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Long-term Structural Change in Plasmid DNA

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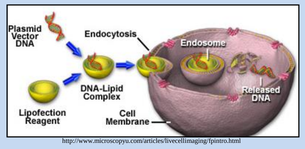
Abstract

Long-term stability of plasmid DNA (pDNA) conformations is critical in many research areas, especially those concerning future gene therapy. Despite its importance, the time-evolution of pDNA structures has rarely been studied at a molecular resolution. Here, the time-evolution of pDNA solutions spanning four years was observed with atomic force microscopy (AFM). The AFM data show that the pDNA molecules evolved from isolated supercoiled structures; to aggregated supercoiled structures; to thin, branched network structures; and finally to wider, branched network structures. Additional topographical analysis of the AFM data suggests the actions of residual proteins could be the main mechanism for the structural changes in our laboratory prepared pDNA.

Introduction

In general, studies using pDNA are performed with little attention paid to its long-term changes in overall structure. However, many studies have found that pDNA structure can be altered by a variety of chemical interactions with its environment, including interactions with enzymes, ions, and drugs. Since stable pDNA structures are necessary for many aspects of biomedical and pharmaceutical research (Figure 1), these chemical interactions could raise a significant barrier to the success of studies using pDNA.

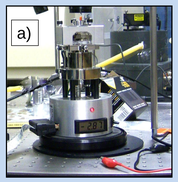
Figure 1: Plasmid DNA is valuable in studies requiring the transfection of a specific gene into the nuclei of cells (depicted here). Much of the research that makes use of the transfection process, such as gene therapy research, require stable pDNA structures for successful transfection.

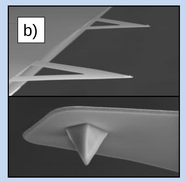


http://www.microscopyu.com/articles/livecellimaging/lpintro.html

The mechanisms of pDNA structural change have been studied using various methods. These methods have limitations in obtaining data on the structural changes occurring at the single molecular level. In contrast, AFM provides single molecular level topographical information about the pDNA, and it accurately and precisely allows direct observation of the molecular structures of pDNA (Figure 2). Despite such high resolution imaging capability of the AFM (Figure 3), there have been few direct observational studies of pDNA long-term structural change using this instrument. In our study, we investigated the structural changes of pDNA over time through AFM to understand the long-term stability of pDNA prepared in a laboratory.

Figure 2: (a). The Nanoscope II AFM used in this study. (b). The silicon-nitride cantilevers and tips used in this AFM study.





http://www.brukerafmprobes.com/index.aspx

Material and Methods

The structures of the pDNA molecules were analyzed using XPM Pro and Kaleidagraph (Figure 4). The dimensions of the pDNA structures were determined through applications of the Pythagorean Theorem to Figures 5 and 6.

Figure 3: Diagram of AFM

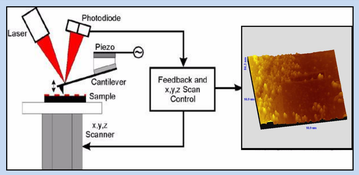


Figure 4: Analysis of AFM data

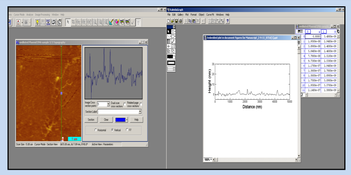
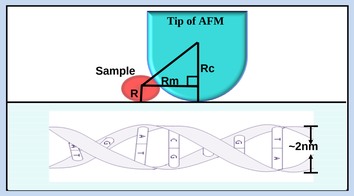
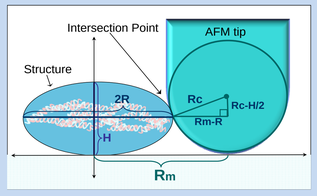


Figure 5: A very large tip can affect the AFM image resolution



$$R_m = 2\sqrt{R \cdot R_c}$$

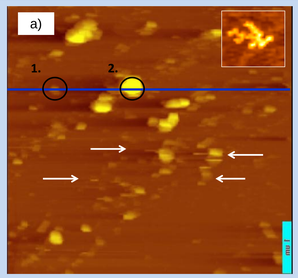
Figure 6: Determining volume of the ellipsoidal structures through the Pythagorean Theorem



$$R_m = R + \sqrt{R_c^2 - (R_c - \frac{H}{2})^2}$$

Results

Figure 7: (a) Contact-mode AFM image (99 days). (b) Sectional profile of (a)



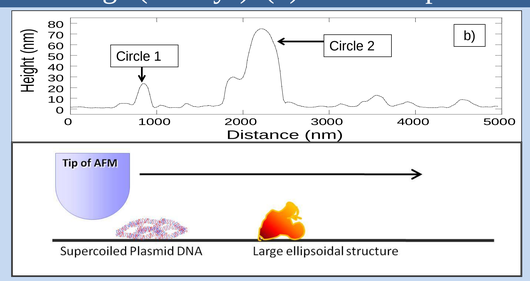
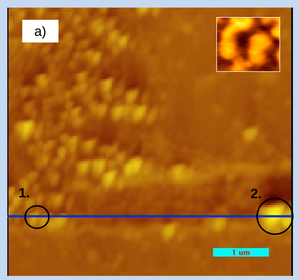


Figure 8: (a) Contact-mode AFM image (110days). (b) Sectional profile of (a)



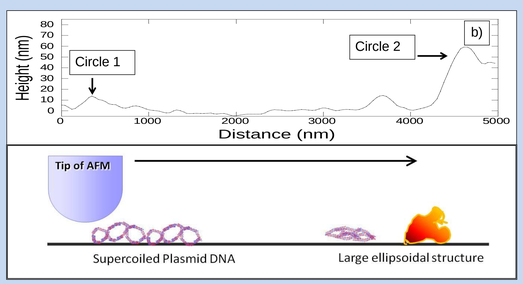
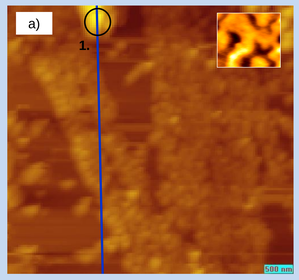


Figure 9: (a) Contact-mode AFM image (110days). (b) Sectional profile of (a)



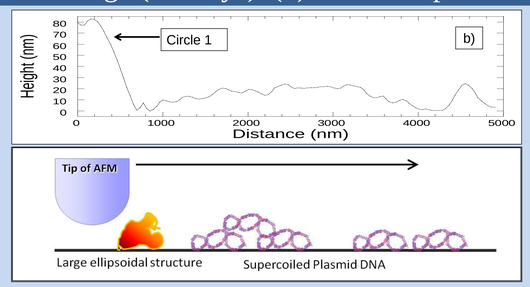
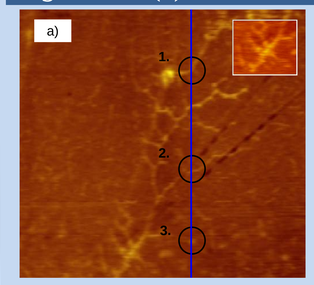


Figure 10: (a) Contact-mode AFM image (130days). (b) Sectional profile of (a)



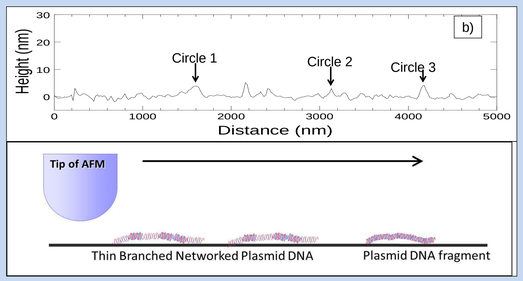
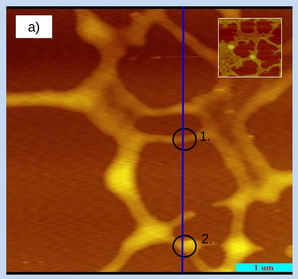
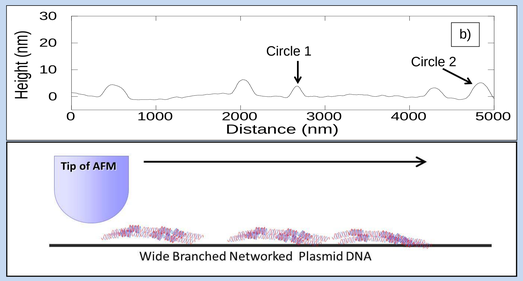


Figure 11: (a) Contact-mode AFM image (4 years). (b) Sectional profile of (a)

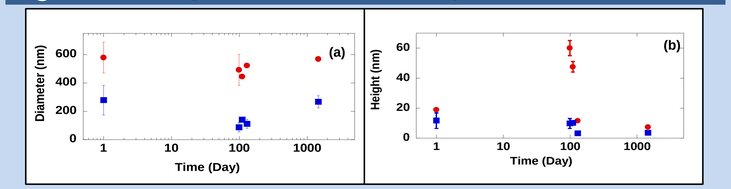




Discussion

The changes in diameter and in height are plotted as a function of time for both larger ellipsoidal structures (red) and smaller ellipsoidal structures (blue), as shown in Figures 12a and 12 b, respectively. The smaller ellipsoidal structures had dimensions similar to pDNA, whereas the larger ones had dimensions too large to be considered pDNA. It is possible that the larger structures were residual proteins in the pDNA solution.

Figure 12: Changes in (a) Diameter and (b) Height over Time

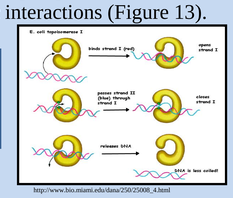


The pDNA structures in the AFM images of the pDNA solutions transformed over time from isolated supercoiled structures (Figure 7); to aggregated supercoiled structures (Figure 8 and 9); to thin, branched network structures (Figure 10); and finally to wider, branched network structures (Figure 11). An illustration of this structural change is shown in Figure 13.

Figure 13: Observed Long-term Structural Change in Plasmid DNA

It is possible that the actions of residual proteins were responsible for the pDNA structural changes. Since the pDNA structural changes observed would continuously require the breakdown and rejoining of the pDNA molecules, the possible residual proteins may have been enzymes related to these processes (Figure 14). The exposure of the modified pDNA molecules, such as the non-polar groups, to water may be the driving force of the aggregations through hydrophobic interactions (Figure 13).

Figure 14: E. Coli topoisomerase is a possible example of a residual protein that could be in the pDNA solution. Topoisomerase's main function is to change pDNA topology in the E.Coli, which could form the pDNA structures seen in the AFM images.



http://www.bio.miami.edu/dana/250/25008_4.html

Conclusions

We observed the structural evolution of laboratory-prepared pDNA, using AFM. The images show that the pDNA molecules evolved from isolated supercoiled structures to wider network structures over a four-year time frame. Additional topographical analysis of the images suggests possible involvement of residual proteins in the modification processes (such as bonding and rupturing) of the pDNA structures during the observed pDNA structural changes. The observed structural changes of the pDNA molecules over time and the proposed mechanism for these changes will contribute to the better understanding of the stability of pDNA, which is of great interest in many progressing fields of research, such as gene therapy.

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