microparticles were modified with 14 different arginine derivatives. These particles were then incubated with interleukin-4 or lipopolysaccharide-stimulated macrophages or naïve macrophages (RAW 264.7). The phenotypic state of the macrophages was assessed by measuring arginase activity, tumor necrosis factor-α (TNF-α) secretion, and nitrite production. Partial least-squares analysis revealed material properties and descriptors that shifted the macrophage phenotype for the three cell conditions in this study. Material descriptors relating to secondary bonding were suggested to play a role in shifting phenotypes in all three macrophage culture conditions. These findings suggest that macrophage responses could be altered through drug delivery vehicles, and this method could be employed to assist in screening potential candidates.

KEYWORDS: macrophage phenotypes, TNF-α, polymer properties

1. INTRODUCTION

Material properties are known to alter cellular responses.1−8 In particular, surface modifications can alter cytokine and molecular secretion profiles.6,8,9 In the case of macrophages, changes in the amount of secreted molecules can signal a shift in their phenotype. Macrophages exist on a spectrum, with one end being pro-inflammatory, denoted M1, and the other being pro-angiogenic, termed M2. Alternatively, these cells can be thought of as occupying a color wheel, as proposed by Mosser and Edwards,12 in which the different activations overlap with each other, blending into “shades” of macrophage phenotypes. Newer nomenclature has been proposed by Murray et al.,13 to more accurately characterize the in vitro state of the macrophage in which the state is characterized by the molecular activator. The pro-angiogenic macrophages can be activated by exposure to interleukin-4, M(IL-4), and the pro-inflammatory cells can be achieved through incubation with lipopolysaccharide (LPS). The exact material properties that promote polarization to specific phenotypes remain poorly defined.

Tumor-associated macrophages (TAMs) can exist anywhere on the spectrum between M1- and M2-like macrophages. However, they are typically polarized toward an M2-like state.14 The phenotype is currently thought to change from an M1 state during cancer initiation to the predominant M2 phenotype during tumor progression.15,16 Creating a permissive environment for the tumor that encourages angiogenesis, matrix remodeling, and metastasis. Altering the phenotype of these cells can be achieved in vitro and in vivo through protein delivery. Systemic delivery of proteins is generally not feasible due to short half-lives and a low maximum tolerated dose of 500 ng/kg in the case of IL-12.17 These high doses led to the development of a local delivery system consisting of the protein encapsulated in biodegradable polylactic acid to polarize M2 TAMs to an M1 phenotype.18,19 On the other side of this coin, excessive M1-like macrophage presence is implicated in inflammatory diseases like arthritis.20,21 Using microspheres to alter macrophage phenotype in combination with delivering chemotherapeutic or immunotherapies may improve outcomes.

Arginine is generally metabolized by two enzymes: nitric oxide synthase (NOS), which produces citrulline and reactive nitrogen intermediates, and arginase, which produces ornithine and urea.22,23 These enzymes are differently up-regulated in polarized mouse macrophages, with NOS being dominant in M1 cells and arginase in M2 macrophages.24 Many researchers used the arginase/NOS ratio to describe the phenotypic population of macrophages.25−28 Several of the intermediate products in the conversion of arginine to nitric oxide or urea have been linked to diseases related to inflammation, which implicate macrophages in these diseases. Cardiomyopathy29 has been associated with carnitine deficiency. Supplementation with...
arginine has improved outcomes for patients with Huntington’s disease\(^\text{20}\) and type 2 diabetes.\(^\text{31}\) At the other end of the spectrum, nitroarginine exhibits neuroprotective effects in Parkinson’s disease.\(^\text{32}\) These are molecules that are intermediate products in the conversion of arginine to nitric oxide or urea. These diseases are related to inflammation, which implicates macrophages in these diseases. Using arginine and its derivatives to alter the phenotypic profile of macrophages has not been explored, particularly in using these molecules as surface modifiers.

In this study, we have examined the effect of arginine derivatives as polymer modifiers in macrophage response. Arginine is known to have a role in immune responses.\(^\text{22}\) Furthermore, several of its derivatives are known to impact NOS production and function. Both 2-amino-3-guanidinopropionic acid and nitroarginine inhibit NOS.\(^\text{33,34}\) Carnitine suppresses the production of NO and the protein expression of inducible nitric oxide synthase (iNOS).\(^\text{35}\) In addition to these molecules, several products in arginine metabolism were studied, including creatine, which is produced through arginine/glycine amidinotransferase and citrulline. Other studied derivatives as polymer modifiers to assess how they affect macrophage activation. All materials were purchased from Sigma (St. Louis, MO) and used as received unless otherwise indicated. Fresh deionized water (Milli-Q, Barnstead Nanopure, Thermo Scientific, Waltham, MA) was used throughout this study.

Figure 1. Chemical structures of all molecules used for the modification of p(NIPAm-co-AAc) particles. The letters are used as labels in the following figures for convenience.

2. MATERIALS AND METHODS

Fourteen chemically unique functional groups were coupled to polymers to assess how they affect macrophage activation. All experiments had at least four replicates, and error bars indicate the standard deviation. All materials were purchased from Sigma (St. Louis, MO) and used as received unless otherwise indicated. Fresh deionized water (Milli-Q, Barnstead Nanopure, Thermo Scientific, Waltham, MA) was used throughout this study.

Modifiers that were coupled to p(NIPAm-co-AAc) particles were 2-amino-3-guanidinopropionic acid, 3-guanidinopropionic acid, nitro-arginine, creatine (Fisher, Pittsburgh, PA), carnitine, citrulline, 5-hydroxylysine, acetylglutamine, N-carbamyl-α-aminoisobutyric acid, acetylcarnitine, 2,4-diaminobutyric acid, acetylornithine, albiziiin, and arginine (Amresco, Solon, OH).

2.1. Polymer Synthesis. 2.1.1. p(NIPAm-co-AAc) Particle Synthesis. Modification of p(NIPAm-co-AAc) particles and their synthesis have been described previously.\(^\text{36}\) Briefly, NIPAm (2.4 g), N,N-methylenebis(acrylamide) (0.16 g), and 157 \(\mu\)L of AAc (J.T. Baker, Center Valley, PA) were dissolved in 100 mL of \(\text{H}_2\text{O}\) and stirred under \(\text{N}_2\) in a 250 mL round-bottom flask for 30 min at 70°C. Next, 15 mL of 13.3 mg/mL \(\text{K}_2\text{S}_2\text{O}_8\) was added to the flask. The reaction was allowed to proceed for 3.5 h, after which the suspension was slowly cooled to room temperature, filtered with P5 grade filter paper, and dialyzed for 48 h in Milli-Q water. The particles were freeze-dried using a lyophilizer (Labconco, Kansas City, MO, 4.5 L).

In a 15 mL tube, 7.5 mL of phosphate buffered saline (PBS, diluted from a 10X solution, Fisher Scientific, to 0.1 M, pH 7.4), 16.7 \(\mu\)L of ethylenediamine (Eda), 1.5 mL of 5% w/v p(NIPAm-co-AAc) particles, and 75 \(\mu\)g of 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC) were vortexed and incubated overnight at room temperature. The particles were dialyzed against PBS and lyophilized. The modifiers in Figure 1 were conjugated to the Eda-p(NIPAm-co-AAc) particles through the carboxylic acid group on the modifiers in Figure 1 by combining 10 mg of Eda-p(NIPAm-co-AAc) particles with 2 mg of modifiers and 10 mg of EDC in 2 mL of PBS at room temperature and stirring overnight. The particles were dialyzed against \(\text{H}_2\text{O}\) and lyophilized.

2.1.2. \(\zeta\)-Potential. Milli-Q water was neutralized to pH 7 with HCl or NaOH to ensure that the ions in water would not interfere with the \(\zeta\)-potential of particles. A low salt concentration was confirmed by measuring conductivities at <0.2 mS/cm. A 100 \(\mu\)L aliquot of 1% w/v of particles was added into 5 mL of water, and \(\zeta\)-potential was measured with a Zetasizer Nano Z (Malvern).

2.2. Cell Viability. RAW 264.7 macrophages were cultured at 37°C with 5% \(\text{CO}_2\) in 10% fetal bovine serum, 100 U/L penicillin, and 100 \(\mu\)g/mL streptomycin in Dulbecco’s modified Eagle’s medium (DMEM high glucose; Thermo Scientific), to be referred to as complete medium (CM). RAW 264.7 cells were seeded in a 24-well plate at 1.25 \(\times\) 10^5 cell/cm^2 in the presence of 5 \(\mu\)g/mL LPS\(^\text{39,40}\) or 25 ng/mL IL-4\(^\text{41}\) (eBioscience Inc., San Diego, CA) and incubated 24 h at 37°C in 5% \(\text{CO}_2\). A control set of experiments was not activated. After activation, the medium was replaced with fresh CM, and particles were added to the wells (100 particles per cell). The cells were incubated for an additional 24 h. All particles were sterilized by washing three times in 70% ethanol, followed by three washes in sterile \(\text{H}_2\text{O}\) and centrifugation at 10 000g for 10 min. In a control experiment, cells were incubated without particles. A control of particle and IL-4 or LPS in the absence of cells was also conducted. The medium in each well was collected and stored at ~20°C.
A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine cell viability in the presence of the modified particles. A total of 50 μL of MTT (5 mg/mL in DI H2O) and 300 μL of CM was added to each well, and the plate was incubated for 2 h at 37 °C in 5% CO₂ after which time 425 μL of media was aspirated. The resulting formazan crystals were dissolved in 500 μL of dimethylsulfoxide (DMSO, Fisher, Pittsburgh, PA). The absorbance was measured at 540 nm with a reference at 690 nm using a plate reader (BioTek Synergy HT Multidetection Microplate Reader, Winooski, VT).

2.3. Measurement of Tumor Necrosis Factor-α Production. Tumor necrosis factor-α (TNF-α) present in the LPS, IL-4, or naive cell supernatant was determined by commercially available immunoassay kits (eBioscience Inc., San Diego, CA) and performed as described by the manufacturer.

2.4. Arginase Activity. After 24 h incubation with particles in section 2.2, cells were washed with 500 μL of PBS. In each well, 100 μL of cell lysis buffer (150 μL protease inhibitor cocktail (Amresco, Solon, OH) and 15 μL of Triton X-100 (Acros Organics) followed by 15 mL with DI water) was added, and the plates were incubated on ice for 10 min. Next, 25 μL of the cell lystate was transferred to a 96-well plate, and 25 μL of 10 mM MnCl₂ (Fisher) and 50 μM Tris (Fisher) was added to each well. Incubation at 55 °C for 10 min activated the arginase. A solution of 50 μL of 1 M arginine (pH 9.7) was added to each well, and the plate was incubated at 37 °C for 20 h. The activity of arginase was determined by measuring the amount of arginine converted to urea. In 96-well plates, 5 μL of either the urea-containing samples or standards was added to 200 μL of a 1:2 mixture of solution 1 (1.2 g of o-phthalaldehyde (Alfa Aesar, Ward Hill, MA), 1 L of H₂O, and 500 μL of HCl (Fisher, Pittsburgh, PA)) and solution 2 (0.6 g of N-(naphthyl)ethylenediamine dihydrochloride (Acros Organics), 5 g of boric acid (Fisher, Pittsburgh, PA), 800 mL of H₂O, and 111 mL of sulfuric acid (Fisher, Pittsburgh, PA), diluted to 1 L with H₂O). The plates were incubated for 30 min, and the absorbance in each well was measured at 540 nm with a reference at 630 nm using a plate reader.

2.5. Griess Reagent Assay. Nitrite concentration in the supernatant collected in section 2.2 was quantified through a Griess reagent assay. A standard curve was generated through serial dilutions of 100 μM NaNO₂. To each well were added 150 μL of sample or standard, 130 μL of DI H₂O, and 20 μL of Griess reagent. The plate was incubated for 20 min, and the absorbance of each well was measured with a plate reader at 448 nm with a reference of 690 nm. TNF-α, arginase, and nitrite concentrations were determined at the same time point of 24 h after introduction of the particles.

2.6. Statistics and Data Analysis. Statistical analysis was performed using XLSTAT statistical software (New York, NY). Statistical significance of the mean comparisons was determined by a two-way ANOVA. Pair-wise comparisons were analyzed with Tukey’s honest significant difference test. Differences were considered statistically significant for p < 0.05. The macrophages responses measured above were analyzed by performing partial least-squares (PLS) regression on the material descriptors shown in Table 1. These descriptors were based on descriptors defined by Bicerano.

3. RESULTS AND DISCUSSION

3.1. Synthesis, ζ-Potential, and Cell Viability. Functionalized p(NIPAm-co-AAC) particles were synthesized and modified with the molecules shown in Figure 1 through the carboxylic acid on the modifier. The ζ-potential of the modified and unmodified p(NIPAm-co-AAC) is in Figure 2. There was a range in ζ-potentials for the particles from −4.2 to −20.8 mV. Cytocompatibility of these materials was tested through an MTT viability assay. Macrophages were activated with LPS or IL-4 to simulate M1- and M2-like cells. After being incubated for 24 h, the medium was replaced and the functionalized particles were added to each well. Metabolic activity of the cell was assessed after an additional 24 h incubation period. Naive cells, denoted M(0), were also assessed. The results of the viability assay are shown in Figure 3. All tested conditions were >70% viable, with most particles and activation conditions resulting in 90% viability of the macrophages. The largest decrease in viability came from B, D, G, I, and J for M(LPS), H for M(IL-4), and L for M(0).

Table 1. Thirty-Eight Molecular Descriptors for the Surface Modifications Used in This Study

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<th>ID</th>
<th>structure descriptor</th>
<th>description</th>
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<tr>
<td>1</td>
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<td>freely rotating bonds</td>
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<tr>
<td>2</td>
<td>H_bond</td>
<td>H-bond donors</td>
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<tr>
<td>3</td>
<td>H_bond_acceptors</td>
<td>H-bond acceptors</td>
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<td>N_sp2</td>
<td>number of sp² carbon atoms</td>
</tr>
<tr>
<td>5</td>
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<td>number of sp³ carbon atoms</td>
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<tr>
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<td>ξ</td>
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<tr>
<td>7</td>
<td>ξ</td>
<td>connectivity index 2, calculated based on the electronic configuration of the molecular graph of the modifier</td>
</tr>
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<td>8</td>
<td>ξ</td>
<td>atomic index 1, calculated based on the number of non-hydrogen atoms to which a non-hydrogen atom is bound</td>
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<tr>
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<td>ξ</td>
<td>atomic index 2, calculated based on the electronic configuration of each non-hydrogen atom</td>
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<td>ξ</td>
<td>intensive connectivity index 1, ξ = \frac{N}{N}</td>
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<td>number of hydrogen atoms to which an atom is bound</td>
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<td>number of hydrogen atoms to which an atom is bound</td>
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<td>38</td>
<td>N_sp2</td>
<td>number of hydrogen atoms to which an atom is bound</td>
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The structure descriptors are defined by Bicerano. *N_number* is the number of methyl groups attached to nonaromatic atoms. *N_diam* is the total number of linkages between amide and nonaromatic atoms.
3.2. TNF-α Secretion from Activated and Naïve Macrophages. Activated and naïve macrophages were exposed to functionalized p(NIPAm-co-AAc) particles, and the concentration of secreted TNF-α was measured (Figure 4A). LPS was used to stimulate a M1-like phenotype. The control in which no particles were added to the cells contained 4.03 ± 0.51 ng/mL. This was statistically higher (p < 0.05) than the unmodified p(NIPAm-co-AAc) particles and the particles modified with ethylenediamine of 2.49 ± 0.29 and 2.55 ± 0.25 ng/mL, respectively. Compared to both the unmodified and Eda-modified particles, modifications D and I–M resulted in statistically higher TNF-α secretion (p < 0.05). Of these, albizziin increased TNF-α secretion ~2-fold. Modifications B, E, G, and H decreased the TNF-α production (p < 0.05). The most significant decrease was observed for 3-guanidinopropionic acid of ~40%. Details of multiple comparisons for these data are in Table S1.

To explore the M2-like phenotype, macrophages were stimulated with IL-4. The control without particles resulted in 2.69 ± 0.08 ng/mL TNF-α measured in the supernatant. The unmodified p(NIPAm-co-AAc) and Eda-modified particles resulted in a decrease in TNF-α by 1.78 ± 0.13 and 1.68 ± 0.12 ng/mL, respectively (p < 0.05), similar to the M(LPS) cells. Modifications A, I–K, and N resulted in increased TNF-α secretion (p < 0.05), while modifications B, C, E, F, and H resulted in a decrease (p < 0.05) compared to the unmodified and Eda particles. Modifications C–G and K resulted in increases in TNF-α secretion, and particles modified with I, J, L, and M decreased the TNF-α concentration detected compared to the unmodified and Eda particles. 2-Amino-3-guanidinopropionic caused the largest increase in TNF-α secretion of ~200%, and 3-guanidinopropionic acid caused the most significant decrease of ~40%. Details of multiple comparisons for these data are in Table S2.

Macrophages without activation were also incubated with the modified particles, and the secreted TNF-α was assessed. The control, unmodified p(NIPAm-co-AAc), and Eda particles were...
not statistically different with 1.04 ± 0.11, 1.31 ± 0.11, and 1.12 ± 0.13 ng/mL TNF-α detected. The largest increase in TNF-α was found for modifications F and K, with an increase of ~350%. The largest decrease was observed for modifications I, J, and L, resulting in an 80% decrease in TNF-α compared with the unmodified or Eda particles. Details of multiple comparisons are in Table S3.

Taken together, modifications I, J, and K lead to increases in TNF-α for both M(LPS) and M(IL-4), while modifications B, E, and H lead to decreases in TNF-α secretion. The most dramatic decreases in TNF-α secretion for both M(LPS) and M(IL-4) were in the presence of the 3-guanidinopropionic acid modification, which reduced TNF-α secretion by ~40% compared to unmodified and Eda-modified particles.

3.3. Urea/Nitrite for Activated and Naïve Macrophages. Arginase activity was measured by exposing cell lysate to arginine and assessing its conversion to urea after 20 h incubation. Increased arginase activity is suggestive of an M2-like phenotype. Increased iNOS indicates an M1-like phenotype. The activity of iNOS was indirectly measured through a Griess assay in which nitrites, the stable form of NO, are quantified. NO results from reactive nitrogen intermediates, which are synthesized by iNOS. Arginase is generally metabolized through one of these pathways, thus the ratio of urea/nitrite can assess the extent of polarization toward either phenotype.

These results are shown in Figure 4B. The nitrite and urea concentrations are shown as Figure S1A,B. M(IL-4) cells were found to have 447 ± 68 mg/μmol urea/nitrite. Cells incubated with A–D and I–L increased the amount of urea/nitrite measured (p < 0.05), while particles modified with E–H decreased the levels of urea/nitrite (p < 0.05). Measured levels of urea/nitrite ranged from 252 to 1023 mg/μmol. There was no statistical difference between the M(IL-4) cells in the absence of particles and M(LPS) cells incubated with unmodified and Eda-modified p(NIPAm-co-AAc) particles: 421 ± 100 and 366 ± 70 mg/μmol urea/nitrite, respectively. Details of multiple comparisons for urea/nitrite for M(IL-4) are in Table S4.

M1-like cells were also studied using M(LPS)-stimulated cells. No statistical differences were observed between the control and cells incubated with unmodified and Eda-modified particles: 62 ± 5, 69 ± 6, and 70 ± 7 mg/μmol urea/nitrite, respectively. Particles E–H, K, and M resulted in elevated urea/nitrite levels. Only particles A and I decreased urea/nitrite (p < 0.05) compared to the positive control, unmodified, and Eda-modified particles. Details of multiple comparisons for urea/nitrite for M(LPS) are provided in Table S5. In comparing trends between M(LPS) and M(IL-4), only modification K resulted in increasing urea/nitrite levels and no modifications were able to decrease urea/nitrite in both M(LPS) and M(IL-4) cells. It is important to note that these results may be particular to murine macrophage cells. Human macrophages transport arginine through system y+L transporters, whereas a cationic amino acid transporter has been suggested as an arginine transporter in mice.

Naïve macrophages were also tested for urea/nitrite levels. The levels of nitrites detected for all M(0) cells were lower than the standard curve. Statistical analysis on these values is not reasonable.

3.4. Material Parameters That Influence Macrophage Phenotypic Shifts. Secretion of TNF-α in the absence of particles followed the expected trend, in which the highest levels were observed for M(LPS), followed by M(IL-4) and M(0). With the exception of modification A, M(LPS) cells secreted more TNF-α than the M(IL-4) cells when they were incubated with particles. The M(0) cells exhibited a range of responses to the particles, secreting less TNF-α in the presence of modifications A, C, J, Eda, and unmodified p(NIPAm-co-AAc) than M(LPS) cells. Increased TNF-α was observed for modification M compared to M(LPS) cells. The increase in TNF-α secretion may be a result of LPS tolerance developed in the M(LPS) cells, which leads to a decreased production of TNF-α. Another possibility is that IL-10, a suppressor of TNF-α, may be secreted. Previous findings have demonstrated a decrease of TNF-α secretion in M(LPS) cells by a factor of 21.4 ± 2.5 in the presence of 0.1 ng/mL IL-10.

Arginine/iNOS is a measure of macrophage polarization, with high levels denoting M2-like states and low levels indicating a more M1-like state. Here, we measure this using urea/nitrite levels. In the case of M(IL-4), both the control and the cells incubated with particles resulted in higher urea/nitrite levels, which is expected.

The aim of this work was to identify material properties that influence M1- and M2-like phenotypes. The trends between material properties and cell responses were quantitatively modeled using material descriptors listed in Table 1. These descriptors were based largely on structural descriptors along with physicochemical properties. PLS was performed on the measured data using principal component analysis values of Figure 4A,B as a function of the molecular descriptors in Table 1. The results of the model are presented in Figure 5, where the horizontal axis represents TNF-α secretion and the vertical axis is urea/nitrite. Cells that secrete high levels of TNF-α and exhibit low levels of urea/nitrite are M1-like, while the converse are M2-like. Both of these regions are outlined in Figure 5. In addition, the specific descriptors that promote either of these two extremes are labeled on the plots. For M(IL-4) cells (Figure 5A), increasing H-bond donors promoted a shift toward an M1-like phenotype, while increasing H-bond acceptors, Nδ, log D (S.S), and log D (7.4) retained the M2-like phenotype. Log D is the octanol–water distribution coefficient, which can be measured at any pH, and Nδ is a correction factor for the dielectric constant. It bears mentioning that the inverse of these material descriptors would result in a shift toward the opposite phenotype. Based on these parameters, polarity of the molecule appears to be an important factor in altering M(IL-4) polarization. It should be noted that surface tension had a negligible effect on either TNF-α or urea/nitrite.

A larger number of material properties were identified as altering M(LPS), shown in Figure 5B. Increasing the number of freely rotating bonds, aliphatic carbons, van der Waals interactions (calculated using Nδ), the number of carbon and hydrogen atoms, and the enthalpy of vaporization promotes an M2-like phenotype. On the other end of the spectrum, primary carbon atoms, the number of sp2 carbon atoms, Nα—a correction term for molar volume, Nα—as a correction term for molar Rao function, Nδ—as a correction term for thermal conductivity, log p, log D (7.4), and log D (S.S) promoted the M1-like phenotype. All of these parameters suggest that hydrophobicity plays a dominant role in altering M(LPS) cells.

Since the levels of nitrites detected for all M(0) cells were lower than the standard curve, material properties influencing phenotypic shifts were not analyzed because statistical analysis on these values is not reasonable.
Interesting similarities exist for the material properties that promote phenotypic shifts in all the conditions examined here. Descriptors related to hydrophobicity, van der Waals interactions, and H-bonding were found to be related to macrophage polarization for M(LPS) and M(IL-4). The conventional dogma surrounding ligand-receptor binding involves all of these interaction forces additively combining to stabilize the ligand-receptor pair. Very likely, serum proteins present in the culture media adsorb in different configurations on the particle surface, thus enabling different interactions with surface receptors. The complexity of macrophage activation and reprogramming through surface receptors prevents predicting which receptors these particles are interacting with; however, examining the impact of material descriptors on altering macrophage response has the potential to improve material systems for implanted biomaterials as well as drug delivery systems.

Based on the observations described above and in Figure 5, three molecules were selected to alter the macrophage phenotype: lysine, ornithine, and ureidopropionic acid. These molecules were conjugated to Eda-modified particles as described in section 2.1.1. The concentration of secreted TNF-α and urea/nitrite in response to incubation with the modified particles is shown in Figure 6.

Figure 5. Analysis of molecular descriptors to identify polymer characteristics that promote M1- or M2-like profiles. Pearson’s correlation between molecular descriptors that promote urea/nitrite (y-axis) and TNF-α (x-axis) for (A) IL-4 and (B) LPS. Relevant descriptors that promote M1- or M2-like responses are labeled on the figure.

Figure 6. Molecular expression of (A) TNF-α and (B) urea/nitrite by macrophages in response to functionalized p(NIPAm-co-AAc) particles and stimulation with LPS or IL-4. Naïve cells are also shown. Data represent the mean value of four replicates for each sample ± standard deviation.

Ureidopropionic acid was predicted to promote urea/nitrite and TNF-α secretion in M(IL-4) cells and decrease secretion in M(LPS). Factors that would increase urea/nitrite and TNF-α secretion in M(IL-4) cells would be increased distribution coefficients at pH 5.5 and 7 and a reduction in H-bond acceptors. Compared to albizziin, ureidopropionic acid has fewer H-bond acceptors and higher distribution coefficients at pH 5.5 (−4.0 vs −2.62, respectively). The distribution coefficients at pH 7 are decreased for ureidopropionic acid (−4.4 vs −4.01). The distribution coefficients at pH 5.5 and 7 are very similar when compared to those of albizziin to citrulline, ergo an increase in urea/nitrite and TNF-α secretion would be expected for ureidopropionic acid compared to citrulline, as is observed in Figure 5A-B, particularly since citrulline has an additional H-bond donor compared to ureidopropionic acid, much like albizziin. In the case of M(LPS) cells, factors that cause cells to secrete decreased levels of urea/nitrite are the distribution coefficients at pH 5.5 and 7. In the
case of M(LPS) cells, the higher distribution coefficient for ureidopropionic acid compared to that of either albizziin or citrulline would cause a decrease in the urea/nitrite and TNF-α levels, as was observed. The number of freely rotating bonds is also lower for ureidopropionic acid compared to albizziin and citrulline.

The difference between lysine and ornithine is an additional aliphatic CH₂ group in lysine, which also results in an additional freely rotating bond for lysine. The other parameters influencing urea/nitrite production are similar for the two modifiers. This resulted in predictions that lysine would result in increased nitrite/urea secretion compared to ornithine. The additional CH₂ group results in a 2-fold increase in urea/nitrite for the two modifiers in the case of M(LPS) cells. In examining the response of M(IL-4) cells, no difference between lysine- and ornithine-modified particles was expected for urea/nitrite and TNF-α levels since the differences in their structures did not result in differences in the material descriptors identified in Figure 5A,B.

4. CONCLUSIONS

In summary, we have examined how a library of 14 surface modifiers alters macrophage response and have correlated those responses to material descriptors that promote shifts in phenotypes. Similarities between all three macrophage treatment conditions exist in that intermolecular nonbonding interactions play an important role in altering macrophage responses. The differences between these three conditions suggest the possibility that material properties can be exploited to manipulate macrophage responses, possibly reprogramming these cells without exogenous protein delivery. Semiquantitative methods such as those employed in this study have the potential to both improve screening for materials that promote M1- or M2-like phenotypes in macrophages and add to the current library of knowledge pertaining to how materials influence cellular responses.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomater.6b00041.

Details of multiple comparisons for TNF-α for M(LPS), M(IL-4), and M(0) cells and for urea/nitrite for M(LPS) and M(IL-4) cells; nitrite and urea measured for M(LPS), M(IL-4), and M(0) cells (PDF)

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Notes

The authors declare no competing financial interest.

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