Supporting Information

Anisotropic Ligand Nanogeometry Modulates the Adhesion and Polarization State of Macrophages

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Materials and Methods

Synthesis of gold nanorods (GNRs) with various anisotropies without altering their nanoscale surface areas.

GNRs with various anisotropies (aspect ratios: ARs), but in similar surface areas were synthesized by precisely tuning \( \text{Au}^{3+} \), \( \text{Ag}^+ \), and binary surfactant mixtures. Maintaining similar surface areas of the GNRs in various anisotropy were critical to control similar surface density of ligand to be subsequently conjugated to the GNRs on the substrate. Hexadecyltrimethylammonium bromide (CTAB)-capped GNRs in various aspect ratio (AR = 2, 4, or 7) were synthesized with the precise control in their dimensions (length and diameter) to obtain the GNRs in various anisotropy, but in similar surface area. We first synthesized gold seed solution. 2.5 mL of 0.5 mM \( \text{HAuCl}_4 \) (Sigma) solution was added into 2.5 mL of 0.2 M CTAB (TCI) solution in 20 mL glass vial. To this mixture solution, 1 mL of fresh 0.006 M \( \text{NaBH}_4 \) (Sigma) was injected. The mixture was subjected to vigorous stirring for 2 min and allowed to age at 29 °C for 30 min to obtain gold seed solution.

To synthesize the GNRs with an aspect ratio of 2 (AR2), a single surfactant of CTAB was used. 0.08 g sodium salicylic acid and 0.9 g CTAB were dissolved in 25 mL of deionized (DI) water. 0.6 mL of 4 mM \( \text{AgNO}_3 \) was added into this mixture, which was then left undisturbed for 15 min. 25 mL of 1 mM \( \text{HAuCl}_4 \) solution was added to this solution and stirred for 15 minutes. 125 \( \mu \text{L} \) of 0.064 M ascorbic acid solution was quickly injected into this reaction mixture and stirred for 30 s. 40 \( \mu \text{L} \) of the gold seed solution was added into this mixture solution. This solution was stirred for another 30 s and then left undisturbed at 29 °C for 12 h to obtain the AR2 GNRs.

To synthesize the AR4 and AR7 GNRs, a binary surfactant mixture (CTAB and sodium oleate) was used for both GNRs, but with different amounts of \( \text{AgNO}_3 \) to prepare different aspect ratio of AR4 vs. AR7 GNRs. 0.1234 g sodium oleate and 0.9 g CTAB were dissolved in 25 mL of DI water. To this mixture solution, 1.6 or 2.4 mL of 4 mM \( \text{AgNO}_3 \) was added to prepare the AR4 or AR7 GNRs, respectively, and then left undisturbed for 15 min. 25 mL of 1 mM \( \text{HAuCl}_4 \) solution was added to this solution and stirred for 90 min. 210 \( \mu \text{L} \) of HCl was added to this mixture and stirred for 15 min. 125 \( \mu \text{L} \) of 0.064 M ascorbic acid solution was quickly injected into this reaction mixture and stirred for 30 s. To this solution, 80 \( \mu \text{L} \) of the gold seed solution was added. This
solution was then stirred for another 30 s and then left the mixture undisturbed at 29 °C for 12 h to obtain the AR4 or AR7 GNRs.

Citrate-capped gold nanospheres (GNSs) were also synthesized as a control to the GNRs. In particular, GNSs were prepared to exhibit similar surface area to that of the GNRs and they were referred to the GNRs in aspect ratio of 1 (AR1). We used the cycle by cycle method to prepare the GNSs in approximate diameter of 45 nm. Briefly, 28.9 mL of DI water was added to a three-necked flask, which was allowed to boil. 1.1 mL trisodium citrate solution was then injected into this boiling water. To this solution, 0.2 mL of 0.025 M HAuCl4 (Sigma) solution was injected and this mixture solution was allowed to react for 15 min under stirring. The reaction mixture was allowed to cool down to 90 °C. To this mixture solution, 0.2 mL of 0.025 M HAuCl4 was injected and this mixture was allowed to react for 30 min. We repeated this step two times. 11 mL of resultant mixture was extracted to which 10.6 mL of DI water was added. When the temperature of this mixture solution reached 85 °C, we added 0.4 mL of 60 mM trisodium citrate into this mixture and subsequently injected 0.2 mL of 0.025 M HAuCl4 three times, which were then allowed to react for 30 min. We repeated this step six times to obtain the GNSs in approximate diameter of 42 nm.

*Transmission electron microscopy (TEM) of the GNRs with various anisotropies.*

TEM imaging (Hitachi) was performed to characterize the GNRs with various aspect ratios (AR = 1, 2, 4, or 7) at an accelerating voltage of 120 kV. The approximate size (length and diameter) and surface area of the GNRs in various aspect ratio (assuming spherical or cylindrical shape) were determined with the Image J software based on 100 different nanoparticles and presented as the mean ± standard error. These quantifications confirmed systematic preparation of the GNRs with various anisotropies without altering their nanoscale surface areas.

*UV-Vis spectrophotometer measurement of the GNRs.*

The extinction spectra of the GNRs with various anisotropies were measured to examine their major absorption peaks that typically exhibit red shifts with increasing aspect ratio of the GNRs. UV-Vis spectrophotometer (Cary 5000, Agilent) was used to characterize the extinction spectra of the GNRs (AR1, AR2, AR4, or AR7) in 1-cm-path length quartz cuvette within the wavelength range from 400 to 1350 nm.
Ligand exchange processes of GNRs with various anisotropies to allow their uniform substrate coupling.

Prior to substrate coupling of the GNRs with various anisotropies, we performed ligand exchange processes of the GNRs to allow their uniform coupling to the substrate. The CTAB-capped GNRs (AR2, AR4, or AR7) underwent ligand exchange processes that serially replace CTAB with polystyrene sulfonate (PSS) and then further with citrate. 160 mL of CTAB-capped GNRs were concentrated to approximately 10 mL in a stirred ultrafiltration cell using a cellulose membrane filter and then diluted with DI water to 100 mL (approximately 168 µg/mL). The citrate-capped GNR suspensions were centrifuged, dispersed in 0.15% Na-PSS to 100 mL (approximately 135 µg/mL), and allowed to sit for 1 h. The PSS-capped GNR suspensions were centrifuged, dispersed in 30 mL of 5 mM sodium citrate, and allowed to sit for 12 h to obtain citrate-capped GNRs (approximately 125 µg/mL), which were subsequently used for uniform substrate coupling.

Zeta potential measurement of GNRs with various anisotropies to characterize their ligand exchange processes.

Zeta potential measurement was performed to determine the changes in surface charges of the GNRs in various anisotropy through their serial ligand exchange processes from CTAB to PSS and then to citrate. Photon correlation spectroscopy (Brookhaven Instruments Corporation) was used for measurements.

Substrate coupling of GNRs displaying anisotropic ligand nanogeometry without changing either macroscale or nanoscale ligand density.

GNRs with various anisotropies after undergoing ligand exchange processes to obtain citrate-capped GNRs were systematically coupled to a substrate (culture-grade glass coverslips in 22 mm x 22 mm) to display anisotropic ligand nanogeometry without modulating macroscale and nanoscale ligand density. The glass substrate was treated with a mixture of HCl and methanol (1:1) for 30 min to wash any organic residues on the substrate surface and was then washed with DI water. The hydroxyl group on the glass substrate was activated by immersing the substrate in sulfuric acid for 1 h and then washing it with DI water. The activated substrate was thiolated by the treatment with 0.5 mM mercaptopropylsilatrane in methanol for 30 min in the dark and
subsequently washed with methanol. The thiol-presenting substrate was then immersed in 1 nM citrate-capped GNR solution and incubated for various time ranging from 30 min to 3 h depending on various anisotropy (i.e. aspect ratio) of the GNRs to obtain similar density of the substrate-coupled GNRs in various nanoscale anisotropy, which was then washed with DI water.

The GNR-coupled substrate was blocked by PEGylation on the remaining area of the substrate that was not covered with the GNRs to minimize the non-specific macrophage adhesion. The GNR-coupled substrate was incubated in 100 µM maleimide-poly(ethylene glycol)-CH₃ in DI water for 2 h in the dark, which was subsequently washed with DI water. The blocked GNR-coupled substrate was incubated in thiol-bearing RGD peptide (GCGYGRGDSPG, Mₙ = 1025.06 Da, GenScript) and 5 mM tris(2-carboxyethyl)phosphine hydrochloride in phosphate-buffered saline (PBS) for 16 h to obtain the RGD-bearing GNR-coupled substrate in various nanoscale RGD ligand anisotropies. As a control, we used a scrambled RGD peptides (RGE peptides, GCGYGRGESPG, GenScript).

*Scanning electron microscopy (SEM) and energy dispersive spectra (EDS) to characterize the substrate decorated with anisotropic ligand-bearing GNRs.*

SEM imaging (SU8000 Series, Hitachi High-Tech Ltd.) was carried out to characterize the substrate coupled with anisotropic RGD ligand-bearing GNRs with various aspect ratios (AR = 1, 2, 4, or 7). The substrate-coupled GNR density was calculated from 10 random areas by using Image J software and presented as the mean ± standard error. These quantifications confirmed systematic substrate coupling of the GNRs in various anisotropy in a monolayer without altering their macroscopic and nanoscale density. EDS analysis was carried out by following a previous report¹ to confirm substrate coupling of the GNRs by detecting gold (Au) element.

*Quantification of RGD ligand conjugated to the substrate-coupled GNRs with various anisotropies.*

The density of the substrate-conjugated RGD ligand was determined by following Ellman’s assay (Sigma). Briefly, the GNR-coupled substrate was incubated in thiol-bearing RGD peptide solution. After this incubation, the remaining solution containing unreacted thiol-bearing RGD peptide (20 µl) was collected and mixed with an assay buffer (100 µl) containing 1 mM EDTA (Sigma-Aldrich) as an antioxidant in sodium phosphate dibasic (0.1 M, pH = 8, Sigma-Aldrich). This mixture
solution was added into a detection buffer (50 µl) containing 0.5 mg/mL Ellman reagent (Sigma-Aldrich) dissolved in an assay buffer for the reaction for 10 min. RGD peptide solution incubated without substrate coupling of the GNRs was included as positive control, whereas DI water was used as a negative control. The reacted mixture solution was subjected to the reading of the absorbance at 412 nm by using UV-absorbance plate reader. The molar concentration of RGD peptide conjugated to the GNRs on the substrate was calculated by subtracting the concentration of RGD peptide in the supernatant from the concentration of positive control. The density of the substrate-coupled RGD was determined by dividing the number of the substrate-coupled RGD molecules by the surface area of the GNRs with various anisotropies on the substrate.

The adhesion and polarization of macrophages in the culture modulated by nanoscale ligand anisotropy.

The modulated adhesion and polarization of macrophages solely by anisotropic ligand nanogеometry were examined. Macrophages at passage 5 (RAW 264.7 from ATCC) were plated onto the substrate displaying nanoscale ligand anisotropy at an approximate density of 50,000 cells/cm². The adhesion of macrophages to the substrate displaying anisotropic ligand nanogеometry was evaluated after their culture in basal medium including high glucose DMEM, 10% (v/v) heat inactivated fetal bovine serum, and 50 U/mL penicillin/streptomycin at 37 °C and 5% CO₂. The polarization of adherent macrophages on the substrate displaying nanoscale ligand anisotropy was evaluated after 36 h in culture, including the 12 h of culture in basal medium (without stimulators) to allow macrophage adhesion, and then 24 h of culture with M1 stimulators [10 ng/mL lipopolysaccharide (LPS) and 10 ng/mL recombinant interferon-gamma (IFN-γ)] or with M2 stimulators [20 ng/mL interleukin-4 (IL-4) and 20 ng/mL interleukin-13 (IL-13)]. The role of adhesive structures of macrophages on the substrate displaying anisotropic ligand nanogеometry in regulating their polarization states was investigated in the presence and absence of 50 µM Y27632 (Abcam), a rho-associated protein kinase (ROCK) inhibitor.

Synthesis of gold nanospheres (GNSs) used for nanoscale immunolabeling.

GNSs used for nanoscale immunolabeling were synthesized in a size (approximately 13 nm) significantly smaller than those of the GNRs with various anisotropy to clearly differentiate the GNSs (used for immunolabeling) from the GNRs. Briefly, 1 mM HAuCl₄·3H₂O in 50 mL of DI
water was boiled with vigorous stirring. To this boiling solution, 1% (w/v) sodium citrate solution in 5 mL of DI water was rapidly added to this boiling solution. The solution was boiled for 15 min and then was cooled to 25 °C to obtain the resultant GNS solution (10 nM) before the GNSs were coated with secondary antibody.

*Dynamic light scattering (DLS) of the GNSs.*

DLS analysis was carried out with photon correlation spectroscopy (Brookhaven Instruments Corporation) to confirm a narrow distribution in the diameter of the GNSs to clearly differentiate the GNSs (used for immunolabeling) from the GNRs with various anisotropies. The DLS measurement was performed for the GNS suspension in DI water.

*High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) of the GNS.*

The HAADF-STEM imaging at atomic resolution (JEOL JEM-ARM200F) was carried out to clearly identify the GNS in high magnification as previously described, which was used for immunolabeling. The GNS was identified by quantifying the lattice spacing between periodic lattice fringes with Image J software.

*Nanoscale immunolabeling to characterize integrin recruitment to the surface of anisotropic ligand nanogometry.*

GNSs (10 nM) synthesized for immunolabeling were incubated in secondary antibody (goat anti-mouse (H+L) IgG, 1:100, Santa Cruz Biotechnology) at 37 °C for 16 h. Secondary antibody-coated GNSs were washed with antibody were washed with DI water. To perform nanoscale immunolabeling of the recruited integrin of macrophages by secondary antibody-coated GNSs, adherent macrophages to the substrate displaying nanoscale ligand anisotropy were washed in 0.1 M 1,4 piperazine bis(2-ethanosulfonic acid) (PIPES) buffer (pH = 7.4) for 2 min. The washed cells were permeabilized with 0.5% Triton X-100 in a buffer containing sucrose, NaCl, MgCl₂, and HEPES in DI water (pH = 7.2) for 1 min four times to remove the cell membrane. The permeabilized cells were then stabilized with 4% paraformaldehyde and 1% sucrose in 0.1 M PIPES buffer (pH = 7.4) for 5 min and washed with 0.1 M PIPES buffer for 2 min. The stabilized cells were treated with a blocking buffer [1% bovine serum albumin (BSA) and 0.1% Tween 20
in 0.1 M PIPES buffer, pH = 7.4] for 15 min to prevent non-specific binding of the secondary antibody-coated GNSs. The blocked cells were then incubated with primary antibody against integrin β1 (mouse) in a blocking buffer at 37 °C for 2 h and washed with a blocking buffer for 2 min six times. Non-specific binding sites were further blocked with 5% goat serum in a blocking buffer for 15 min.

The cells were subsequently labeled with secondary antibody (goat anti-mouse)-coated GNSs in PIPES buffer at 25 °C for 12 h and then washed with PIPES buffer for 2 min six times. The labeled cells with the GNSs were permanently fixed with 2.5% glutaraldehyde in PIPES buffer for 5 min and washed with PIPES buffer for 2 min three times. The GNSs that labeled cells were enhanced with silver through their incubation in a silver-developing solution for 7 min (BBI International) and washed with DI water. To enhance the contrast of the labeled cells, they were stained with 1% osmium tetroxide in PIPES buffer for 1 h and washed with PIPES buffer for 2 min six times. To prepare samples with nanoscale immunolabeling before SEM imaging, we performed critical point dehydration to dry all the substrate samples including macrophages, which were recruited to the GNRs and subsequently labelled with antibody-coated gold nanospheres. Afterwards, we mounted the samples onto copper studs and sputtered them with gold/palladium approximately for 30 s with rotation to obtain 2-3 nm-thick conductive layer. SEM imaging was subsequently performed to characterize the recruitment of integrin of macrophages to the surface of the anisotropic RGD ligand-bearing GNRs on the substrate. In the SEM images, red pseudocolor was assigned to the cells, whereas yellow pseudocolor was assigned to the GNSs used for nanoscale immunolabeling.

**Immunofluorescent staining of adherent macrophages.**

The adhesion and polarization of macrophages modulated by anisotropic ligand nanogometry were analyzed by immunofluorescent staining by following previously reported procedures. In brief, the macrophages were fixed with 4% (w/v) paraformaldehyde at 25 °C for 10 min and thoroughly washed with PBS. The treated macrophages were permeabilized with 0.25% (v/v) Triton-X-100 (Sigma Aldrich) in PBS at 25 °C for 10 min. The permeabilized macrophages were incubated in a blocking buffer containing 3% (w/v) bovine serum albumin (BSA) in PBS at 25 °C for 30 min. The treated macrophages were incubated in the blocking buffer including primary antibodies against vinculin (1:400, Sigma Aldrich), iNOS (M1-specific indicator, 1:100, Santa
Cruz Biotechnology), Arg-1 (M2-specific indicator, 1:200, Abcam), or ROCK2 (1:200, Abcam) at 4 °C for 16 h and thoroughly washed with PBS containing 0.5% Tween 20. The adherent host neutrophils were incubated in the blocking buffer containing primary antibody against NIMP-R14 (Neutrophil-specific indicator, 1:100, Santa Cruz Biotechnology). The cells were then incubated in the blocking buffer containing secondary antibodies (1:250, Thermo Scientific) with phalloidin (1:100, Molecular Probes) at 25 °C for 45 min. The cells were subsequently washed with PBS containing 0.5% Tween 20. The nuclei of macrophages were stained by incubating the treated cells in PBS containing DAPI (1:1000, Molecular Probes) at 25 °C for 10 min, which were then washed with PBS. The stained cells were mounted onto glass slides and their images were acquired using confocal microscope (Nikon) under the same exposure conditions between the compared groups. The background of images was identically subtracted from the acquired images between the compared groups by using ImageJ software (rolling ball radius of 750 pixels).

Quantitative cell analysis based on immunofluorescent staining.
The adhesion and polarization of macrophages modulated by nanoscale ligand anisotropy were quantified for immunofluorescently stained images by using ImageJ software. To determine the adherent macrophage density, the number of macrophages was counted from four different DAPI-stained images. To determine the area and aspect ratio (major axis/minor axis) of the adherent macrophages, F-actin images of approximately 30 cells from four different images were used. The density, area, and aspect ratio of the adherent host macrophages were calculated from F-actin images of host cells expressing either M1-specific indicator (iNOS) or M2-specific indicator (Arg-1). To calculate the density of the adherent host neutrophils, host cells expressing neutrophil-specific indicator (NIMP-R14) from four different images were counted.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR).
The polarization state of macrophages modulated by anisotropic ligand nanogeometry was quantitatively analyzed by the qRT-PCR by following a previous report. After macrophages were cultured in either M1 or M2 stimulators, they were collected to analyze quantitative expression levels of M1-specific indicators (iNOS and CD80) or M2-specific indicators (Arg-1 and Ym2). RNA from macrophages were extracted with TRIzol by following the manufacturer’s protocol. For each sample, 800 ng of RNA was subjected to reverse transcription to cDNA using the
RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The Applied Biosystems 7300 Real Time system was used to run real-time PCR reactions with TaqMan assays. The expression of each target gene was presented after it was normalized to that of corresponding housekeeping gene (GAPDH). The expression level of macrophages was subsequently normalized to that of the control group as described in figure captions and presented as relative fold expression.

*The adhesion and polarization of host macrophages modulated by anisotropic ligand nanogeometry.*

The adhesion and polarization of host macrophages modulated by the substrate displaying nanoscale ligand anisotropy were examined. 20 three-month-old BALB/c mice were used for surgery after the approval of the Institutional Animal Care and Use Committee at the Chinese University of Hong Kong. Each mouse was anesthetized by the intraperitoneal supply of ketamine (100 mg/kg) and xylazine (10 mg/kg). A 1.5 cm-long incision was then made in the back of each mouse. Each substrate displaying nanoscale ligand anisotropy was implanted into the subcutaneous pocket and the skin was closed. For 10 mice, we subsequently supplied each 100 ng of IL-4 and IL-13 (M2 stimulators) onto the implanted substrates (groups with M2 stimulator supply), whereas the other 10 mice had no supply of these stimulators. The mice were then housed in the cages and sacrificed at 24 h after surgery. The implanted substrates were retrieved for immunofluorescent staining analysis for the adhesion and polarization of host cells as well as qRT-PCR analysis for their state.

*Statistical analyses.*

All the experiments shown in this study were repeated at least twice independently. All the quantitative statistical analyses were carried out with Graphpad Prism 5.00 software. In the statistical analyses, p-values less than 0.05 were considered statistically significant differences between the compared groups, to which different alphabetical letters (a, b, c, or d) were assigned. Same alphabetical letters represent statistically non-significant differences between the compared groups.
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Supplementary Figure S1. Characterization of the GNRs with various anisotropies. Extinction spectra of the GNRs with various aspect ratios (“AR1”, “AR2”, “AR4”, or “AR7”).
Supplementary Figure S2. Characterization of the ligand exchange processes of the GNRs that aided their uniform substrate coupling. (A) Schematic and transmission electron micrographs of GNRs with various anisotropies (“AR2”, “AR4”, or “AR7”) corresponding to their ligand exchange processes detailing the Figure 1A, and (B) corresponding zeta potential (n=3) of the GNRs with various anisotropies. Scale bar: 100 nm.
Supplementary Figure S3. The adhesion of macrophages was elevated by high ligand anisotropy. The densities, areas, and aspect ratios of adherent macrophages shown in the staining micrographs in the Figure 2A were accordingly determined. AR represents various aspect ratio (“AR1”, “AR2”, “AR4”, or “AR7”) of the substrate-conjugated GNRs. Data indicate mean ± standard deviations (n=30). Different alphabetical letters (a or b) indicate statistically significant differences (p < 0.05) between the compared groups. Same alphabetical letters represent statistically non-significant differences between the compared groups.
Supplementary Figure S4. RGD coating on the GNRs is crucial to support macrophage adhesion. (A) Actin and nuclei stained in adherent cells on the substrate decorated with GNRs (“AR1”) with or without RGD coating. Scale bar: 50 µm. (B) The adherent macrophage densities were accordingly determined. Data indicate the means ± standard error of the mean (n=30). Different alphabetical letters (a and b) represent statistically significant differences (p < 0.05) between the compared groups.
Supplementary Figure S5. RGD conjugation to the GNRs is functional to support macrophage adhesion. (A) Actin and nuclei stained in adherent cells on the substrate decorated with AR7 GNRs with RGD peptide or scrambled RGD (RGE) peptides. Scale bar: 50 µm. (B) The adherent macrophage densities were accordingly determined. Data indicate the means ± standard error of the mean (n=30). Different alphabetical letters (a and b) represent statistically significant differences (p < 0.05) between the compared groups.
Supplementary Figure S6. Characterization of the gold nanosphere (GNS) used for nanoscale immunolabeling. Dynamic light scattering analysis of the distribution in the diameter of the GNSs. High-angle annular dark-field scanning transmission electron micrograph (HAADF-STEM) of the GNS at atomic resolution. The lattice spacing between the periodic lattice fringes was determined and labeled. Scale bar: 5 nm.
Supplementary Figure S7. The expression of M1-specific indicators in adherent macrophages after culture with M2 stimulators with or without ROCK inhibition. Macrophages were cultured without stimulators for 12 h and then with M2 stimulators for 24 h. M1-specific indicators include iNOS and CD80. AR represents various aspect ratio (“AR1” or “AR7”) of the substrate-grafted GNRs. The “AR7” was also cultured in the presence of the ROCK inhibitor, Y27632 (“AR7 + Y27632”). Data indicate means ± standard deviations (n=3). N.S. represents statistically non-significant differences between the compared groups.
Supplementary Figure S8. The expression of M1-specific indicators in adherent macrophages after culture in M1 stimulators with or without ROCK inhibition. Macrophages were cultured without stimulators for 12 h and then with M1 stimulators for 24 h. M1-specific indicators include iNOS and CD80. AR indicates various aspect ratio (“AR1” or “AR7”) of the substrate-conjugated GNRs. The “AR7” was also cultured in the presence of the ROCK inhibitor, Y27632 (“AR7 + Y27632”). Data indicate means ± standard deviations (n=3). Different alphabetical letters (a, b, or c) represent statistically significant differences (p < 0.05) between the compared groups.
Supplementary Figure S9. The expression of M2-specific indicators in adherent macrophages after culture in M1 stimulators with or without ROCK inhibition. Macrophages were cultured without stimulators for 12 h and then with M2 stimulators for 24 h. M2-specific indicators include Arg-1 and Ym2. AR represents various aspect ratio (“AR1” or “AR7”) of the substrate-coupled GNRs. The “AR7” was also cultured in the presence of the ROCK inhibitor, Y27632 (“AR7 + Y27632”). Data indicate as means ± standard deviations (n=3). N.S. indicates statistically non-significant differences between the compared groups.
Supplementary Figure S10. M2 state of adherent host macrophages on the substrate displaying anisotropic ligand nanogeometry. Actin, Arg-1, and nuclei stained in adherent host cells at 24 h after surgery. AR indicates various aspect ratio (“AR1” or “AR7”) of the substrate-grafted GNRs. Scale bar: 20 µm.
Supplementary Figure S11. The adhesion of host neutrophils to the substrate displaying nanoscale ligand anisotropy. (A) NIMP-R14 and nuclei stained in adherent host cells at 24 h after surgery. AR indicates various aspect ratio (“AR1” or “AR7”) of the substrate-conjugated GNRs. Scale bars: 20 µm. (B) The densities of host neutrophils were accordingly determined. Data are shown as means ± standard error of the mean (n=30). Different alphabetical letters (a or b) were assigned to statistically significant differences with p-values less than 0.05 between the compared groups.
Supplementary Figure S12. M1 state of adherent host macrophages was restrained by high ligand anisotropy with M2 stimulators. Actin, iNOS, and nuclei stained in adherent host cells at 24 h after surgery and subsequent supply of M2 stimulators (IL-4 and IL-13). AR represents various aspect ratio (“AR1” or “AR7”) of the substrate-grafted GNRs. Scale bar: 20 µm
Supplementary Figure S13. The adhesion of host neutrophils to the substrate displaying anisotropic ligand nanogeometry in the presence of M2 stimulators. (A) NIMP-R14 and nuclei stained in adherent host cells at 24 h after surgery and subsequent supply of M2 stimulators (IL-4 and IL-13). Scale bars: 20 µm. (B) The densities of host neutrophils were determined. AR represents various aspect ratio (“AR1” or “AR7”) of the substrate-conjugated GNRs. Data indicate means ± standard error of the mean (n=30). Different alphabetical letters (a or b) were assigned to statistically significant differences with p-values less than 0.05 between the compared groups.