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Directional Movement of Dendritic Macromolecules on Gradient Surfaces

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ABSTRACT

A gradient-driven methodology has been developed to manipulate the movement of dendritic macromolecules. Poly(propyleneimine) dendrimers, labeled with rhodamine B, are attached to glass substrates via multiple imine bonds. The dendrimers are able to move on the surface by the hydrolysis and re-formation of these imine bonds. In the absence of an external stimulus, this random movement results in a two-dimensional diffusion on the substrate. We are able to bias the movement of these nanoparticles by means of an aldehyde gradient on the glass substrate.

Chemotaxis,[1] the response of cells to chemical gradients by directed movement, is a universal phenomenon that is vital to the survival of both microorganisms and multicellular organisms. For example, bacteria are able to sense changes in concentrations of certain chemicals and move toward or away from this chemical by means of altering its tumbling frequency.[2] Likewise, multicellular organisms are able to fight off bacterial infections using white blood cells (neutrophils) that are able to sense chemicals left by bacteria to find and destroy the invading microorganisms. Hence, directional sensing and response play a central role in health and diseases.[3] Fundamentally, the same principles should apply for particles of any size that are able to sense and respond to a gradient, although the mechanism may be completely different.[4] In this Letter, we present the directed movement of dendritic macromolecules on glass substrates.

Dendrimers have been used in a variety of applications; in this case it is the multivalent, globular nature and their ability to deform their shape that is essential for gradient sensing. For our purposes, the dendrimers need to be attached to the substrate, yet have some freedom to move about on the surface, to "sense" and respond to their environment. This freedom requires that their attachment to the surfaces be reversible, making use of the dynamic covalent chemistry,[5] to allow the dendrimer to move across the substrate.

An amine-terminated dendrimer will attach to a surface containing aldehyde groups by imine condensation. One or more of these imine bonds can hydrolyze,[6] giving the dendrimer additional freedom to move about and attach itself via imine formation with another aldehyde on the surface. The net result is random motion on the surface, resembling a random walk. In the presence of a gradient, it is more likely for the dendrimer to move in one direction (with the gradient) as it is statistically more likely to form imine bonds where more aldehyde groups are present.

The number of imine bonds formed between the two will depend on the availability and proximity of amine and aldehyde groups. On a fifth generation dendrimer there are 64 amine groups, some (approximately 8) of which are used to attach rhodamine labels, so it is possible to follow by fluorescence. Although dendrimers do deform on surfaces, flattening to maximize favorable interactions, not all of the amine groups are accessible at the same time for attachment to the surface; hence when the dendrimer moves around, those free amine groups are free to react with aldehyde groups on the substrate.

Reactive aldehyde substrates were prepared by reacting glass slides with trimethoxysilylalkylaldehyde. *Supplier: Fluorochem UK.* The aldehyde gradients are achieved by a modified reverse dip-coating procedure,[7] whereas the control substrates were prepared by full immersion of the substrate for a specified amount of time. The dendrimers are labeled with rhodamine B by reacting the fifth generation poly-propyleneimine dendrimer with 8 equiv of rhodamine B isothiocyanate. The resulting labeled dendrimers were applied onto the substrate by microcontact printing.[8] The printed substrates[9] were fully immersed[10] in water overnight and then analyzed using confocal microscopy.

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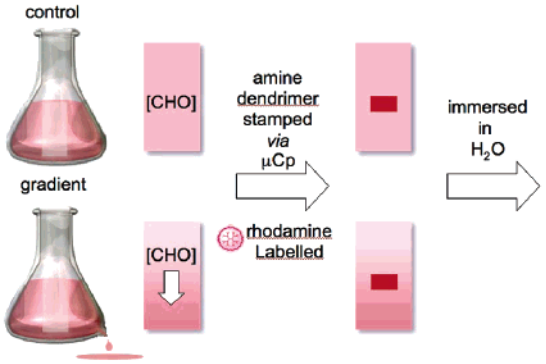

Figure 1. Substrate preparation by modified dip coating and printing.

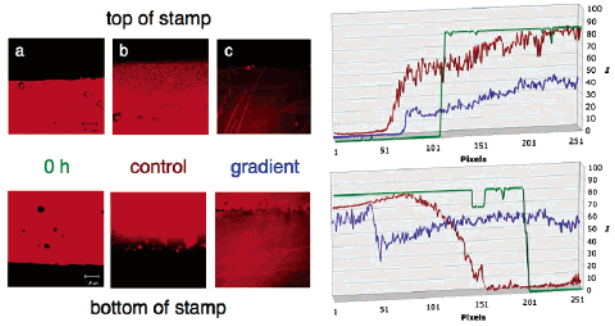

Figure 2. Confocal microscopy images of the top and bottom edges of stamped dendrimer regions (a) before immersion on a uniform aldehyde substrate, (b) after 16 h immersion in water, and (c) after 16 h immersion on a 15 min gradient substrate. The graphs on the right show the averaged fluorescence intensity profile[11] of each picture from top to bottom ((a) green, (b) red, (c) blue).

The confocal microscope images of the top and bottom of a rectangular pattern of dendrimers printed on a glass substrate before and after overnight immersion in water are shown in Figure 2.

Before immersion, the image is sharp as can be observed by the edges of the pattern as well as defects in the stamp. This can be seen clearly in the fluorescence intensity graphs, where sharp slopes can be seen at the edges of the stamp. After immersion, the patterns are blurred as a consequence of diffusion of the dendrimers. In the absence of a gradient, we see that the edges are no longer clear, there is migration of dendrimer away from where they were originallly stamped, and the amount of migration is similar on all edges. The fluorescence intensity is lower after immersion which can be attributed to loss of dendrimers either that were attached to other dendrimers or that were weakly bound. Dendrimers on the gradient substrate, however, appear to move with the gradient. On the top of the stamp, the edge remains sharp, whereas at the bottom we see a large amount of fluorescence past the edge of the stamp.

To observe the behavior of dendrimers on surfaces of different aldehyde concentrations, we also printed smaller patterns using these fluorescent-labeled dendrimers. Figure 3 shows one such example where circles of approximately100 μm in diameter are printed. The pattern is less defined after immersion, the circles are less intense in fluorescence, and fluorescence can be detected between the circles. The

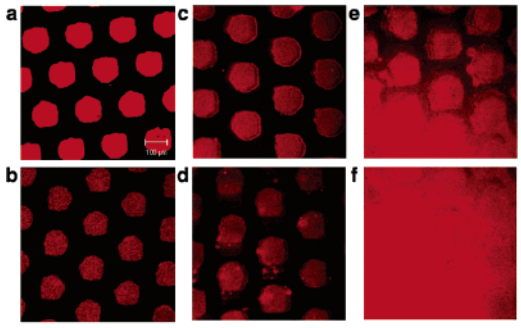

Figure 3. Confocal microscope images of dendrimers stamped using microcontact printing with circles of ~100 μm in diameter: (a) before immersion; (b) a uniform aldehyde substrate; (c−f) gradient substrates with increasing aldehyde concentrations, after 16 h of immersion in water.

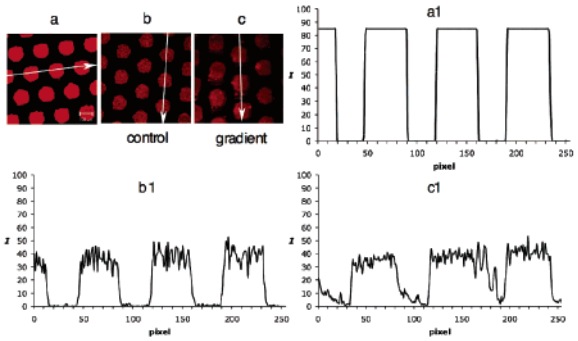

Figure 4. Averaged fluorescence profiles and their corresponding confocal microscope images of dendrimers (a) before immersion and (b) control and (c) gradient substrates, after 16 h of immersion.

behavior of the dendrimers is very different and is dependent upon the local aldehyde concentration of the gradient. Toward the top of the stamp with the least aldehyde density, depicted in Figure 3c, there is minimal diffusion of the dendrimers from their starting positions. Further down the gradient (Figure 3d) the movement of dendrimer primarily along the gradient was observed, resulting in an elongation of the pattern. With higher aldehyde concentration, the pattern becomes less defined and diffusion appears to be faster at these concentrations (Figure 3e,f).

We see accumulation of dendrimers at higher aldehyde concentration and observe an almost even distribution[12] of dendrimer in the region of highest local aldehyde concentration.

The fluorescence intensity profiles (Figure 4) show the preference of dendrimers to migrate toward higher aldehyde concentrations. The edges of the patterns are sharp (Figure 4a) before immersion and become less defined after immersion for either the control (Figure 4b) or gradient (Figure 4c) substrates. In the absence of a gradient (Figure 4b) there is some migration in all directions as seen by the slightly shallower slope. With a gradient, the dendrimers exhibit directional preference as indicated by the steep slope on the edges against the gradient and a more gradual change[13] in fluorescence intensity when the dendrimers are moving with the gradient.

From this series of images, it appears that the diffusion rate rises with increasing aldehyde concentrations. This may

initially seem unexpected since a smaller number of attachments to the surface should allow for greater dendrimer mobility. At "low" aldehyde concentration, the dendrimer does have a larger degree of freedom but there are fewer aldehyde groups available around the dendrimer to allow movement. In addition, once an imine bond is hydrolyzed, there is a relatively high local amine concentration (since few imine bonds are formed, there are many free amine groups) and an imine bond can form between the same aldehyde with another amine on the same dendrimer. The converse should be true for very high aldehyde concentrations. In this case, when an imine bond is hydrolyzed, the free amine can react with another nearby aldehyde group. At this aldehyde concentration, there are probably so many imine bonds between the dendrimer and the surface that there is limited dendrimer movement. Hence, biased migration can only be observed with a certain aldehyde concentration/gradient range. The size and number of available amines at the periphery of the dendrimer will determine this range. Detailed molecular parameters are needed to fully understand the dynamics of this directional movement at the molecular scale.

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(6) The reversible formation and hydrolysis of imines in self-assembled monolayers is described in: Rozkiewicz, D. I.; Ravoo, B. J.; Reinhoudt, D. N. *Langmuir* **2005**, *21*, 6337−6343.
(7) The substrates were immersed vertically in an Erlenmeyer flask containing a solution of trimethoxysilylalkylaldehyde in hexanes. The reagent is removed from the flask via a drip spout; hence the top of the substrate will have the least reaction time and the bottom of the slide the most reaction time, to form the gradient. The steepness of the gradient will depend on the rate of reactant removal. The slower the solution is drained, the steeper the gradient, since the top and bottom of the substrate will have a larger difference in reaction time.
(8) Microcontact printing was carried out using conventional PDMS stamps. The ink solution was 1 mM dendrimer in ethanol, and the printing time was 1 min.
(9) Another control experiment was done with an amine containing Lucifer Yellow dye (containing only one amine) directly printed onto a gradient substrate and then immersed in water. No movement of the dye was observed.
(10) The angle that the substrate is placed at while immersed in water does not appear to make any difference in the direction or amount of diffusion. All the images presented in this Letter are immersed horizontally.
(11) The graphs were calculated by taking the average fluorescence of six lines in the images from the top to the bottom of the images. The maximum intensity is 255 for white light; since only red light is emitted, the relative maximum is 85. Defects in the stamp and/or the substrate are also taken into account as evidenced by regions of reduced fluorescence.
(12) The original circular patterns can probably only be seen in Figure 3f because of deformations caused by the PDMS stamp, as fluorescence is higher outside of the circles.
(13) We do not see a large change in the movement of dendrimers in the middle of the printed spots since, presumably, most of the aldehydes will be reacted where we print the dendrimers. The lack of free aldehydes will result in limited mobility of dendrimers since those dendrimers will not "sense" a gradient. Dendrimer movement will therefore occur mostly at the edges of the printed areas and the fluorescence intensity between the printed spots.

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