Particle Stiffness and Surface Topography Determine Macrophage-Mediated Removal of Surface Adsorbed Particles

Aaron Lee, Dedy Septiadi, Patricia Taladriz-Blanco, Mauro Almeida, Laetitia Haeni, Miguel Spuch-Calvar, Wildan Abdussalam, Barbara Rothen-Rutishauser, and Alke Petri-Fink*

Cellular surface recognition and behavior are driven by a host of physical and chemical features which have been exploited to influence particle–cell interactions. Mechanical and topographical cues define the physical milieu which plays an important role in defining a range of cellular activities such as material recognition, adhesion, and migration through cytoskeletal organization and signaling. In order to elucidate the effect of local mechanical and topographical features generated by the adsorption of particles to an underlying surface on primary human monocyte-derived macrophages (MDM), a series of poly(N-isopropylacrylamide) (pNIPAM) particles with differing rigidity are self-assembled to form a defined particle-decorated surface. Assembly of particle-decorated surfaces is facilitated by modification of the underlying glass to possess a positive charge through functionalization using 3-aminopropyltriethoxysilane (APTES) or coating with poly(L-lysine) (PLL). MDMs are noted to preferentially remove particles with higher degrees of crosslinking (stiffer) than those with lower degrees of crosslinking (softer). Alterations to the surface density of particles enabled a greater area of the particle-decorated surface to be cleared. Uniquely, the impact of particle adsorption is evinced to have a direct impact on topographical recognition of the surface, suggesting a novel approach for controllably affecting cell-surface recognition and response.

1. Introduction

Material cues such as mechanical properties and surface topography have been shown to be powerful assets in directing cell adhesion, migration, organization, and communication.[1–5] The combination of advanced lithographic approaches alongside substrates with controllable mechanical compliance has enabled a deeper appreciation for the centrality of cellular mechanosensing in governing gene and protein regulation.[6] Moreover, mechanical properties have also been implicated in modifying the interaction between particulate matter and cells.[7] Principally, the integration of physical cues such as rigidity and membrane curvature-inducing features with cell behavior are expressed through changes in cytoskeletal organization and presents an attractive target for material-driven programming of biological responses.[8,9]

Earlier work has shown that colloidal assembly mediated by electrostatic and van der Waals interactions[10,11] are being used as a facile and flexible approach for altering surface characteristics and is capable of modulating cell adhesion and migratory activity.[12] Nevertheless, the concomitant effects of particle mechanical features such as stiffness and the role of...
particles in the production of surface topography are rarely considered, despite the clear implications for cell adhesion and subsequent cellular responses. To our knowledge, there is no corresponding study which examines both the role of particle stiffness in surface adsorbed systems as well as the potential impacts related to topographical changes generated by their presence and their role in determining mechanobiological responses. In the following work, the relevance of particle mechanical and topographical response in governing cell behavior was assessed on poly(N-isopropylacrylamide) (pNIPAM) microgel particles with differing degrees of stiffness adsorbed to glass treated with either (3-aminopropyl)triethoxysilane (APTES) or poly(L-lysine) (PLL). Amongst its many properties, pNIPAM has attracted a great deal of attention for being both biocompatible and thermo-responsive with a lower critical solution transition temperature for each synthesis was approximately 32 °C and is characteristic of pNIPAM. Data presented as mean ± standard deviation, n = 10.

2. Results and Discussion

In this work, fluorescent pNIPAM particles exhibiting differential mechanical response were prepared by controlling the relative amounts of crosslinking agent (N,N'-methylenebisacrylamide, BIS) in the formulation. Three different amounts of BIS (1 mol%, 5 mol%, and 10 mol%) were used to prepare spherical pNIPAM particles, namely, pNIPAM-1, pNIPAM-5, and pNIPAM-10, respectively (Figure 1a–c). Production of pNIPAM-decorated surfaces was performed by Layer-by-layer deposition on a glass surface positively-modified either covalently (APTES-functionalization) or through adsorption of a cationic polyelectrolyte (PLL-functionalization) (Figure 2). This technique is commonly employed for particle stabilization on solid substrates. Particle fluorescence was a necessary feature for tracking particle internalization and fate through confocal laser scanning microscopy and was imparted through co-polymerization of NIPAM with an acrylate bearing rhodamine B (RhodB). The concentration of NIPAM and RhodB monomers in the formulations used were kept constant (see Experimental Section for details).

Morphology, size and size distribution of synthesized pNIPAM particles were determined by transmission electron microscopy (TEM) for pNIPAM-1 (d_{TEM} = 535 ± 22 nm) (Figure S1a,b, Supporting Information), pNIPAM-5 (d_{TEM} = 497 ± 54 nm) (Figure S1c,d, Supporting Information), and pNIPAM-10 (d_{TEM} = 465 ± 14 nm) (Table 1 and Figure S1e,f, Supporting Information). Notably, the introduction of RhodB into the NIPAM network does not alter the temperature-dependent swelling behavior.
Figure 2. Schematic representation of particle assembly on polyelectrolyte functionalized glass to produce decorated surfaces. The interaction between the mechanical response of the particle and cells is examined through surface clearance of adsorbed particles. On PLL functionalized glass, MDMs exhibit stiffness-dependent clearance. Contrastingly, on APTES-functionalized glass, no internalization behavior was observed independent of pNIPAM particle compliance.

Table 1. Physico-chemical properties for the pNIPAM particles synthesized with different concentrations of BIS. Particle size was determined by TEM. Data are presented as mean ± standard deviation for n > 500 particles. The hydrodynamic diameter of pNIPAM particles was measured at 20 and 40 °C to calculate the volumetric swelling ratio \( \left( \frac{V_{20°C}}{V_{40°C}} \right) \). Zeta-potential of pNIPAM particles was determined at 37 °C in ultrapure water (pH = 7.0). Data are presented as mean ± standard deviation, \( n = 10 \). Adhesive forces of pNIPAM particles to APTES- and PLL-functionalized surfaces was calculated based on the sum total of the electrostatic force and van der Waals interactions.

| Crosslink [mol%] | TEM [nm] | DLS-20°C [nm] | DLS-40°C [nm] | Swelling ratio | Zeta potential [mV] | Adhesive force [nN] APTES | Adhesive force [nN] PLL |
|------------------|----------|----------------|----------------|----------------|---------------------|--------------------------|--------------------------|
| pNIPAM-1         | 1        | 535 ± 22       | 881 ± 26       | 323 ± 8        | 20.4                | -46 ± 4                 | 0.32                     | 0.38                     |
| pNIPAM-5         | 5        | 497 ± 54       | 686 ± 23       | 310 ± 6        | 10.8                | -45 ± 3                 | 0.33                     | 0.39                     |
| pNIPAM-10        | 10       | 465 ± 14       | 678 ± 23       | 416 ± 10       | 4.3                 | -42 ± 4                 | 0.30                     | 0.36                     |

of pNIPAM (Figure 1d). Following the introduction of particles to protein-rich cell culture medium, it was observed by DLS that the temperature-induced phase transition resulted in the formation of micron-sized agglomerates following incubation which was also observable in phase contrast (Figure S2, Supporting Information).

Zeta-potential analysis was performed for pNIPAM particles as a dilute suspension in ultrapure water at 37 °C. For all pNIPAM particles, strongly negative potentials were observed with all particles possessing a surface charge of −40 mV and colloidal stability was not compromised by the addition of RhodB (Table 1). Although the monomer is itself neutral, use of a persulfate ion as an initiator results in a persistent negative charge which is required to further assemble the particles on APTES- and PLL-functionalized surfaces (Figure 2). The hydrodynamic diameter of synthesized pNIPAM particles was determined in both the swollen (20 °C) and collapsed (40 °C) states to derive the volumetric swelling ratio. As swelling behavior in hydrogel networks is strongly dependent on the degree of chemical crosslinking, an increase in the number of chemical crosslinks which acts as network constraints results in a more restricted network, and a lower swelling ratio (Table 1).

Consequently, the swelling ratio of pNIPAM-1, pNIPAM-5, and pNIPAM-10 decreases as the amount of BIS increases and is indicative of a commensurate increase in the mechanical modulus of the particle;[20,21] that is, pNIPAM-1 containing the lowest number of chemical crosslinks is the softest formulation and pNIPAM-10 possessing the greatest stiffness. In this manner, we dictate the bulk mechanical response of the pNIPAM particles through the initial concentration of crosslinker (BIS) and observe behavior consistent with existing reports in that greater crosslink content produces stiffer particles.[22–24] The tendency for the particles to aggregate under cell culture conditions necessitated an alternative approach to consider the impact of particle mechanics on cell behavior which was achieved by suppressing particle–particle interactions through surface adsorption. Implementation of this design allowed the generation of a parameterizable surface capable of presenting distinct local stiffness and topographical cues for investigating and altering cell responses.

Physical determinants of cell behavior such as mechanical response and surface topography can have a profound impact on macrophage behavior through alterations in attachment, spreading, and adhesion.[25–27] Given the central role that macrophages play in maintaining tissue homeostasis and pathogen

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**Figure 2.** Schematic representation of particle assembly on polyelectrolyte functionalized glass to produce decorated surfaces. The interaction between the mechanical response of the particle and cells is examined through surface clearance of adsorbed particles. On PLL functionalized glass, MDMs exhibit stiffness-dependent clearance. Contrastingly, on APTES-functionalized glass, no internalization behavior was observed independent of pNIPAM particle compliance.

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surveillance, understanding how mechanical forces and interactions alter cell phenotype and function is critical.[28] By evaluating the relationship between particle stiffness in conjunction with topographical variation, a clearer description of macrophage mechanosensing, and physical regulation of phagocytosis can be obtained.

Implementation of covalent (APTES-functionalization) and non-covalent (PLL-functionalization) approaches to glass surface modification give rise to distinct substrate topographies given the difference in molecular size between APTES and PLL.[29,30] Although island-like features are apparent in both film preparations, the features observable on APTES-modified glass are more uniformly distributed than those produced by PLL and is reflected in the root mean square surface roughness of produced films, which was found to be 1.3 nm for APTES-modified glass and 2.4 nm for PLL-modified glass (Figure S3, Supporting Information). Given that traction forces exerted by cells on substrates and surface-bound particles represent a theoretical upper limit to clearance, we examined whether synthesized pNIPAM particles exhibiting adhesive forces defined by fundamental van der Waals and electrostatic interactions would play a substantial role in driving particle uptake. Importantly, the strength of the adhesive force between particles and the underlying substrate must be lower than the force which can be exerted by the cell as a precondition for particle removal. The average adhesive force determined between pNIPAM and APTES-treated glass (0.3 nN) was marginally smaller than the average adhesive force calculated between pNIPAM and PLL-treated glass (0.4 nN) (Table 1). MDMs have been shown to be able to apply up to 1.2 nN of traction force,[12] rendering them theoretically capable of removing adsorbed pNIPAM particles irrespective of the approach used to facilitate attachment. To account for the potential role of surface chemistry in determining protein adsorption and effects on cell behavior, contact angle measurements were performed on untreated, APTES-treated, and PLL-treated glass. It was noted that both APTES and PLL were able to reduce the water contact angle to differing extents, determined to be 41.9° and 69.9° respectively (Figure S4, Supporting Information). Interestingly, MDMs cultured on APTES-functionalized glass exhibited no discernible removal of adsorbed particles within the 24 h culture period (Figure S5, Supporting Information). In contrast, PLL-mediated attachment of pNIPAM particles facilitated particle removal in a stiffness-dependent manner (Figure 3a–c). Z-stack imaging performed by confocal laser scanning microscopy reveals that particle removal leads to intracellular accumulation of fluorescent signal from the particles (Figure S6, Supporting Information). Unlike chemical modification of glass with APTES which allows a covalently fixed positive charge to be maintained on the surface, removal of pNIPAM particles from PLL-functionalized glass may be facilitated by partial detachment of PLL from glass while remaining adsorbed to the pNIPAM particle surface. Partial removal of PLL following particle detachment was confirmed by performing zeta potential measurements on mechanically removed particles which were found to be −13.6 mV compared to particles removed from APTES-treated surfaces which had a zeta potential of −22.7 mV and freely suspended particles with a zeta potential of −26 mV at 25 °C in ultrapure water (Table S1, Supporting Information). The partial neutralization of the inherent negative charge of pNIPAM following detachment from the surface is indicative that some loss of polyelectrolyte from the underlying glass and adsorption to the particle surface may occur and may facilitate particle removal.

To probe stiffness-mediated surface uptake behaviors, remaining experiments focused on pNIPAM particles (100 µg mL⁻¹) adsorbed to PLL-functionalized glass to allow examination of the salient interactions in a labile system (Figure 3). Weakly adsorbed systems were deemed to better reflect the key aspects which contribute to particle adsorption to surfaces in protein-rich environments. As cells remove particles from the surface, there is a depletion of RhodB fluorescent signal in the vicinity of neighboring active cells. Concomitantly, fluorescence within the cells corresponding to internalization of RhodB-labelled pNIPAM increases (Figure S6, Supporting Information). Removal of the softest particles (pNIPAM-1) from the surface is limited within the 24-hour timeframe with some cells showing no apparent uptake (Figure 3a,d). Particle clearance is enhanced when the crosslinking degree is increased (pNIPAM-5) (Figure 3b,e). Additionally, pNIPAM-10 particles which carry the highest degree of chemical crosslinking are easily removed from the surface and coupled with strong intracellular fluorescence (Figure 3c,f). Membrane spreading behavior was more filamentous in the presence of particle features (Figure 3d–f) than on featureless glass (Figure 3g).

No substantial changes in cell area arose from surface topography and particle decoration (Figure 3h). The preferential surface clearance of stiffer particles (Figure 3i) mirrors pre-existing reports regarding mechanosensitivity of phagocytosis in planktonic or freely suspended particles.[7,31] Despite a similar size to pNIPAM-coated polystyrene latexes produced by Kruger et al., mechanically-dependent internalization activity runs in opposition to observed trends, that is, softer particles promoted internalization by macrophages.[23] Unlike freely suspended particles, adsorption to a surface introduces additional interactions which can alter the ease of particle wrapping while being opposed by particle–substrate adhesive forces. Adhesive forces can increase the force threshold exerted by the cell in order to internalize material. While increasing the relative interaction strength between cells and particles can help to drive uptake, proximity between the membrane and the particle has been posited to be sufficient and necessary for wrapping.[12] Notably, the presence of particles appears to have a strong influence on cell morphology as radial membrane spreading is inhibited by topographical cues.

 Alterations in substrates particle density modify cell behavior through greater exposure of the PLL-conditioned surface and minimized topographical stimulus. Consequently, topographically-modulated cell behaviors such as adhesion, spreading, and migration are expected to manifest in changes to particle clearance. Cell spreading, morphological polarization as well as particle clearance were assessed on PLL-substrates decorated with different pNIPAM particle surface density (Figure 4 and Figure S8, Supporting Information). The latter was controlled by using an initial pNIPAM deposition concentration of 25 or 50 µg mL⁻¹ resulted in fewer adsorbed particles (Figure S7 and Table S2, Supporting Information). The degree of surface adsorption and coverage are a byproduct of the deposition conditions and have been shown to alter the total surface hydrophobic/hydrophilic balance.[31] As particle density increases, the mean inter-particle spacing decreases to provide a means through which surface topography can be controlled.
The principal effects associated with the density of surface particles were observed in the manifestation of the cleared area which tended to be greatest when the particle density was lowest and stiffness-correlated effects remained (Figure 4a–f). Minimal particle clearance was observed for pNIPAM-1 particles (Figure 4a,d,g), while greater clearance areas were determined for pNIPAM-5 at 25 µg mL\(^{-1}\) (Figure 4b) compared with surfaces prepared with 50 µg mL\(^{-1}\) (Figure 4e) and similar trends are present for pNIPAM-10 (Figure 4c,f). As particle density on the surface decreased, the cleared area quantified for pNIPAM-5 and pNIPAM-10 increased (Figure 4h,i). High-density particle density arrays were observed to promote cell spreading behavior (Figure S8a–c, Supporting Information) while no clear trends were observed in cell elongation driven by changes in surface topography (Figure S8d–f, Supporting Information). Particle association for the stiffest particles (pNIPAM-10) decreased from 1300 particles per cell on high-density arrays (0.5 particles µm\(^{-1}\)) to 600 particles per cell when the surface density was reduced to 0.1 particles µm\(^{-2}\) (Table S2, Supporting Information). This is suggestive of two contributing effects: that the arrangement of objects on a surface suppresses migration behavior and that the reduction in their number density does not provoke a commensurate response in migration and particle seeking behavior. These mechanically-dependent observations are consistent with earlier work finding an average of 2000 associated particles per cell for 480 nm silica particles.\(^{[12]}\) Unlike previous reports investigating the effect of colloidally-generated topography on cell adhesion and spreading behaviors, the greatest surface spreading was observed when particle spacing was at a minimum.\(^{[33,34]}\) As aforementioned, macrophage surface attachment and spreading is driven by a combination of topographical and mechanical characteristics. Macrophage preference for spreading on rougher surfaces appears to be offset by a reduction in migration speed and distance which may explain the propensity for spreading and in situ clearance on high-density arrays.\(^{[35–37]}\)

Similarities in membrane curvature which are generated by substrate topography and cellular endocytic mechanisms have prompted investigations into the salient molecular mechanisms
underlying these fundamental cell behaviors. To address the possible signaling pathways responsible for differentiating internalization proficiency based on pNIPAM particle stiffness, we utilized immunolabeling for cellular adhesion proteins to assess the influence of particle-substrate topography on cellular recognition of pNIPAM particles adsorbed to PLL-functionalized glass. In contrast to many adherent cells, macrophages are migratory and require rapid adhesion turnover in order to facilitate motion, mediated by structures known as podosomes. Formation of podosomes has been associated with stiffness sensing in substrates and mechanoregulation of polarization. When cultured on untreated glass surfaces, MDMs present F-actin clustering surrounded by vinculin rings characteristic of podosome assembly. Strikingly, when cultured on a surface coated with pNIPAM particles, there is clear adaptation of substrate proximal elements such as F-actin and vinculin around the topographically distinct particles. In the case of removable particles, the loss of topographic features in the substrate restores the formation of podosome-like actin and vinculin organization. It is also evident that the presence of a surface-bound particle is sufficient to drive F-actin assembly which serves as an organizational center for establishing further physical interactions such as phagocytosis.

The effect of particle-decorated surfaces on cell viability was assessed by selective dye exclusion following 24 h of culture. A marginal decrease in cell viability was observed for pNIPAM particle-decorated surfaces correlating with an increasing degree of crosslinking compared with cells grown on untreated glass, suggesting that harder particles may lead to greater cytotoxicity due to an enhanced affinity for particle removal. One unintended consequence of greater particle clearance is presentation of the underlying PLL-layer which may also contribute to perturbed membrane integrity. Regulation of macrophage behavior by physical cues has been found to have important ramifications for cell phenotype and function. Crucially, M1-like and M2-like polarization remain a key design aspect in tailoring the in vivo response to biomaterials. To address the consequences of pNIPAM particle stiffness on cytokine expression, we performed an enzyme-linked immunosorbent assay for tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) as primary indicators of phenotypic identity.
Increased secretion of TNF-α is observed on all particle-decorated surfaces, with the greatest production associated with moderately stiff particles (pNIPAM-5) (Figure 6a). Interestingly, there is reduced secretion of TNF-α when the surface is decorated with pNIPAM-10 particles which suggest that the degree of particle removal and uptake is not the principal driver of inflammatory cytokine release. Although pNIPAM-1 particles do not appear to be internalized to any great extent, increased release of TNF-α is also observed. Production of IL-10, which is typically associated with anti-inflammatory signaling is only marginally increased on soft particle substrates (pNIPAM-1) (Figure 6b). Mechanical induction of differential cytokine expression has been described as a priming agent in guiding inflammatory behavior as well as preferences in particle uptake with a general tendency for softer materials to support M2-like functions.\[^{40,43}\] Cytokine induction in cells grown on pNIPAM-1 decorated surfaces on which there is minimal to no uptake and surface clearance suggests that a substantial component of the response is driven through a combination of surface topography and local material stiffness. To the extent that cationic surfaces contribute to cytokine expression, PLL surface modification of poly(L-lactic acid) fibers has been shown to increase IL-10 secretion and reduce TNF-α secretion from MDMs, which does not account for observed secretory profiles.\[^{44}\] Regarding chemistry-driven polarization by pNIPAM itself, it has been observed that culture of THP-1 macrophages on thermoresponsive surfaces leads to an increase in mRNA expression for both TNF-α and IL-10.\[^{45}\] Substrate-induced polarization behavior in macrophages is thought to occur through the alteration of integrin-controlled signaling pathways such as PI3K/Akt or Src.\[^{46,47}\] Altered organization of adhesion-associated proteins such as vinculin and actin to pNIPAM particles highlights how local morphology can alter expression of proteins involved in mediating material interactions. Microgel-based coatings have shown some promise in
reducing in vivo fibrosis of polyethylene terephthalate discs as well as reducing glial adhesion in vitro.\(^{48,49}\) While softer materials are typically associated with the promotion of anti-inflammatory and tissue repair behavior, further work is needed to understand the interplay between force transduction and perturbations to membrane curvature through surface topography as well as their contributions to cell mechanosensing.

Our findings underscore the importance and centrality of mechanical properties of sub-micron objects in directing immunological cell function. The broad palette of roles which are performed by macrophages and their phenotypic diversity reflects the necessity for them to dynamically modulate activity in response to mechanically distinct stimuli; whether this entails phagocytosis of bacteria and other pathogens of different size, shape, and mechanical response, sensing alterations to the extracellular matrix and tissue integrity or the interaction with engineered biomaterials. The combination of mechanical properties such as stiffness with corresponding membrane perturbations associated with cell-material contact, drive observed mechanoresponsive changes in cytoskeletal organization and signaling required for modulating cellular response and fulfillment of relevant biological functions.

3. Conclusion

Physical cues play an indispensable role in cellular decision-making and subsequent behavior. We have shown that sub-micron pNIPAM particles with distinct mechanical properties can adsorb to a surface to produce a biorelevant substrate capable of altering the organization of cell-surface contacts. The use of a covalent charge stabilization (APTES-modification) compared with adsorption of PLL was a vital consideration to the ease of particle removal from the surface, favoring non-covalent interactions. Surface particle decoration results in biorecognition by cultured macrophages, which are able to remove material in a stiffness-dependent manner. Surface particle density was found to alter the strength of mechanosensing by distributing preferential adhesion sites. These effects likely act in concert to increase the local mobility of the cell to optimize surveillance of potential phagocytic targets. Consequently, adsorption of particles or similarly sized objects to biological surfaces can lead to unexpected particle–cell interactions when compared to freely suspended material. Interactions arising from transient surfaces may provide greater insight into how surface topography and endocytic machinery integrate mechanoresponsive inputs to drive cell behavior in clinically relevant situations such as in change of extracellular matrix stiffness, wound-healing, or in governing biomaterial responses.

4. Experimental Section

Materials: N-isopropylacrylamide (NIPAM, 99% pure, stabilized) was purchased from Acros Organics (USA). N,N’-methylenebisacrylamide (BIS), potassium persulfate (KPS, 99%, ACS Reagent), and Triton X-100 were supplied by Fluka chemicals (USA). PLL (MW = 150000–300000, 0.1 wt% w/v solution in H2O), hexamethyldisilazane (HMDS, 99.9% Reagent Plus), APTES (99%), bovine serum albumin (BSA, 98% heat shock fraction, protease-free, fatty acid-free, essentially globulin free, pH 7) and 4’,6’-diamidino-2-phenylindole (DAPI) were obtained from Sigma-Aldrich (Switzerland) while acryloxyethyl thiocarbamoyl rhodamine B was purchased from Polysciences Inc (USA). Phosphate buffered saline (PBS 1x, Germany) and cellulose dialysis membrane (MWCO 14,000 Da) were purchased from Carl Roth GmbH. All reagents were used as received without further purification. Ultrapure water (resistivity of 18.2 MΩ cm) was used for all preparations. Cells were cultured in Roswell Park Memorial Institute (RPMI-1640) medium was supplemented with 10% (v/v) foetal bovine serum, 2 mM L-glutamine, and penicillin/streptomycin (100 units mL\(^{-1}\) and 100 µg mL\(^{-1}\), respectively). Cell culture reagents were purchased from Gibco, Thermo Fisher Scientific (Switzerland). Recombinant anti-vinculin antibody and goat anti-rabbit IgG conjugated with Alexa Fluor 488 were obtained from Abcam (UK). Alexa Fluor 488 Phalloidin used for staining F-actin cytoskeletal elements was sourced from Gibco Life Technologies, Thermo Fisher Scientific (Switzerland).
Synthesis of pNIPAM Particles: Polymerization of NIPAM by free-radical polymerization was performed by adapting the protocol reported by Pelton et al.[50] PolyNIPAM (pNIPAM) particles were prepared by dissolving 2 mmol of NIPAM, a corresponding amount of BIS (0.02, 0.1, or 0.2 mmol) in 19 mL of water containing 8 mM of acryloylthiocarbarnoyl rhodamine B. The solution was magnetically stirred at 300 rpm in a 50 mL round bottom ask and continuously purged with nitrogen gas. The flask was heated to 70 °C in an oil bath for thermal initiation of polymerization and equilibrated for 40 min. Polymerization was initiated with the addition of 1 mL of potassium persulfate (0.02 mmol) dissolved in water. The reaction was allowed to proceed for two hours as the clear and transparent solution gradually became milky white, before dialysis of the product in a dialysis membrane (MWCO 14,000 Da) against ultrapure water with frequent changes over one week until residual conductivity was below 1.0 mS cm⁻¹. Particles were subsequently stored in water at 4 ºC.

Dynamic Light Scattering and Zeta Potential Analysis: Hydrodynamic diameter, polydispersity, and zeta potential of synthesized particles were performed using a Brookhaven 90Plus Particle Size Analyzer with a 2048 pixel wide-angle lens (numerical aperture 1.4, oil). Fluorophores were excited sequentially in two minutes of 50 W air plasma (Diener, USA). Freshly activated surfaces were spin-coated at 2000 rpm for 5 min and rinsed twice with ultrapure water and dried under nitrogen flow. A pNIPAM particle suspension was then prepared in water to remove unattached particles. A comparison was drawn against the relevant charges are given by q = 4πε₀εrσ.

Electrostatic forces were calculated through the following equation where the negative value represents an attractive interaction. The natural frequency ν = 1 GHz, k_F = 1.38 064 852 × 10⁻²¹ m² kg⁻¹ s⁻² K⁻¹, temperature T = 298.14 K and Planck constant h = 6.62 607 004 × 10⁻³¹ m² kg⁻¹ s⁻¹ were used to yield the adhesive force with a magnitude of −0.32971203 nN. Calculations were performed for PLL-treated glass with consideration of the surface potential φ_s = 50 mV, permittivity ε_r = 83 C m⁻¹, refractive index n_p = 1.3325, n_i = 1.4225, n_s = 1.52 and distance r_s = 10⁻⁶ m, r_i = 10⁻⁷ nm and approximated r_d = 10⁻⁹ nm yields electrostatic force of F ≈ −0.39 nN and an adhesive interaction with a magnitude of 0.39311209 nN. Total adhesive forces (W) were likewise determined for the remaining particles as previously reported as the sum of electrostatic (F_e) and van der Waals forces (F_vdw).

Cell Culture: Work involving primary human MDMs was approved by the committee Federal Office for Public Health Switzerland (reference number: 611-1, Meldung A110635/2). Macrophages were prepared from whole buffy coat following a previously developed protocol.[51] Briefly, peripheral blood mononuclear cells were isolated from buffy coats provided by the Swiss Transfusion Centre (Bern, Switzerland). Magnetic beads (Milteny Biotec GmbH, Germany) were used to select for CD14⁺ monocytes which were seeded in a 6-well plate (Corning, USA) at 10⁶ cells mL⁻¹ and cultured with 3 mL per well of supplemented culture medium and 10 ng mL⁻¹ of macrophage colony-stimulating factor (M-CSF) (10 ng mL⁻¹, Milteny Biotec, Germany) for seven days. MDMs were harvested by cell scraping and seeded on prepared particle-decorated substrates at 8000 cells cm⁻² for 24 h at 37 ºC, 5% CO₂ in a humidified incubator in supplemented culture medium.

Cell Viability: Cell viability was determined by using a LIVE/DEAD assay. MDMs were cultured on prepared surfaces for 24 h. Two drops of reagent A and reagent B (ReadyProbes Cell Viability Imaging Kit, Blue/Green, Invitrogen, USA) were added to each dish and incubated for 20 min prior to imaging with a Zeiss LSM710 confocal microscope. Cell viability was determined by counting the number of live and dead stained cells from five representative areas selected at random for three biologically independent replicates. Viability was determined using the following equation:

Viability = \frac{\text{live}}{\text{live} + \text{dead}} × 100

Determination of Particle Clearance: In order to determine particle uptake by MDMs was confirmed with fluorescence microscopy. Cells were washed twice with PBS (Gibco, ThermoFisher Scientific, Switzerland) and fixed with 4% para-formaldehyde in PBS. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, Switzerland) while F-actin was stained with Alexa Fluor 488 Phalloidin (Thermo Fisher, Switzerland). Images were acquired using a Zeiss LSM 710 (Zeiss, Germany) confocal laser scanning inverted microscope using a 20x (numerical aperture 0.8) or 63x objective lens (numerical aperture 1.4, oil). Fluorophores were excited sequentially at 405, 488, 561, and 633 nm collected with a field of view of 425.10 µm.
x.425.10 μm with at a pixel density of 512 × 512 and a pixel dwell time of 2.55 μs. Optical slicing was performed during z-stack acquisition with a slice thickness of 0.15–0.3 μm. Image processing (maximum intensity projection) was performed in ImageJ (NIH, USA). Cleared area determination based on manual boundary marking in-plane for the rhodamine channel (Excitation 561 nm) referenced against the particle carpet as an indicator of the substrate for n = 100 cells. Mean and standard error of the mean for cleared area were determined for each particle-decorated surface. Statistical comparison performed using a one-way ANOVA with Tukey’s test and considered significant (*) when p < 0.05.

In addition to fluorescence microscopy of cells on particle substrates, scanning electron microscopy (Mira3 LM FE, Tescan) was performed to investigate morphological features associated with cellular surface recognition. Following 24 h of culture, cells were rinsed twice with PBS and fixed with 2% paraformaldehyde and 0.5% glutaraldehyde (Sigma Aldrich, USA) in PBS for 1 h. Samples were subsequently rinsed with PBS and water before being processed through an ethanol series and treatment with hexamethyldisilazane (Sigma Aldrich, USA). To avoid charging, 3 nm of gold was sputter-coated and images were acquired with an accelerating voltage of 2.0 kV with an Everhart–Thornley type secondary electron detector.

Cytokine secretion Determination by Enzyme-Linked Immunosorbent Assay: Cytokine release by MDMs cultured on particle-decorated surfaces for 24 h was determined by enzyme-linked immunosorbent assay. Supernatant from cultures was collected and stored at −80 °C and assayed in triplicate for the presence of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10). Macrophage polarization was assessed through exposure to lipopolysaccharide (LPS) at a concentration of 1 mg mL⁻¹ in culture medium. TNF-α was detected using the Human TNF-alpha DuoSet, while IL-10 was detected using the Human IL-10 DuoSet ELISA kit. Both kits were provided by R&D Systems (USA).

Adhesion Staining: The capacity of MDMs to adhere and respond to mechanical surface cues was examined by staining vinculin as a known adhesion protein. Cells were fixed with 0.1 M glycine solution followed by permeabilization with 0.1% Triton X and 1% BSA. Primary anti-vinculin (Abcam, UK) was incubated with permeabilized cells overnight at 4 °C. Samples were rinsed twice with 0.1% BSA solution followed by incubation with goat anti-rabbit secondary antibody (Alexa Fluor 647, Abcam, UK) for 2 h. Subsequently, samples were rinsed twice with 0.1% BSA solution and stained with Alexa Fluor 488 Phalloidin to visualize F-actin. All preparations were made in 1x phosphate buffered saline (Carl Roth, Germany).

Statistical Analysis: Particle sizes and zeta potential measurements presented as mean ± standard deviation. Characterization of cell spreading, morphology, and clearance areas as well as cytokine secretion were performed with three biologically independent replicates from distinct donors. Statistical significance between conditions was determined using a one-way ANOVA with Dunnett’s and Tukey’s post hoc tests for multiple comparisons with a significance level of p < 0.05. Analysis was performed in Prism 8 (GraphPad Software).

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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[1] A. Engler, S. Sen, H. L. Sweeney, D. E. Discher, Cell 2006, 126, 677.
[2] C. M. Lo, H. B. Wang, M. Dembo, Y. L. Wang, Biophys. J. 2000, 79, 144.
[3] Y. Hou, L. Yu, W. Xie, L. C. Camacho, M. Zhang, Z. Chu, Q. Wei, R. Haag, Nano Lett. 2019, 20, 748.
[4] E. B. Lomakina, G. Marsh, R. E. Waugh, Biophys. J. 2014, 107, 1302.
[5] D. Septiadi, W. Abdussalam, L. Rodriguez-Lorenzo, M. Spuch-Calvar, J. Bourquin, A. Petri-Fink, B. Rotthen-Rutishauser, Adv. Mater. 2018, 30, 1806181.
[6] P. Bahmannejad, B. L. Cavanagh, M. Ahearn, Colloids Surf., B 2020, 190, 110971.
[7] A. Anselmo, M. Zhang, S. Kumar, D. R. Vogus, S. Menegatti, M. E. Helgeson, S. Mitragotri, ACS Nano 2015, 9, 3169.
[8] H. Y. Lou, W. Zhao, X. Li, L. Duan, A. Powers, M. Akamatsu, F. Santoro, A. F. McGuire, Y. Cui, D. G. Drubin, B. Cui, Proc. Natl. Acad. Sci. U. S. A. 2019, 116, 23143.
[9] Q. Li, B. Zhang, N. Kasoju, J. Ma, A. Yang, Z. Cui, H. Wang, H. Ye, Int. J. Mol. Sci. 2018, 19, 2344.
[10] R. A. Bowling, Particles on Surfaces 1, Springer, New York 1988.
[11] F. L. Leite, C. C. Bueno, A. L. Da Róz, E. C. Ziemath, O. N. Oliveira, Int. J. Mol. Sci. 2012, 13, 12773.
[12] D. Septiadi, A. Lee, M. Spuch-Calvar, T. L. Moore, G. Spiaggia, W. Abdussalam, L. Rodriguez-Lorenzo, P. Taladriz-Blanco, B. Rotthen-Rutishauser, A. Petri-Fink, Adv. Funct. Mater. 2020, 30, 2002630.
[13] S. Zhang, J. Ozdemir, L.-C. Xu, P. Butler, C. A. Siedlecki, J. L. Brown, S. Zhang, J. Biomed. Mater. Res., Part B 2016, 104, 488.
[14] Y. Xia, X. He, M. Cao, C. Chen, H. Xu, F. Pan, J. R. Lu, Biomacromolecules 2012, 13, 3635.
[15] F. Crippa, T. L. Moore, M. Mortato, C. Geers, L. Haeni, M. A. Hirt, B. Rotthen-Rutishauser, A. Petri-Fink, Magn. Magn. Mater. 2017, 427, 212.
[16] G. Decher, Science 1997, 277, 1232.
[17] S. Schmidt, M. Zeiser, T. Hellweg, C. Duschl, A. Fery, H. Möhwald, Adv. Funct. Mater. 2010, 20, 3235.
[18] L. Scheidegger, M. A. Fernández-Rodríguez, K. Geisel, W. Abdussalam, L. Rodriguez-Lorenzo, P. Taladriz-Blanco, B. Rotthen-Rutishauser, A. Petri-Fink, ACS Appl. Bio Mater. 2018, 1, 12654.
[19] Z. L. Yao, N. Grishkewich, K. C. Tam, Soft Matter 2013, 9, 5319.
[20] C. Echeverria, N. A. Peppas, C. Mijangos, Soft Matter 2012, 8, 337.
[21] T. R. Matzelle, G. Geuskens, N. Kruse, Macromolecules 2003, 36, 2926.
[22] A. Auerhorst-Roberts, D. Baker, R. J. Foster, O. Cayre, J. Mattsson, S. D. Connell, Nanoscale 2018, 10, 16050.
[23] T. M. Kruger, B. E. Givens, T. I. Lanssara, K. J. Bell, H. Mohapatra, A. K. Salem, A. V. Tsvankin, L. L. Stevens, ACS Appl. Bio Mater. 2018, 1, 1253.
[24] B. Sierra-Martin, J. A. Frederick, Y. Laporte, G. Markou, J. J. Lietor-Santos, A. Fernandez-Nieves, Colloid Polym. Sci. 2011, 289, 721.
[25] C. F. Guimarães, L. Gasperini, A. P. Marques, R. L. Reis, Nat. Rev. Mater. 2020, 5, 351.
[26] A. Upadhyaya, Semin. Cell Dev. Biol. 2017, 71, 137.
[27] N. Jain, J. Moeller, V. Vogel, Annu. Rev. Biomed. Eng. 2019, 21, 267.
[28] S. Gordon, L. Martinez-Pomares, Eur. J. Appl. Physiol. 2017, 469, 365.
[29] E. Metwalli, D. Haines, O. Becker, S. Conzone, C. G. Pantano, J. Colloid Interface Sci. 2006, 298, 825.
[30] K. Colville, N. Tompkins, A. D. Rutenberg, M. H. Jericho, Langmuir 2009, 26, 2639.
[31] X. Banquy, F. Suarez, A. Argaw, J. M. Rabanel, P. Grutter, J. F. Bouchard, P. Hildgen, S. Giasson, Soft Matter 2009, 5, 3984.
[32] T. Wiegand, M. Fratini, F. Frey, K. Yserentant, Y. Liu, E. Weber, K. Galior, J. Ohmes, F. Braun, D. Herten, U. S. Schwarz, K. Salaita, E. A. Cavalcanti-Adam, J. P. Spatz, Nat. Commun. 2020, 11, 32.
[33] D. Tang, Z. Zeng, Y. Xia, B. Chen, S. Gao, M. Cao, S. Wang, D. Li, J. Appl. Polym. Sci. 2019, 137, 48773.
[34] D. Wang, K. M. Bratlie, ACS Biomater. Sci. Eng. 2015, 1, 166.
[35] A. Rich, A. Harris, J. Cell Sci. 1981, 50, 1.
[36] D. Kosoff, J. Yu, V. Suresh, D. J. Beebe, J. M. Lang, Lab Chip 2018, 18, 3011.
[37] S. Lee, J. Choi, S. Shin, Y. M. Im, J. Song, S. S. Kang, T. H. Nam, T. J. Webster, S. H. Kim, D. Khang, Acta Biomater. 2011, 7, 2337.
[38] H.-Y. Lou, W. Zhao, Y. Zeng, B. Cui, Acc. Chem. Res. 2018, 51, 1046.
[39] F. Y. McWhorter, C. T. Davis, W. F. Liu, Cell. Mol. Life Sci. 2015, 72, 1303.
[40] R. Sridharan, B. Cavanagh, A. R. Cameron, D. J. Kelly, F. J. O’Brien, Acta Biomater. 2019, 89, 47.
[41] C. A. Hong, H. Y. Son, Y. S. Nam, Sci. Rep. 2018, 8, 7738.
[42] R. Sridharan, A. R. Cameron, D. J. Kelly, C. J. Kearney, F. J. O’Brien, Mater. Today 2015, 18, 313.
[43] S. Ghrebi, D. W. Hamilton, J. D. Waterfield, D. Brunette, J. Biomed. Mater. Res., Part A 2013, 101A, 2118.
[44] C. R. Correia, J. Gaiem, M. B. Oliveira, R. Silvestre, J. F. Mano, Biomater. Sci. 2017, 5, 551.
[45] V. Malheiro, Y. Elbs-Clatz, M. Obzarzanek-FOjt, K. Maniura-Weber, A. Bruinink, Sci. Rep. 2017, 7, 42495.
[46] Z. Piedra-Quintero, C. Serrano, N. Villegas-Sepulveda, J. Maravillas-Montero, S. Romero-Ramirez, M. Shibayama, O. Medina-Contreras, P. Nava, L. Santos-Argumedo, Front. Immunol. 2019, 9, 3118.
[47] X. Hu, H. Wang, C. Han, X. Cao, Cytokine 2018, 111, 209.
[48] A. W. Bridges, R. E. Whitmire, N. Singh, K. L. Templeman, J. E. Babensee, L. A. Lyon, A. J. Garcia, J. Biomed. Mater. Res., Part A 2010, 94, 252.
[49] S. M. Gutowski, K. L. Templeman, A. B. South, J. C. Gaulding, J. T. Shoemaker, M. C. La Placa, R. V. Bellamkonda, L. A. Lyon, A. Garcia, J. Biomed. Mater. Res., Part A 2014, 102, 1486.
[50] R. H. Pelton, P. Chibante, Colloids Surf. 1986, 20, 247.
[51] S. N. Ramakrishna, P. C. Nalam, L. Y. Clasohm, D. N. Spencer, Langmuir 2013, 29, 175.
[52] K. Yamada, S. Yoshii, S. Kumagai, I. Fujiwara, K. Nishio, M. Okuda, N. Matsukawa, I. Yamashita, Jpn. J. Appl. Phys. 2006, 45, 4259.
[53] H. Barasova, B. Drasler, A. Petri-Fink, B. Rothen-Rutishauser, J. Visualized Exp. 2020, 159, e61090.