Thermal Imaging of Bacterial Cells

AFM Studies Using Heated Nanoprobes

Since its invention Atomic Force Microscopy has established itself as a versatile tool for imaging material systems & associated dynamic processes. In the early 90s the technique was further developed to incorporate the use of heated probes as means of scanning the sample surface. In this study E.coli bacterial cells were used in an attempt to not only further develop and optimize the use of thermal AFM, but also to try and gain further insight into their interactions with pharmaceutical materials.

Introduction

Atomic Force Microscopy (AFM), which is based on the use of a tip or a probe that interacts with the specimen surface, creates images by "feeling" rather than "looking" at samples, much like one would read Braille [1]. This novel imaging method results in magnification range spanning that associated with both the Optical and the Electron Microscopes. Since its invention in 1986 by Binning et al. [2] the AFM has established itself as a versatile instrument for imaging material systems and associated dynamic processes not only in real time, but also under natural conditions [3]. In the early 90s the technique was further developed to incorporate the use of heated probes as means of scanning the sample surface. Pylkki et al. (1994) used a probe made of Wollaston wire, whereby the tip could be used not only as a thermometer, but also as a heat source. In this way it was possible to perform not only Scanning Thermal Microscopy, but also Localized Thermal Analysis [4]. This experimental methodology was further developed incorporating it with Modulated Thermal Differential Scanning Calorimetry and the newly invented instrument was commercially launched in 1998 by TA Instruments as a Micro Thermal Analyzer [5].

Undoubtedly, in the world of bacteria, the spotlight for biologists has for a long time been Escherichia coli (E.coli). The bacteria are a normal part of the gut flora and are commonly found in the lower intestines of animals and humans. It can be easily grown in a simple nutrient broth (e.g. Luria Broth) in a culture bottle. Certain strains of E.coli are toxigenic and produce a toxin very similar to that seen in dysentery, which can cause food-poisoning usually associated with eating
contaminated meat.

The severity of the illness varies considerably; but can be fatal [6]. In this research study E.coli bacterial cells were used as a model biological system in an attempt to not only further develop and optimize the use of the above described Atomic Force Microscopy techniques, but also to try and provide further insight into the inner structure and interactions of the biological system with pharmaceutically relevant materials.

Materials and Methods

E.coli bacterial cells were grown from overnight cultures with mild shaking in Luria broth medium to stationary growth phase. These were then harvested by centrifugation and then washed with phosphate-buffer saline. 50 μL of the cell suspension was placed on a glass slide coated with poly-L-lysine solution followed by incubation, rinsing and drying. The treatment of the E.coli cells was done by using Ampicillin (concentration of 100 mg/ml). Approximately 1 ml from the freshly prepared solution was then simply poured over the clearly seen colony of bacterial cells. Specimens from the cells treated with antibiotics were examined after different periods of time. Imaging was carried out using a Veeco diCaliber AFM equipped with conventional Wollaston wire (fig.1) and novel nano-probes, linked to a Nano-TA controller.

Results and Discussion

It was found that the sample preparation technique, based on the use of poly-L-lysine, gave reproducible results and presented the opportunity to generate images of excellent quality in the AFM modes used. The images obtained in thermal mode using the classical Wollaston wire probes (fig. 2a) although informative, appeared quite noisy and difficult to interpret, when compared to those obtained using the nano-probes (fig. 2b).
This was attributed to the shape and size of the tip, which is directly related to the spatial resolution that can be achieved. Moreover, it was found that image quality of the samples obtained at higher temperatures was better than the ones obtained at lower. The origins of this effect are yet to be fully understood, but are believed to be linked to the way the tip operates at elevated temperatures. The heated nano-probes were also used for imaging of bacterial cells treated with antibiotics, in our case Ampicillin. Examples from the images obtained from these are presented in figure 3. It was found that after 12 hours of treatment the degradation of the cell wall and membrane had progressed to such an advanced stage so that it became difficult to distinguish individual cells. The actual process of degradation is thought to be associated to the production of nanopores and further leakage from the inside of the cell wall. In the example provided, this is further confirmed by the presence of detached flagellum of the *E.coli* cells.

**Conclusions**

AFM in combination with Micro/Nano-Thermal Analysis has been shown to be a unique set of experimental techniques that can be used not only for high-resolution imaging, but also to simultaneously study interactions between different material systems. These techniques have been successfully applied in the study of the interactions of bacterial cells (*E.coli*) with pharmaceutically relevant materials (Ampicillin). It is believed that the results discussed here represent the first attempt to utilise the above described techniques in the study of bacterial cells.

**References**


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