The Characterization of High Generation Poly(amidoamine) G9 Dendrimers by Atomic Force Microscopy (AFM)

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SUMMARY: Scanning force microscopy (AFM) has been employed to characterize the generation-9 (G9) poly(amidoamine) (PAMAM) dendrimer packing on a mica surface under various conditions. Well ordered 2-D arrays from hexagonally packed particles of PAMAM (G9) dendrimers (11.4nm in diameter) were deposited on the mica surface. This may be one of the smallest regular monolayer arrays ever observed. The mechanism considered to be responsible for this 2-D array packing is the interaction of forces between the dendrimer and the mica surface and between dendrimer molecules as well. Other factors such as molecular interpenetrating and the rigidity of the branch structure obviously play an important role in the 2-D array formation.

Introduction

Assemblies of colloidal particles can be used to prepare a two-dimensional (2-D) array packing over the entire surface of a planar substrate[1)]. Such submicron-size particle pattern may be considered an optical storage medium. These arrays function by concentrating the evanescent light from a sub-wavelength aperture into an area equivalent to the sphere diameter[2)]. To date, these phenomena have been investigated primarily with classical polymer latices. Micro-emulsion polymerizations allow the synthesis of ultra-fine latex particles in the size range of 5 to 50 nm with relatively narrow size distributions[3)], however, the deposition of an ordered monolayer of ultra-fine spheres is known to be increasingly difficult as the diameter of such particles decreases[4)]. This is due primarily to strong Brownian motion and capillary effects which create a state of disorder in the system that is difficult to control. Presently, one of the smallest 2-D arrays of latex spheres prepared has a periodicity of 42 nm[5)].

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However, we now present the successful AFM imaging of 2-D array packing of G9 PAMAM dendrimers which posses periodicities of nearly four times less than the smallest latex arrays (theoretical diameter: 11.4 nm). This exceptional packing phenomenon is undoubtedly due to the unique physical and chemical properties of PAMAM dendrimers.

PAMAM dendrimers are members of the most structure-controlled subset of dendritic polymers. They are synthetic, highly branched, spherical molecules with precisely defined chemical functionality [6] as illustrated in Figure 1.

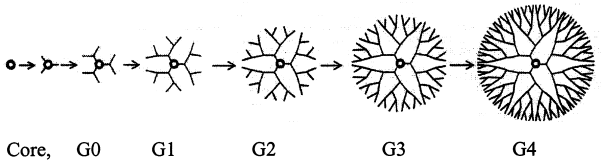

Figure 1. Two dimensional Model for PAMAM dendrimer.

They are core-shell macromolecules which synthetically grown from a small core where in each complete iterative reaction sequence results in a new dendrimer shell or "generation". These core-shell structures are enhanced systematically at each generation to produce a larger molecular diameter, twice the number of reactive surface sites, and approximately twice the molecular weight of the preceding generation (see Table 1)[7].

Table 1: The theoretical properties of starburst PAMAM dendrimers

Generation	Measured* Diameter(nm)	Surface Groups	Molecular Weight
0	1.5	4	517
1	2.2	8	1430
2	2.9	16	3256
3	3.6	32	6909
4	4.5	64	14215
5	5.4	128	28826
6	6.7	256	58048
7	8.1	512	116493
8	9.7	1024	233383
9	11.4	2048	467162
10	13.5	4096	934720

Ethylenediamine core *: Determined by light scattering

These water-soluble macromolecules are not only uniform in size and three-dimensional shape, but are also systematically hyperbranched with mathematically defined number of functional surface groups[8]. They manifest versatile nano-scaffolding properties, which may be designed to meet specific requirements. Recent research has shown that PAMAM dendrimers have a variety of prospective applications in the fields of photosensitive materials, adhesives, functional coatings, chemical sensors, drug delivery and gene transfection[9-10].

In this paper, AFM has been employed to study G9 PAMAM dendrimer packing on a mica substrate. The organizational properties of the G9 dendrimer 2-D array packing can clearly be visualized on the mica surface. The combinatorial variety of packing organizations obtained with different conditions may be considered to be a dendrimer-packing library. The mechanism of the particle packing is also discussed.

Experimental

Ethylenediamine (EDA) core poly(amidoamine) G9 dendrimer was obtained from Dendritech Inc. (Midland, MI) in a methanol solution. After thorough drying, the solid G9 dendrimer was weighed and re-dissolved in distilled water to produce a 0.1% (w/w) stock solution. The solution was stored at 4°C until use. Samples were prepared by placing various amounts of dilute aqueous solution of G9, conc. $5x10^{-3}$ % (w/w) on a freshly cleaved mica surface and allowing the film to dry slowly at room temperature. The different conditions of sample preparation are described in the appropriate figures. The samples on mica were examined with a Nanoscope III MultiMode AFM instrument (Digital Instrument, Santa Barbara, CA), using the tapping mode under ambient conditions. A 12μm scanner was used in every experiment. Silicon tapping probes having a spring constant of ca. 50N/m with about 5-10nm radius were used for tapping scans.

Results and Discussion

High generation poly(amidoamine) dendrimers (G9) readily form 2-D packing arrays on a freshly cleaved mica surface, as shown in Figure 2. The sample was prepared by placing 6 μl of a dilute aqueous solution of G9, conc. $5x10^{-3}$ % (w/w), on a freshly-cleaved mica surface, and allowing the film to dry slowly at room temperature for a few hours. This top-view AFM image reveals well-ordered particle packing except for some vacancies, dislocations and grain boundaries. Most areas show very dense packing of globular G9 dendrimer, exhibiting a clear hexagonal order as indicated in the square of Figure 2. The size of these densely packed G9 dendrimer molecules as determined by AFM measurement is approximately 12.8nm in diameter. This suggests that hexagonal dense packing of G9 dendrimer molecules may reduce the deformation, which normally occurs with single isolated G9 molecules on a mica surface[11].

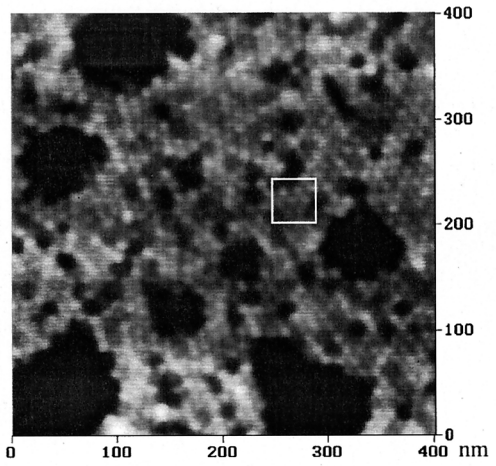

Figure 2. Tapping Mode AFM image of G9 PAMAM dendrimer molecules on a mica surface.

It is interesting to note, however, that G9 dendrimers tend not to form 2-D packing arrays on the highly orientated pyrolytic graphite (HOPG) surface as shown in Figure 3. This sample was prepared with the same procedure as that on mica. We can see that

the G9 particles are aggregated together to form clusters, hills and many domains. One may observe some small particles, but no ordered packing can be observed. This result suggested that the interactive force between the G9 dendrimers and the substrate might play an important role in these 2-D packing arrays.

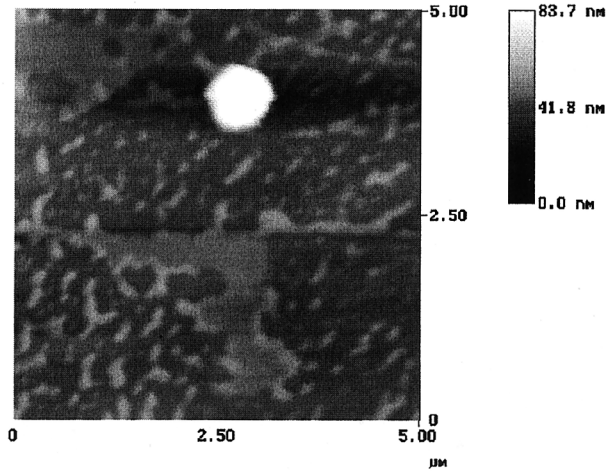

Figure 3. AFM image of G9 packing on the HOPG surface.

The interactive forces (in terms of adhesion force) have been measured using AFM force distance curve techniques. In this experiment, a plot of the force interaction between the AFM tip and the surface as a function of relative tip-sample separation constitutes a force curve and is often referred to as force spectroscopy. The designed experiments are shown in Figure 4.

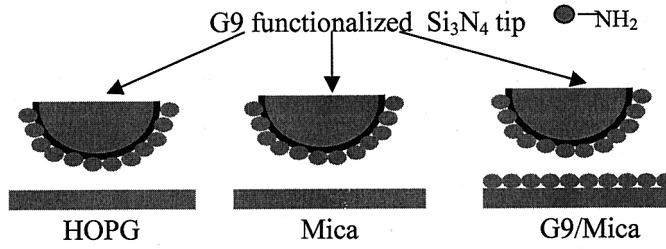

Figure 4. Cartoon model of the adhesion force measurement between the G9 functionalized tip and surfaces.

The interactive forces between G9 dendrimers and the surface may be measured if the AFM Si_3N_4 tip is functionalized with G9 dendrimer molecules. The tip functionalization was performed by immersing the tip in $4x10^{-3}$ % (w/w) G9 aqueous solution for 5 mins, then dried the tip using a light stream of nitrogen gas. It was found that the G9 dendrimer could be strongly adsorbed on the Si_3N_4 AFM tip surface, as determined by FE-SEM (Field Emission Scanning Electron Microscopy) techniques shown in Figure 5 (a, b, c, d). Figure 5a shows the standard Si_3N_4 tip surface. After functionalization by G9 dendrimer molecules, a very thin film formed on the tip surface, Figure 5b. This film can be seen much more clearly at higher magnification, Figure 5 (c, d).

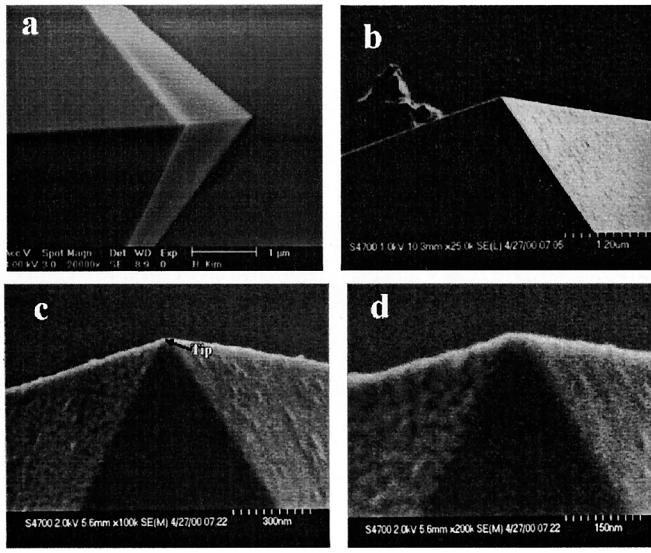

Figure 5. FE-SEM images of AFM contact tip. a: Standard tip, b: G9 functionalized tip, c: High magnification of b, d: High magnification of c.

PAMAM G9 dendrimer molecules were uniformly adsorbed on the tip surface to form very dense uniform packing. This is believed to be caused by an electrostatic adsorption forces between the positive charged amino groups on the PAMAM dendrimer surface and the negative charged Si_3N_4 AFM tip surface. Using the same

G9 modified tip, force distance curves between G9/tip and mica, G9/mica and HOPG surfaces have been performed respectively. Figure 6 shows the force-distance curves between the G9 functionalized tip and the surfaces in air. Each curve represents a minimum of 50 repeat experiments. The results are very reproducible.

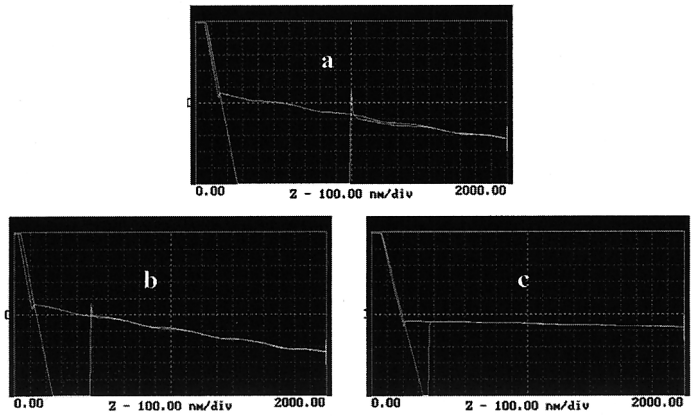

Figure 6. Comparison of force distance curves between the same G9 modified tip and different surfaces. a: Mica surface, b: G9 film on mica surface, c: HOPG

The relative adhesion forces can be calculated from each force distance curve by measuring the distance between the tip pull-on and pull-off and applying Hooke's Law:

$$F = K * \Delta x$$

with K representing the spring constant and Δx the displacement of the tip pull-on to pull-off from the surface. In this case, the average value of K is about 0.12N/m. The calculated adhesion forces are listed in Table 2.

Table 2. Comparison of adhesion forces between the same G9/tip and different surfaces

Surface	Displacement of Piezo (nm)	Adhesion Force (nN)
Mica	850	102.0
G9/mica	353	42.4
HOPG	170	20.4

One can see that the adhesion force between the G9/tip and mica is substantially larger than the adhesion force between the G9/tip and G9/mica. Therefore, G9 dendrimer molecules deposited on a mica surface might be expected to form defined 2-D packing arrays. On the other hand, the adhesion force between the G9/tip and the HOPG surface is substantially smaller than the adhesion force between the G9 dendrimers. Therefore, G9 dendrimer particles appear not to form well-ordered 2-D packing arrays on the HOPG surface. Instead they tend to aggregate into clusters as shown in Figure 3. This may be the result of the hydrophobic property of the HOPG surface. These results suggest that strong adhesion forces between the G9 molecules and substrates play an important role in the development of 2-D packing arrays.

Unlike latex particles, the G9 dendrimer molecular surfaces may be expected not to be smooth and rigid but spatially diffuse and dynamically rough. Therefore, the 2-D array packing on the mica surface might be described as meshing of adjacent dendrimer surfaces much like gears to produce a dendrimer-dendrimer interaction much as shown in Figure 7.

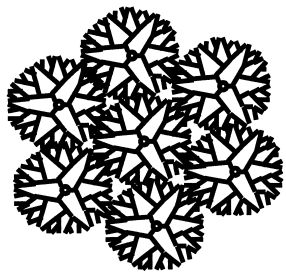

Figure 7. A possible dendrimer packing model.

It was also found that the deposition patterns change if the amount of G9 solution placed on the mica surface is decreased from 6ul to 3ul, as illustrated in Figure 8. It can be seen that the molecules assembled to form an inter-linked chain texture. Some hexagonal patterns can also be observed within these arrays. Very few isolated G9 dendrimer molecules were found even after multiple scans of several areas. These

results indicate that the interface forces between dendrimer molecules dominated the particle pattern. The chemical and physical characteristics of G9 PAMAM dendrimer molecules such as their spherical shape, the high functional surface densities with hydrogen bonding characteristics and the relative rigidity of the dendrimers play very important roles in this interface behavior.

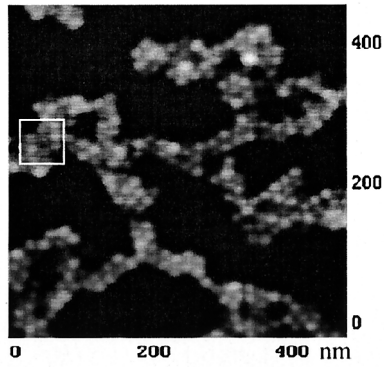

Figure 8. Tapping Mode AFM image of G9 dendrimers on mica surface.

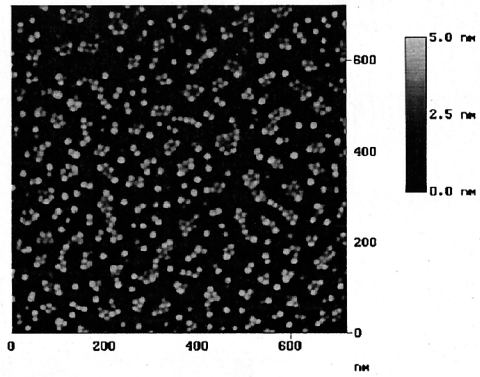

Figure 9. Tapping Mode AFM images of G9 PAMAM dendrimer molecules on mica surface.

Figure 9 shows the striking changes that occur in G9 PAMAM packing when a nitrogen gas stream is allowed to flow at an approximate 35 degree angle to the sample surface, while applying 3ul of G9 solution, $5x10^{-3}$% (w/w) on a freshly cleaved mica

surface at room temperature. A variety of packing assembles, which may be considered to be a G9 PAMAM dendritic packing library formed by this perturbation condition. With this library, one can observe single isolated G9 dendrimer molecules, dimers, trimers and other packing patterns. These patterns may be seen more clearly in the high magnification images shown in Figure 10a. A single G9 is enclosed by the circle, the dimer by the oval and the trimer by the square.

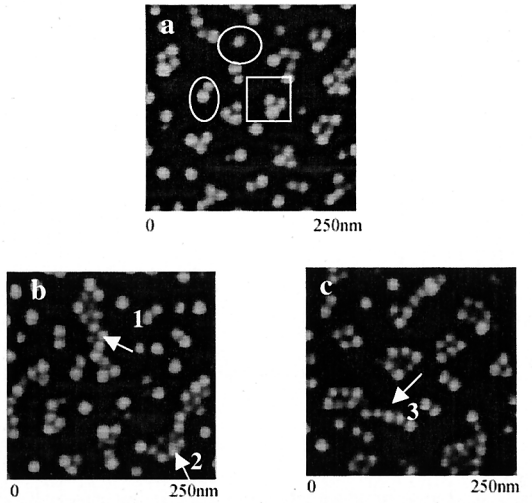

Figure 10. Magnified images from Figure 9.

Another surprise realized from the application of small nitrogen flow forces is that, in addition to the dimer and trimer configurations of G9 molecules in Figure 10a, changes in the shape and size of the G9 dendrimer can also be observed, which are dependent on the G9 particle location in the array. Some of the G9 molecules have the appearance of having been squeezed together, their shape contorted into irregular, asymmetrical forms instead of their characteristic round form, indicated by arrows 1 and 2 in Figure 10b. A large variety of packing assemblies is also present, some resembling curved linear catenations as in Figure 10c arrow 3, and others such as barbell-type dimers, pyramidal trimers, parallelogram-type tetramers, and hexamers.

Size changes in this packing can be measured by AFM line scans; examples are listed in Table 3.

Table 3. Size comparison of the G9 dendrimers after drying on the mica surface

State	Shape	Size (x/y/z)nm
Isolated		25.3/ 25.3 / 3.63
In a row		12. 8 / 24.7 / 4.92
As a center Particle		12.8 / 12.8 / 6.81

One can see that isolated particles have the largest diameter and smaller height value, which suggests they are deformed completely on the mica surface. On the other hand, as a center particle in a 2-D array, the dendrimer molecule shows the smallest diameter and the largest height value, which suggests the surrounding particles provide vertical support for it and reduce the deformation. It is also possible that a certain degree of molecule interpenetration may also occur. The important feature is that a G9 dendrimer will show irregular shape when the particles are in a row. They seem to be squeezed by each other. These results suggest that dendrimers may be soft, spongy and elastic, and that their shape can be changed by the application of very small flow forces.

Conclusion

Assemblies of G9 PAMAM dendrimer molecules have been studied by AFM and observed to form a widely variety of 2-D packing arrays. The architecture of G9 dendrimers, the interactive forces between the dendrimers, and the interaction force between the dendrimers and the substrate are believed to be the major influences on 2-

D array dendrimer film formations. In addition, a large variety of packing assemblies is also present, dependent on sample preparation. These unique nano-size packing libraries of G9 dendrimers may have potential applications as energetic storage medium, antibody mimic and nano-device designs.

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