Interactions between Colloids Induced by a Soft Cross-Linked Polymer Substrate

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Using video-microscopy imaging we demonstrate the existence of a short-ranged equilibrium attraction between heavy silica colloids diffusing on soft surfaces of cross-linked polymer gels. The inter-colloid potential can be tuned by changing the gel stiffness or by coating the colloids with a polymer layer. On sufficiently soft substrates, the interaction induced by the polymer matrix leads to large-scale colloidal aggregation. We correlate the in-plane interaction with a colloid-surface attraction.

Interactions between colloids confined to surfaces [1–3] or interfaces [4] are key to the fundamental understanding of many physical phenomena. For instance, self-assembly of ordered phases [5] can be induced, which finds applications in the engineering of photonic crystals [5–7]. Recent studies have shown that aspects of cellular morphology [8, 9], mechanical properties [9, 10], mobility [9, 11] and differentiation [9, 12] are sensitive to the elastic response of the environment. Experiments in this field often employ cross-linked polymer-gel surfaces [8, 10, 11] as a model for biological tissues. We demonstrate that soft polymer substrates may themselves actively induce non-negligible interactions between inanimate, micrometer-sized, objects. It is vital to recognize that the same phenomena may affect living systems of similar dimensions, and therefore must be taken into account when studies such as those above are carried out. In this Letter we explore the nature of such substrate-induced inter-colloidal forces and show how they are modified by changing the physical properties of the soft environment. After a brief introduction of our system, we report qualitative observations on the collective behavior of sedimented colloids. Then, we describe quantitative measurements of in-plane colloid-colloid interactions using two complementary techniques. Finally we report colloid-surface potential energy measurements, and micro-rheology results on the viscoelastic properties of the substrate, which help us understand the observed in-plane interactions.

We used plain silica colloids with diameter $\sigma = 1.16 \pm 0.05$ μm (Microparticles GmbH, Berlin) and a nominal density of 1.5–2 g cm^{-3} suspended in 100 mM tris(hydroxymethyl)aminomethane (TRIS) buffer at pH 8. Under these conditions the silica colloids have a net negative charge with an average ζ-potential of -42±1 mV. As soft surfaces we used 150 μm thick polyacrylamide (PAA) cross-linked gel films deposited on a microscope coverslip. The polymerization of PAA was triggered by

adding tetramethylethylenediamine (TEMED) and ammonium persulfate to a solution containing acrylamide monomers and the cross-linker bis-acrylamide (bis-AA), all in phosphate buffered saline buffer (PBS, pH 7.4). For control experiments, we coated the same silica colloids with positively charged poly-L-lysine-poly(ethylene glycol) (PLL-PEG, Surface Soultions, Switzerland) that is easily adsorbed onto silica and provides steric stabilization. PLL-PEG coated beads were almost neutral with a ζ-potential of -2.2±0.2 mV. Non-adhesive hard surfaces were obtained by coating the glass bottom of 8 mm diameter incubation wells (Sensoplate, Greiner bio-one) with PLL-PEG. Using a PAA concentration of 5 % we tuned the stiffness of the material by changing the bis-AA fraction. The observations of weak attractive interactions between colloids were qualitatively and quantitatively similar for gels with elastic shear modulus G' [13] between 55 and 522 Pa, thus, we focused on a substrate prepared with 4×10^{-4} v/v bis-AA resulting in a $G' = 240 \pm 5$ Pa — we refer to this gel as the "soft" substrate. In contrast, colloids on "ultra-soft" gels produced with a bis-AA concentration of 1×10^{-4} v/v and with $G' = 12 \pm 3$ Pa exhibited stronger interactions.

On soft gels, sedimented colloids did not undergo large scale aggregation. Instead, we observed the formation of dimers, triplets, and small chains and clusters, as shown in Fig. 1(a) and 1(b) for two different surface densities. On ultra-soft gels, sedimented beads initially formed small clusters that were still mobile and merged into larger ones. The aggregation stopped after about one hour as the reduced mobility of big clusters kinetically hindered the process. A sequence of images of the aggregation is shown in Fig. 1(c). The larger aggregates could be either amorphous or ordered in a hexagonal close-packed lattice [see Fig. 1(d)]. The mobility of colloids within the aggregates was small and we only observed rearrangement of the beads located at the outer perimeter. Using PLL-PEG coated colloids instead of plain ones, the aggregation still took place on the same timescale but the clusters showed liquid-like behavior with a continuous rearrangement of single colloids in the bulk of the
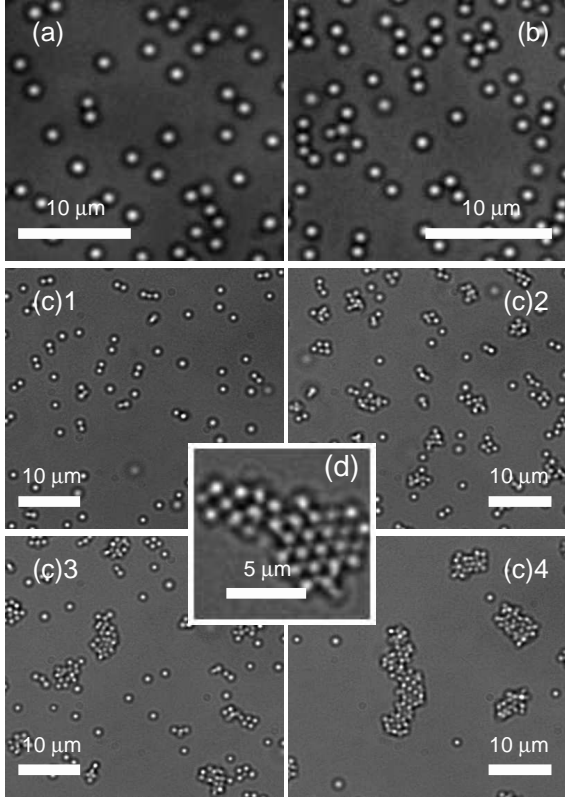
FIG. 1. Panels (a) and (b): microscope images of plain silica colloids sedimented on a PAA soft gel with different surface coverage. Panels (c)1–4: aggregation process of plain silica colloids sedimented on ultra-soft PAA gel surface at $t = 0$ (1), $t \approx 15$ min (2), $t \approx 30$ min (3) and $t \approx 60$ min (4). Panel (d): highlight of an ordered aggregate on the ultra-soft gel.
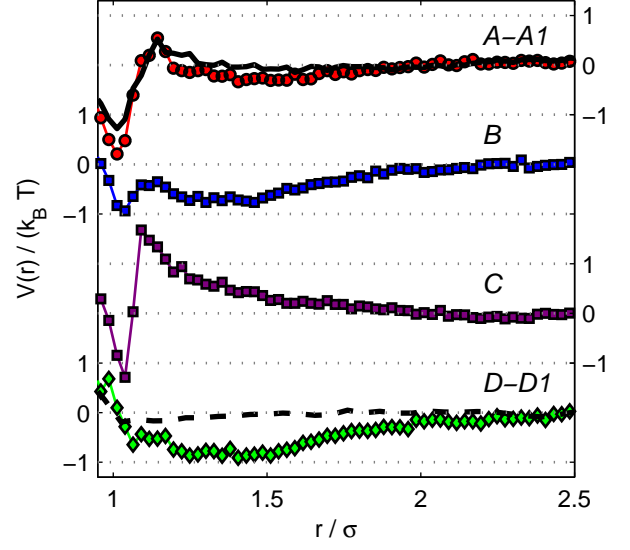
FIG. 2. (Color online) Pair potentials of colloids sedimented on gels and glass surfaces measured with the blinking-tweezers (BT) or pair-distribution [$g(r)$] methods. Bare silica colloids sedimented on soft PAA gel surface measured with BT (A ○) and with $g(r)$ ($A1$ solid line); PLL-PEG coated silica colloids sedimented on a soft PAA gel surface measured with BT (B); PLL-PEG coated silica colloids sedimented on a ultra-soft PAA gel surface measured with BT (C); PLL-PEG coated silica colloids sedimented on a PLL-PEG coated rigid glass surface measured with BT (D, ◇) and with $g(r)$ ($D1$, dashed line). The curves have been vertically shifted for clarity.

aggregates.

We measured the effective pair-potential of supported beads with two complementary techniques. For the first one, referred to as "blinking optical tweezers" (BT) [14, 15], we positioned two isolated colloids at separations between 1.5 and 10 μm using optical tweezers [16]. After releasing the colloids, movies were taken at 30 frames per second, and the separation r between beads was obtained tracking their positions with conventional algorithms [17]. After discretizing r with a mesh size of 25 nm we sampled the transition matrix elements P_{ij} that express the probability for r to evolve from bin i to bin j in the time between two consecutive frames. The probability distribution of r is $\rho_i^{\mathrm{s}} = P_{ij}\rho_j^{\mathrm{s}}$ and the effective pair-potential is given by $V(r)/(k_{\mathrm{B}}T) = -\log(\rho^{\mathrm{s}})$. The second method consisted of extracting $V(r)$ from the equilibrium radial distribution function $g(r)$, which was estimated for every sample taking a few hundred snapshots from an area of about 1.5×10^{-2} mm^2 with a surface coverage of 0.5–1 %. At these densities we could neglect many body effects and extract the pair-potential as $V(r)/(k_{\mathrm{B}}T) \approx -\log[g(r)]$. In both techniques, superposition of colloid diffraction-images can

bias the measurement of the inter-colloid distance in microscopy images, leading to systematic errors in the estimate of $V(r)$ [2, 18]. We corrected our data following the method proposed by Polin et al. [2]. The potential extracted using the BT method arises from forces that may not have an underlying equilibrium potential, including hydrodynamic coupling [3]. The evaluation of $V(r)$ from $g(r)$ is generally preferable, being unaffected by non-equilibrium effects. However, due to rapid aggregation, $g(r)$ measurements on the ultra-soft gels in the low-density limit were unfeasible. BT technique was applicable to all of our surfaces, and was therefore adopted as reference technique, using $g(r)$ measurements to spot non-equilibrium artifacts.

For the case of plain silica colloids supported by soft PAA surfaces, the apparent $V(r)$ measured with BT evidences a sharp minimum for $r \approx \sigma$, as shown in Fig. 2, curve A. The same measurement done using PLL-PEG coated colloids on the same soft gels is displayed in Fig. 2 curve B and demonstrates a weakened short-range attraction. In Fig. 2, curve C, we show $V(r)$ measured with BT for PLL-PEG coated colloids on ultra-soft gels [19]. In this case, the short-range attraction is stronger than for soft gels. In Fig. 2 curves A, B and C show a maximum in the potential at $r \approx 1.2\,\sigma$. This cannot be ascribed to screened Coulomb repulsion as the Debye screening length is estimated to be only a few nanometer

[20]. As a control we measured $V(r)$ with BT for the case of PLL-PEG coated colloids sedimented on a PLL-PEG coated rigid glass surface. The potential, plotted in Fig. 2 curve D, does not show the short-range minimum observed on PAA surfaces. The broader, longer ranged minimum observed in BT measurements of $V(r)$ for both soft gels (Fig. 2 curves A and B) and rigid glass surfaces (Fig. 2 curve C) can be ascribed to non-equilibrium artifacts [3]. This hypothesis is confirmed by equilibrium measurements of $V(r)$ from $g(r)$ for plain colloids settled on a soft gel surface and PLL-PEG coated colloids settled on a rigid glass surface, shown in curves $A1$ and $D1$ of Fig. 2. In both cases, the long-range minimum is absent, consistent with the explanation that this attraction was due to hydrodynamic coupling. As demonstrated with both BT and equilibrium measurements from $g(r)$, the short-range attraction is only present on PAA gels but not on the rigid surface, indicating that it is an equilibrium effect mediated by the polymer gel substrate. The same argument applies to the repulsive maximum in the colloid pair-potential located at $r \approx 1.2\,\sigma$ that is present when the colloids are on soft gels, but absent when colloids are on a hard glass interface. We can deduce that the short-range attraction is responsible for the small aggregates on the soft gel substrates and for the large-scale clustering on the ultra-soft ones, where it became stronger. The weakening due to the PLL-PEG coating accounts for the liquid-like behavior of clusters on the ultra-soft gels.

The range and shape of the short-range attractive well are compatible with a description in terms of depletion [21]. The PAA gel can in principle release free polymers in solution that may act as depletants. However, the absence of aggregation on bare glass in the same sample cell that contains gels with associating colloids excludes this possibility. Chen and Ma [22] predicted that a combination of excluded volume effects and polymer-colloids interfacial energy can give rise to an interaction between colloids embedded in a polymer brush. A similar effect can occur in our system assuming a partial inclusion of the colloids in a weakly cross-linked polymer layer on top of the substrates.

We tested this possibility by measuring the colloid-surface interaction energy and the effective viscoelastic properties of the gel surfaces using micro-rheology. We tracked fluctuations of sedimented beads in z-direction by video-microscopy measurements [23]. The diffraction image of a colloid changes as a function of the distance between the bead and the focal plane of the objective lens. We took a set of a few hundred pictures of an immobile colloid by moving the focus with steps of 25 nm, and associating an intensity $I(z)$ to each reference image by integrating the pixel value within a circle concentric with the bead. Fitting the intensity as a function of z we obtained a calibration curve that is monotonic within an interval of a few microns. Working with the same illumination conditions we took movies of single freely diffusing colloids, measured $I(t)$ for each frame
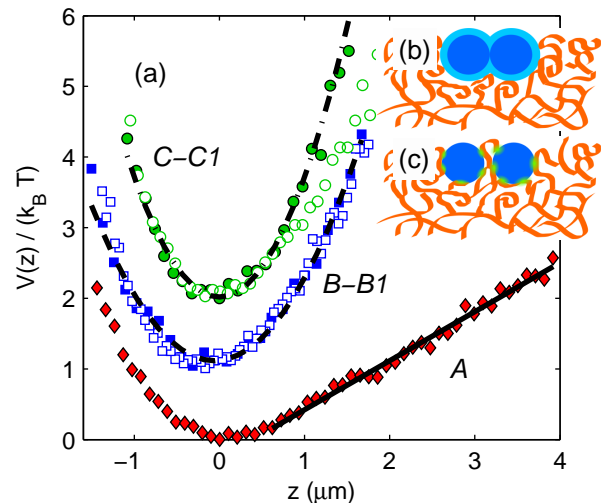

FIG. 3. (Color online) (a) Experimental colloid-surface interaction potentials for PLL-PEG coated silica colloids confined by PLL-PEG coated glass surface (A); plain (B, filled symbols) and PLL-PEG coated ($B1$, empty symbols) silica colloids confined by a soft PAA gel surface; plain (C, filled symbols) and PLL-PEG coated ($C1$, empty symbols) silica colloids confined by an ultra-soft PAA gel surface. The solid line is a linear fit. Dashed and dot-dashed lines are quadratic fit. z is the direction normal to the surface. The curves are shifted vertically and the minima aligned horizontally for clarity. Insets: not-to-scale sketch of two beads partially included in the gel surface and interacting through excluded volume effects (b) and colloid-polymer adhesion (c).

and found the corresponding $z(t)$ using the calibration curve. We sampled the probability distribution $\rho(z)$ by taking a histogram of $z(t)$. The colloid-surface potential is given by $V(z)/(k_B T) = -\log[\rho(z)]$. As a control we measured $V(z)$ for PLL-PEG coated colloids sedimented on PLL-PEG coated glass surface. As expected, the only potential attracting the bead towards the surface is the gravitational one, as shown in Fig. 3(a), curve A. Fitting the linear region of $V(z)$ we estimated the weight of the colloid $mg \approx 2.6$ fN, which is in good agreement with the nominal value $\gtrsim 3$ fN. For plain silica colloids settled on the surface of a soft PAA gel, $V(z)$ reveals an additional attraction (see Fig. 3(a), curve B). We quantify this attraction with a parabolic fit that gives an effective spring constant of 4.1 nN m^{-1}. For the case of an ultra-soft gel, Fig. 3(a) curve C shows that the colloid-surface attraction is even stronger with an effective spring constant of 7.2 nN m^{-1}. The attraction points to a partial inclusion of the beads into the gel surface that should be deeper for the case of ultra-soft gels.

Further insight on colloid-surface interactions was obtained by analyzing the horizontal fluctuations of a single sedimented colloid applying micro-rheology techniques [24]. Note that we only report effective values about the viscoelastic properties of the surfaces, as conventional micro-rehology techniques are derived for probe colloids

embedded in an isotropic environment [25]. The effective surface viscosity (η_s), estimated from the effective loss modulus G_s'' [24], was 1.7 mPa s and 5.5 mPa s for soft and ultra-soft gels respectively. For both substrates η_s was larger than the value expected for water at room temperature, even in presence of hydrodynamic coupling with a boundary [26]. This indicates a coupling between the probe bead and the PAA gels, compatible with partial inclusion. Bulk measurements of the viscosity show that the soft gel has a higher viscosity ($\eta = 24 \pm 0.5$ Pa s) than the ultra-soft gel ($\eta = 1.7 \pm 0.5$ Pa s), which is expected as the soft gel has a higher cross-link density. The opposite trend between the effective surface and bulk viscosities can be explained as follows. The bead penetrates more deeply in the ultra-soft gel than the soft gel because of the former's lower cross-link density. This results in a larger contact area between bead and gel and thus larger adsorption energy, which in turn, diminishes the diffusion of the particle on the ultra-soft gel accounting for its larger effective loss modulus.

We suggest two possibilities to explain the observed inter-particle attraction of colloids on PAA gels. One, partial encapsulation accentuates a depletion-like colloid-colloid interaction [22], as sketched in Fig. 3(b). Two, direct polymer bridging mediated by the colloid-polymer surface adhesion can be responsible for the in-plane attraction, as shown in Fig. 3(c) [27]. For the ultra-soft gels, the deeper penetration of the beads explains the stronger in-plane attraction either by increasing the excluded volume between two adjacent colloids or increasing the bead-gel contact surface. The repulsion found for $r \approx 1.2\,\sigma$ can be interpreted as the elastic response of polymer coils squeezed between two adjacent beads. We also measured $V(z)$ on the soft and the ultra-soft gels using PLL-PEG coated beads, as shown in Fig. 3 curves *B1* and *C1*. In the first case, steric stabilization does not affect $V(z)$ while in the case of ultra-soft gels the potential is slightly less attractive.

In summary, we demonstrated that silica colloids sedimented onto soft cross-linked polymer-gel surfaces interact through a strong short-range equilibrium attractive potential that can be tuned either by changing the substrate stiffness or bead-surface properties, and, for the case of ultra-soft substrates, produces large scale aggregation, eventually leading to the formation of ordered phases. The in-plane interaction correlates with a colloid-gel attraction. All the experimental observations are consistent with the in-plane interactions arising from either depletion effects or direct polymer bridging. Further investigations are needed to determine which of these proposed mechanisms account for the aggregation of colloids on surfaces of soft polymer gels.

[1] G. M. Kepler and S. Fraden, Phys. Rev. Lett. **73**, 356 (1994).
[2] M. Polin, D. G. Grier, and Y. Han, Phys. Rev. E **76**, 041406 (2007).
[3] T. M. Squires and M. P. Brenner, Phys. Rev. Lett. **85**, 4976 (2000).
[4] A. Domínguez, M. Ottel, and S. Dietrich, J. Phys.: Condens. Mat. **17**, S3387 (2005).
[5] S. H. Im, Y. T. Lim, D. J. Suh, and O. O. Park, Adv. Mater. **14**, 1367 (2002).
[6] Y. Xia, B. Gates, Y. Yin, and Y. Lu, Adv. Mater. **12**, 693 (2000).
[7] I. I. c. Tarhan and G. H. Watson, Phys. Rev. Lett. **76**, 315 (1996).
[8] P. Moshayedi, L. da F Costa, A. Christ, S. P. Lacour, J. Fawcett, J. Guck, and K. Franze, J. Phys.: Condens. Mat. **22**, 194114 (2010).
[9] D. E. Discher, P. Janmey, and Y.-l. Wang, Science **310**, 1139 (2005).
[10] A. Engler, L. Bacakova, C. Newman, A. Hategan, M. Griffin, and D. Discher, Biophys. J. **86**, 617 (2004).
[11] T. A. Ulrich, E. M. de Juan Pardo, and S. Kumar, Cancer Res. **69**, 4167 (2009).
[12] A. L. Zajac and D. E. Discher, Curr. Opin. Cell Biol. **20**, 609 (2008).
[13] Measured using Physica MCR bulk rheometer (Anton Paar, Austria).
[14] J. C. Crocker and D. G. Grier, Phys. Rev. Lett. **73**, 352 (1994).
[15] J. C. Crocker and D. G. Grier, J. Colloid Interf. Sci. **179**, 298 (1996).
[16] Home-made optical tweezers based on a 2W 1064nm laser (CrystaLaser) mounted on a Nikon Eclipse Ti-E microscope with a Plan Apo VC 60x WI 1.2 N.A. objective and a Pike F-100B CCD camera (Allied Vision Technologies).
[17] We used home-made software based on open source MATLAB routines available at http://physics.georgetown.edu/matlab.
[18] J. Baumgartl and C. Bechinger, Europhys. Lett. **71**, 487 (2005).
[19] Tracking the distance between plain colloids on ultra-soft gels was not reliable due to rapid aggregation.
[20] For practical reasons our samples could not be sealed. Resulting slow evaporation could possibly increase the buffer concentration leading to further reduction in the Debye screening length. No measurable flow at the level of the surface was observed.
[21] S. Asakura and F. Oosawa, J. Chem. Phys. **22**, 1255 (1954); J. Polym. Sci. **33**, 183 (1958).
[22] K. Chen and Y.-q. Ma, J. Phys. Chem. B **109**, 17617 (2005).
[23] G. M. Kepler and S. Fraden, Langmuir **10**, 2501 (1994).
[24] T. Yanagishima, D. Frenkel, J. Kotar, and E. Eiser, J. Phys.: Condens. Mat. **23**, 194118 (2011).
[25] B. Schnurr, F. Gittes, F. C. MacKintosh, and C. F. Schmidt, Macromolecules **30**, 7781 (1997).

[26] J. Happel and H. Brenner, *Low Reynolds Number Hydrodynamics* (Kluwer Academic Publishers Group, Dordrecht, The Netherlands, 1983).

[27] F. Lafuma, K. Wong, and B. Cabane, J. Colloid Interf. Sci. **143**, 9 (1991).