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Supporting Information

Nanostickers for cells: a model study using hybrid cell nanoparticles aggregates

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Glossary

α	numeric coefficient relating G to C_m
C_e	NPs bulk concentration
C_e*	internalization threshold concentration
C_e**	surface saturation threshold concentration
C_i, C_{is}	NPs internal concentration (s: saturating)
C_L	Langmuir concentration
C_m, C_{ms}	NPs surface concentration (s: saturating)
G	sticking efficiency parameter
k_BT	thermal energy
k_i	rate constants
l	characteristic absorption length $=\sqrt{k_1/k_4}$
l_s	sedimentation length
N	number of groups
N_0	initial number of cells
n*	number of particle per adhesive contact
$N_{control}$	number of cluster without NPs
N_{NP}	number of cluster with NPs
$\tilde{N}_{NP}^{-1}$	$N_{control}$ / N_{NP}
r_p, d_p	NP radius, diameter
U	adhesion energy due to NPs
v	NP volume

Unit conversions

Nanoparticles	Volume fraction Φ (vol / vol)	Number concentration C (NPs per mL)	Mass concentration (ug per mL)
Carbo20	9.5×10^{-8}	8.9×10^{9}	0.1
	9.5×10^{-7}	8.9×10^{10}	1
	9.5×10^{-6}	8.9×10^{11}	10
	9.5×10^{-5}	8.9×10^{12}	100
Carbo200	9.5×10^{-8}	3.1×10^{7}	0.1
	9.5×10^{-7}	3.1×10^{8}	1
	9.5×10^{-6}	3.1×10^{9}	10
	9.5×10^{-5}	3.1×10^{10}	100
Carbo1000	9.5×10^{-8}	1.4×10^{5}	0.1
	9.5×10^{-7}	1.4×10^{6}	1
	9.5×10^{-6}	1.4×10^{7}	10
	9.5×10^{-5}	1.4×10^{8}	100
Amine200	9.5×10^{-8}	2.3×10^{7}	0.1
	9.5×10^{-7}	2.3×10^{8}	1
	9.5×10^{-6}	2.3×10^{9}	10
	9.5×10^{-5}	2.3×10^{10}	100
SiO_2	3.7×10^{-8}	5.8×10^{9}	0.1
	3.7×10^{-7}	5.8×10^{10}	1
	3.7×10^{-6}	5.8×10^{11}	10
	3.7×10^{-5}	5.8×10^{12}	100

Experimental

Materials: Fibronectin, phosphate-buffered saline (PBS), and silica nanoparticles were purchased from Sigma-Aldrich Co. Trypsin-EDTA, penicillin-streptomycin, Dulbecco's Modified Eagle Medium (DMEM), and polystyrene nanoparticles (FluoSpheres®) were obtained from Life Technologies Co. Their physical properties are listed in Table S1.

Table S1. Physico chemical characteristics of the nanoparticles employed

NPs	Material (density)	TEM Diameter [nm] [a]	Sedimentation length [mm] [b]	Functionalization of NP surface (pH 7.4)	Zeta potential in PBS [mV] [c]
Carbo20	Polystyrene (1.05)	28 ± 5	729	COO^-	-37.2 ± 2.5
Carbo200	Polystyrene (1.05)	180 ± 12	2.7	COO^-	-35.9 ± 1.3
Carbo1000	Polystyrene (1.05)	1100 ± 36	0.01	COO^-	-53.3 ± 2.7
Amine200	Polystyrene (1.05)	200 ± 10	2.0	NH_3^+	0.8 ± 0.5
SiO_2	Silica (2.7)	23 ± 4	39		- 22 ± 4

[a] Provided by the supplier.

[b] The sedimentation length was calculated with $l_s = \frac{k_B T}{v(\rho - \rho_{H_2O})g}$, where k_BT is the thermal energy, v the NP volume, g the gravitational acceleration, ρ the density of NPs and ρ_{H2O} the density of water

[c] The zeta potential of the nanoparticles in solution is measured with a Delsa Nano C instrument (Beckman Coulter, USA) equipped with a flow cell. The measurements are performed at 20 °C in a phosphate-buffered saline pH 7.4

Cell culture and aggregates preparation: Murine sarcoma cells S180[1]were cultured at 37 °C under a 95% air / 5% CO_2 atmosphere in culture medium consisting of Dulbecco's Modified Eagle Medium (DMEM)

supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and antibiotics (100 μm mL^{-1} streptomycin and 100 U mL^{-1} penicillin). Cell/NP aggregates were prepared following a modified hanging droplet method. Upon reaching confluence, cells were detached from the flask using trypsin and dispersed in DMEM with a concentration of 4×10^4 cells mL^{-1}. Nanoparticles were added to the cell suspension to reach the desired concentration. Droplets (15 μL) of the cell/NP suspension in cell culture medium were deposited on the lid of a Petri dish. The lid was inverted and placed on top of a Petri dish filled with PBS, such that the droplets containing the cells in the medium and hanging from the lid were maintained under a high humidity atmosphere. Due to gravity, cells fall to the bottom of the droplets and start to adhere to each other. After a 2 day incubation at 37 °C under a 95% air/5% CO_2 atmosphere, aggregates were obtained. For control experiments with cells alone, the same protocol was followed except that the addition of NPs was omitted. For each condition (NP type and NP concentration) 21 identical droplets were used for aggregate formation in order to obtain statistically significant data.

Aggregates observation by bright field microscopy: The droplets were removed from the lid with a pipette and paced on the surface of a non-adhesive, coated, Petri dish (Iwaki) and observed with an inverted microscope (TIRF AF 6000LX, Leica) equipped with a ×10 0.3 NA objective. Images were recorded with a CCD camera (Photometrics Cascade 512B, Roper Scientific), exported from the instrument software in TIFF format and visualized using the ImageJ software package v.1.46r (National Institutes of Health, Bethesda, MD).

Observation of S180 cells by confocal fluorescence microscopy. Upon reaching confluency, cells were detached from the flask using trypsin and dispersed into DMEM supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and antibiotics (100 μm mL^{-1} streptomycin and 100 U mL^{-1} penicillin) at a concentration of 2.5×10^5 cells mL^{-1}. An aliquot of this dispersion (200 μL) as deposited on a petri dish coated with collagen. After a 24 hr-incubation, a NP suspension in DMEM supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and antibiotics (100 μm mL^{-1} streptomycin and 100 U mL^{-1} penicillin) (22 μL) was added to the cells to reach a final concentration in NPs of 12 μg mL^{-1}. The cells were incubated

with the NPs for 4 hr. The cells were washed twice with NPs-free DMEM supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and antibiotics (100 µm mL^{-1} streptomycin and 100 U mL^{-1} penicillin). Aggregates formed were observed with an inverted confocal microscope (TCS SP5, Leica Microsystems) equipped with a ×63 1.4 NA objective with oil immersion. Images were exported from the instrument software in TIFF format and visualized using ImageJ. Representative micrographs of S180 cells treated with NPs are presented in Figure S1. Figure S1 shows that within 4 hours, cells internalize all 4 nanoparticles types. In the case of Carbo200, Amine200 and Carbo1000, individual nanoparticles are visible. As for Carbo20 NPS, two states seem possible: free in the cytoplasm (appearing as a diffuse cloud) or gathered inside endosomes (appearing as brighter round shapes, smaller than 1 µm).

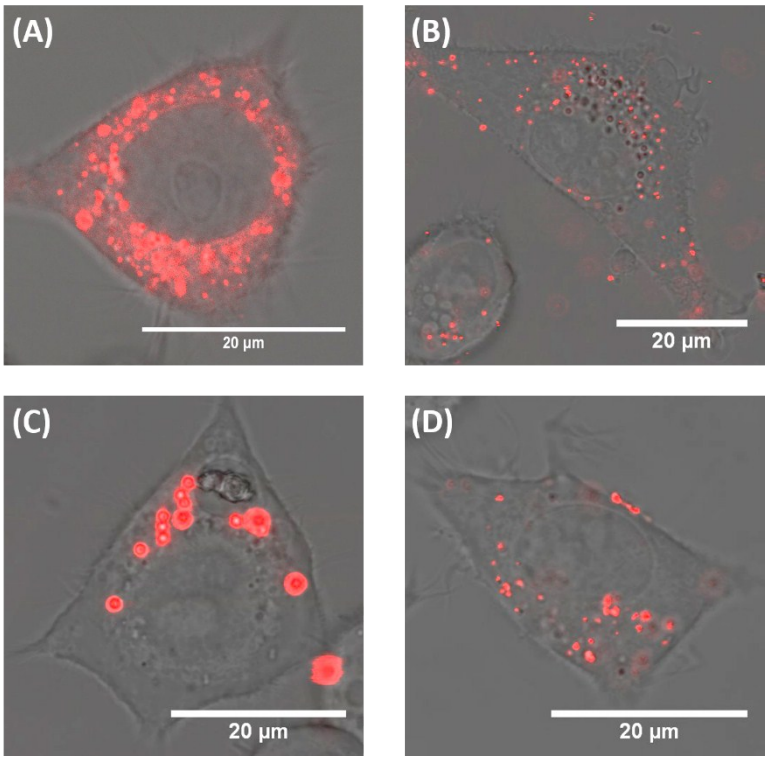

Figure S1. Micrographs recorded by confocal fluorescence microscopy of S180 cells incubated for 4 hr with NPs (Volume fraction $\Phi = 1 \times 10^{-5}$) and washed with PBS (A) Carbo20, (B) Carbo200, (C) Carbo1000 and (D) Amine200.

Additional data in support of the three-state model

The model was designed to account for several other observations. The existence of a critical concentration is explained by assuming a saturating concentration of nanoparticles inside the cells. The fact that this saturation is related to the volume fraction of the nanoparticles can be explained by the fact that cells can absorb a maximal volume of nanoparticles whatever their size. In the description of the model, we supposed that NPs located in the solution were in excess in comparison to the NPs inside the cells and on the cell membrane. If the nanoparticles were not in excess, increasing the number of cells for a determined volume of dispersion should decrease the final concentration of nanoparticles. The experiment was done for concentrations near the critical concentration (1.2 µg mL^{-1}), where the changes of external concentration of NPs produce the largest variations. As seen in Figure S2, *G* does not depend on the number of cells. This result means that the nanoparticles are indeed in excess in the drops.

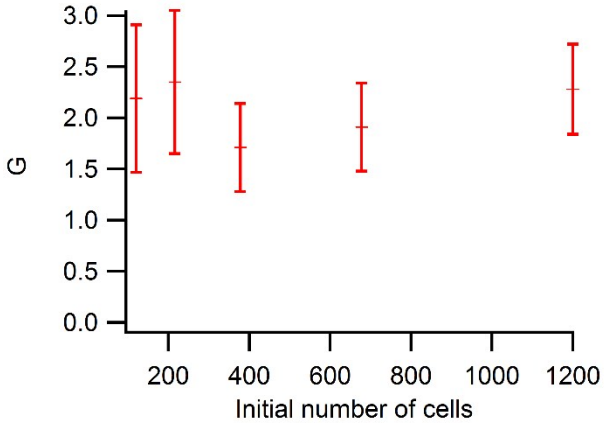

Figure S2. *G* as a function of the initial number of S180 cells in the drops, for Carbo20 at 1.2 ug mL^{-1}

Dynamics of spheroid formation for LCAM cells. To demonstrate that indeed *nanostickers* may replace the missing cellular adhesion molecules CAMs, we have applied the same technic as showed in Figure 2 to LCAM cells, which are S180 cells transfected to express the highest level of cadherins.

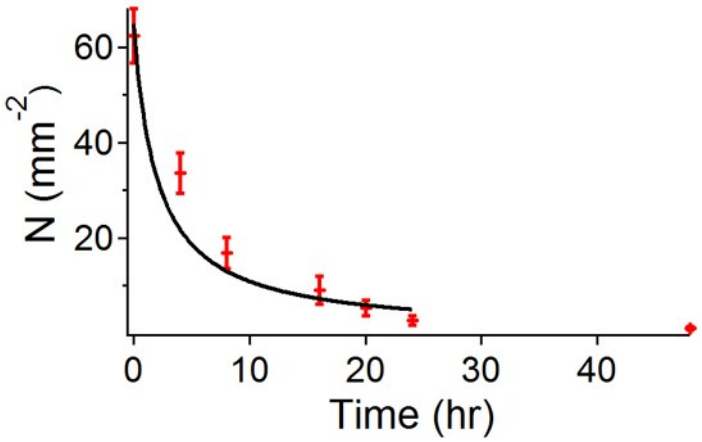

Figure S3. Number N of groups of LCAM cells inside a droplet as a function of time. Experimental points are fitted using equation (1)

Fitting with equation (1), we found the parameters: $N_0 = 65 \pm 6$ and $P_{LCAM}K = (4.2 \pm 0.6) \times 10^{-12}$ m^2 s^{-1}. Using the value previously found for S180 cells without nanoparticles, we deduce $G = P_{LCAM} / P_{control} = 2.1 \pm 0.3$. This means the adhesion induced by nanoparticles is comparable to the one due to cadherins, and is even higher for Carbo20 NPs.

Reference

1 Y.-S. Chu, W. A. Thomas, O. Eder, F. Pincet, E. Perez, J. P. Thiery and S. Dufour, *J. Cell Biol.*, 2004, **167**, 1183–94.