Polysaccharide based Nanoparticles and Nanoporous matrices

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ABSTRACT

In this paper we present an Atomic Force Microscopy (AFM) study of a biopolymer which is capable of forming different phases. We observed a wide range of phases varying from condensed polymer strands, forming networks on a support, to nano particles of different size distributions to porous matrices with a flat surface. The formation of these different phases is depending on the concentration of the biopolymer. At high concentrations it is self organizing into a regular gel like matrix, whereas at lower concentrations nano particles are being the dominant structures together with a network of condensed self organized strands, covering the support. Due to its unusual properties and its high water retention this material has a broad application potential both in pharmaceutical and cosmetic industry as well as in the field of tissue engineering.

Keywords: Biopolymer, Polysaccharide gel, Atomic force microscopy, surface coating, Biocompatibility

1 INTRODUCTION

One of the major research areas in nanotechnology is the production of novel nanometer sized particles. Apart from the inorganic nano crystalline material, such as gold or silver nano particles, nano particles based on biopolymer material are gaining more and more interest. The research in this area has been spurred in the beginning mainly by the cosmetic industry, whose interest was to find novel material with high water retention for their skin care products, but the focus has shifted now also towards the pharmaceutical industry. There the focus is on novel delivery systems. The application of biopolymers in this field has many advantages, since these polymers are found virtually in any organism, their application to organisms is safe. They do not exhibit toxic effects as for example is the case for the quantum dot materials, which have to be masked to make them more biocompatible and if the masking material is stripped off during the transport to or into the cell, they are toxic again. Nano particles based on biopolymers can be stabilized by chemical cross linking of the single particles and various strategies for their functionalization can be employed, such as inclusion of drugs or therapeutic proteins inside the nano particles or surface derivatisation of these nano particles.

Polysaccharide material is found throughout all living organisms in nature. Every organism is using one or another form of polysaccharides for energy storage, as cell stabilizing environment surrounding the cells, or as scaffold material, e.g. cartilage, or in skin. These polysaccharides when used as carriers for drugs, or in the form of functionalized nano particles can be easily incorporated into organisms and easily metabolized when necessary. Polysaccharide material has a huge advantage over metallic nano particles because of the low antigenicity of the material. Therefore it is very unlikely that people will develop allergic reactions or trigger immune responses upon incorporation of biopolymer based nano structured material. These biopolymers have found their first applications in the cosmetics industry because of their water retention properties, and derivatized polysaccharides based on starch have found a huge market in the food industry. The food industry has just recently begun to recognize the possibilities of nano structured material in their applications and have coined a totally new field within this area of research called nano food. Here also nano structured food additives which have to be safe for human consumption will find their applications. Among the first candidates for applications will be biopolymers, because of their properties mentioned earlier in this section.

Polysaccharides due to their water solubility in contrast to the metallic nano particles are able to dissolve again in aqueous environments, therefore the polysaccharides have to be either chemically modified to increase their hydrophobicity, which will influence greatly their water retention capabilities, which in many cases will be disadvantageous, or they have to be chemically cross linked to stabilize their structure in solution, which can be achieved by cross linking with glutaraldehyde, or other suitable chemical modifiers. Polysaccharides naturally have a tendency to self organize themselves in aqueous environments because of their biphasic structure with a hydrophilic and a hydrophobic site. The hydrophobic side of the polysaccharide has a tendency to self aggregate with other polysaccharides chains to expose the least hydrophobic surface towards the solvent environment. This has been seen for many polysaccharides when they undergo chain condensation [1]. During this process single chains intertwine and coil up to minimize their hydrophobic surface. The resulting hydrophilic surface is then in contact with the solvent.

In this paper we present an Atomic force microscopy investigation of the aggregated phase of polysaccharide based biopolymers and their different stages of aggregation which are depending upon the biopolymer concentration in

solution. These different aggregation stages can be exploited in a wide variety of applications.

2 MATERIALS AND METHODS

The biopolymer has been dissolved in a sodium phosphate buffer solution, containing 100mM sodium phosphate at pH 7.5. The Biopolymer has been dissolved at room temperature with constant shaking at 150 rpm on a shaking table for at least 3 hours. In between the solution has been vigorously mixed on a vortex to disrupt larger Polymer particles and allow for a homogenous solution to establish. The Polysaccharide used in this study was Hyaluronic acid from *B. subtilis* with an average MW of 600.000 kDa. The concentrations used here have been 10mg/ml and 100µg/ml.

The Atomic force measurements have been performed on a Veeco Multimode atomic force microscope with a Nanoscope IIIa controller and a Quadrex extension. The samples have been prepared on cut silicium wafer plates, which have been cleaned with ethanol in an Ultrasound bath for 20 minutes and dried under a stream of nitrogen. 5µl of sample has been placed on the support and after 5 minutes of incubation the liquid has been rinsed off the support with deionized water and the wafer has been dried under a stream of nitrogen. The support has been mounted on a metal sample holder for imaging in the microscope. The cantilevers used for imaging have been ultrasharp diamond-like carbon (DLC) tips NSG01_DLC (NTMDT) with a resonance frequency of 150 kHz and a force constant of 5 nN. The images have been obtained in tapping mode in air.

3 RESULTS

Hyaluronic acid is a Biopolymer with a very high solubility in water and also possesses strong water retention capabilities. Upon dissolution in a buffer solution the Hyaluronic acid displays swelling and in the presence of bulk water, begins to dissolve. This dissolution of Hyaluronic acid has been done at room temperature under gentle shaking. Over a period of 3 hours a highly viscous but clear solution is obtained. This high viscosity of the solution can be attributed to the formation of a gel like Hyaluronic acid matrix being formed in the solution. This gel like matrix behaviour which has been observed qualitatively, has been verified by AFM imaging and is shown in figure 1. The AFM scan of a region of 6.9x6.9 µm revealed a flat but nor featureless surface of the Hyaluronic acid matrix.

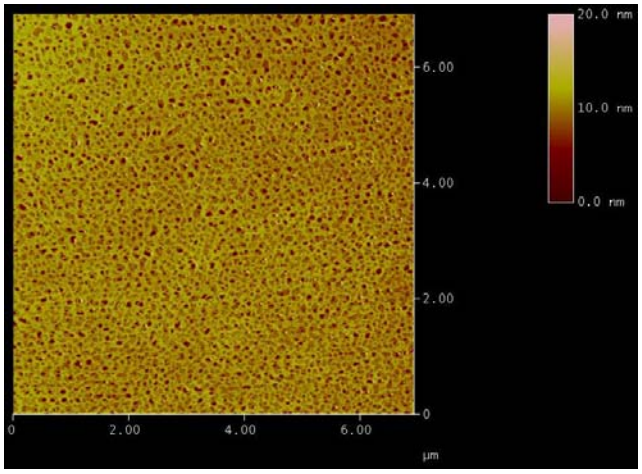

Fig 1: Height image of a 6.9x6.9 µm scan of a 10mg/ml Hyaluronic acid solution deposited on a Silicium wafer.

The surface was porous with deep pores into the matrix as can be seen in a zoomed image of the same matrix shown in figure 2. The pore depth for some of the pores is deeper then 20nm. The thickness of the gel matrix on the support is exceeding the penetration depth of the cantilever into the pores and the support could never be reached by the cantilever. As can be seen in figure 2, the pores are formed by a network of condensed strands of Hyaluronic acid overlaid in a gel like fashion forming the porous structure.

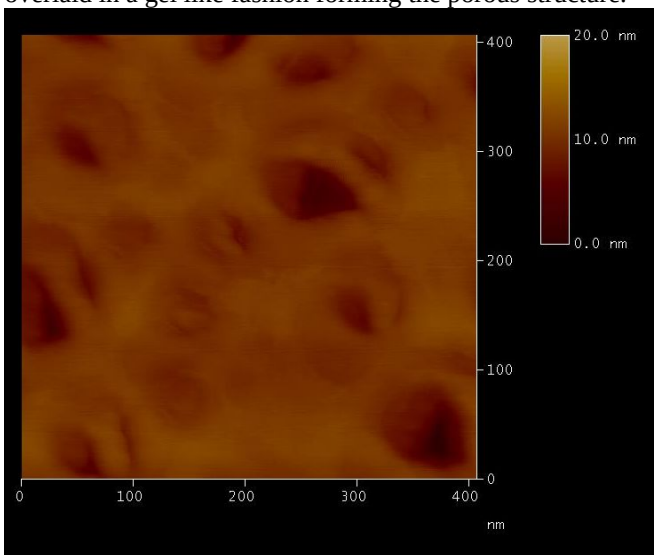

Fig 2: Height image of a 407 x 407 nm scan area taken on the same sample as shown in Fig1.

Upon dilution of the 10mg/ml gel like solution down to a concentration of 100µg/ml, the gel like matrix structure did not dissolve, but due to the further swelling of the gel like matrix which has been faster than the dissolution of the preformed porous gel, the matrix ruptured and resulted in fragments of the condensed strands forming the gel. The result of such a dilution experiment is shown in figure 3.

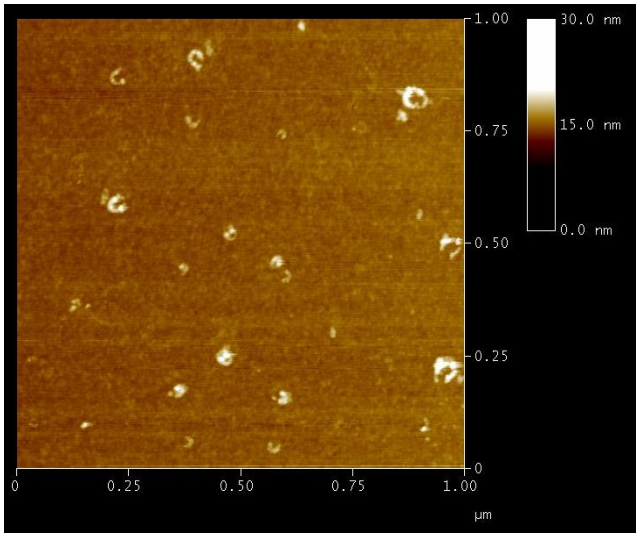

Fig 3: Height image of a 1 x 1 µm scan of a diluted 10mg/ml Hyaluronic acid gel solution deposited on a silicium wafer.

A more detailed image of such a donut-shaped fragment is represented in figure 4. In figure 4 it can be seen that the ruptured fragments of the gel matrix are composed of condensed intertwined strands of Hyaluronic acid. Two fragments of different degrees of strand condensation can be clearly seen in this image.

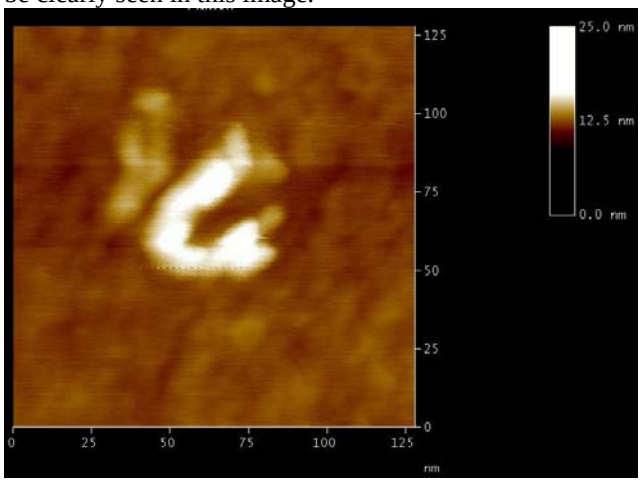

Fig 4: Height image of a 130x130nm scan area of the sample shown in Fig3.

In order to verify the structures seen in the AFM images of the of the 100µg/ml diluted solution a fresh Hyaluronic acid solution of 100µg/ml has been prepared and imaged on the AFM microscope. These images revealed that a solution of Hyaluronic acid at a concentration of 100µg/ml in the same buffer solution as the 10mg/ml solution forms particles of varying sizes in solution. Figure 5 represents a 5x5 µm scan area of such a nano particle containing solution with a broad particle size distribution. The wide size distribution can be more clearly seen from the the 2.5 x2.5 µm scan of a different area of the same preparation as is shown in figure 6.

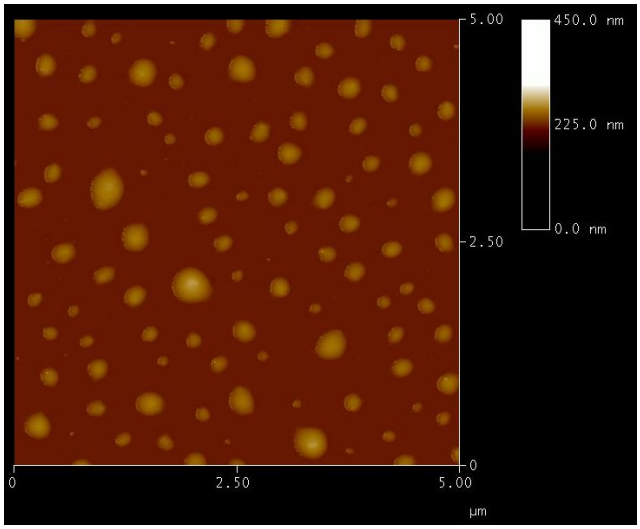

Fig 5: Height image of a 5x5µm scan of a 100µg/ml Hyaluronic acid solution deposited on a Silicium wafer plate as support.

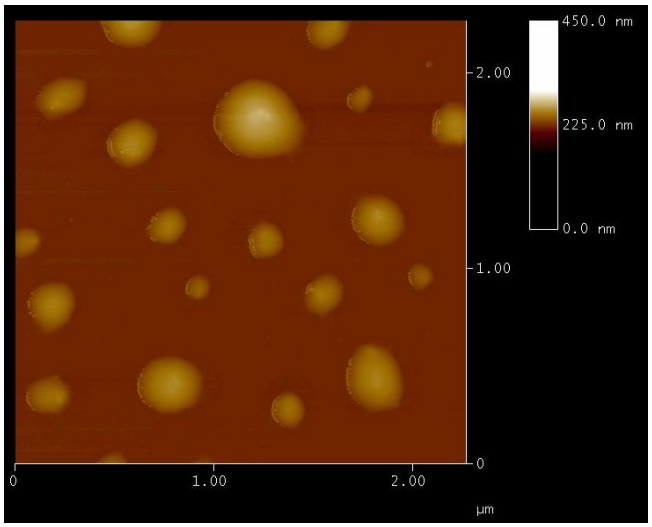

Fig 6: Height image of a 2.5x2.5 µm scan area of a 10µg/ml Hyaluronic acid solution.

Figure 7 reveals the underlying architecture of the nano particles formed in the 100µg/ml solution of Hyaluronic acid. The 158 x 158nm scan shows clearly that in this solution not all of the polymer is found as nano particles but the polymer is coating the substrate in a network like fashion being composed of condensed strands of Hyaluronic acid. This mesh of condensed strands forms the scaffold onto which the remaining larger particles deposit. At the base of these particles smaller particles associate with the larger particles and are being incorporated into these larger structures. Figure 8 represents a 3D view of the same scanning area as is depicted in Figure 7.

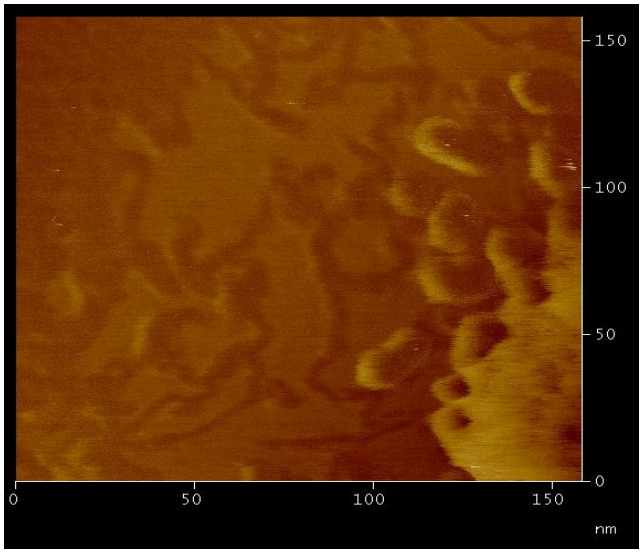

Fig 7: Detail zoom af a 158 x 158 nm area of the image shown in Fig6.

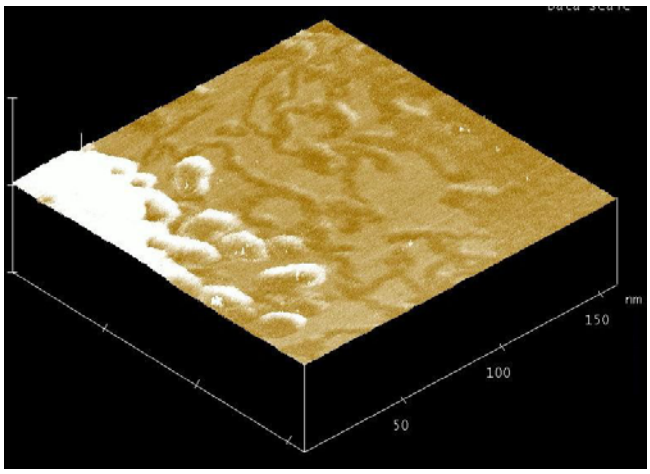

Fig 8: 3D view of the 158x158 nm image of a 100µg/ml Hyaluronic acid solution deposited on a Silicium wafer shown in Fig 7.

4 DISCUSSION

Hyaluronic acid of a MW of 600.000kDa has the ability to organize itself into a gel-like matrix structure with varying pore sizes. The hydrogel-like matrix, when deposited onto a flat support, displays a flat surface with open pores in its surface. The pores display varying sizes and depths. The gel forms macroscopically as can be seen by eye at room temperature in concentrated solutions (10mg/ml) (fig.1 and 2). Upon dilution of such a stabilized hydrogel the gel is swelling. The incorporation of additional water molecules into the gel matrix leads to a swelling of the preformed gel matrix. But since the dilution from 10mg/ml to a final concentration of 100µg/ml was too dilute for the gel to retain its structure and only absorbing the additional water molecules, the gel matrix ruptured leading to fragments of condensed Hyaluronic acid strands in this solution. These condensed rupture fragments did not further dissolve in the solution and have been found as donut like fragments when imaging this solution (fig 3).

This formation of condensed short strands was only a result of the gel rupture as could be verified by the imaging of a dilute solution of 100µg/ml obtained by the dissolution of dry material. This equilibrated dilute solution of Hyaluronic acid revealed the concentration dependent formation of nano particles in solution in addition to the formation of condensed strands of Hyaluronic acid, forming an underlying network on the support upon which the the larger particles are attached. The particles formed in this solution are not of uniform size. The wide size distribution seen in figure 5 and 6 can be attributed to the fact that Hyaluronic acid is very well soluble in water and that the formation of a more uniform particle distribution at this concentration could be achieved by much longer equilibration times of the solution, since the formation of the particles is concentration dependent and the dissolution of Hyaluronic acid form a particle is a very slow process.

The underlying Hyaluronic acid network as seen in figures 7 and 8 reveal that at this concentration not all of the Hyaluronic acid is incorporated into particles and that a prerequisite of particle formation is the condensation of single Hyaluronic acid strands. Only when the condensation of the strands reaches a certain thickness the strands start to coil up into smaller particles which then reorganize themselves into larger structures. These larger structures attach to the underlying network of Hyaluronic acid strands covering the support and one can speculate that the larger particles are being kept together by a network of such condensed polymer strands.

5 CONCLUSION

In this paper we present the concentration dependent formation of a gel matrix and the formation of nano particles from the same sized biopolymer. The formation of these different structures is solely dependent of the concentration of the biopolymer. Once the gel-matrix has been established and stabilized, the interconversion into the more dilute equilibrium structure is a slow process and fast a one step dilution process will lead to rupture of the polymer strands.

The ability of Hyaluronic acid to form large variety of structures depending on concentration makes it an amazing material suitable for applications ranging from pharmaceutical drug delivery systems to novel supports for cells being cultivated at surfaces with a built-in delivery system for hormones and nutritional substances.

6 REFERENCES

[1]Jacoboni I, Valdre U, Mori G, Quaglino, Jr D and Pasquali-Ronchetti I Journal of Structural Biology, Vol 126, Issue 1, p 52-58, 1999.

7 ACKNOWLEDGMENTS

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