Atomic Force Microscopy – Scanning Electron Microscopy: comparative evaluation on solid surfaces

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Abstract

This work aims to characterize 4 groups of samples through SEM and AFM in order to show how these 2 techniques are complementary. The group 1 consists of polypropylene spheres used in down flow anaerobic fluidized bed reactor. Group 2, polypropylene and silicone lenses that were immersed in specific broth. Group 3, metallic samples formerly used in electrochemical process in a media of organic compounds of complex chemical structure. Finally, group 4 consists of Brazilian clay also formerly submitted to a copper extraction. Both techniques have shown that they are excellent tools to observe differences in bacteria morphology with or without biofilm.

Keywords: AFM, bacteria, stainless steel, clays, anaerobic reactor.

Introduction

Spectroscopic techniques as Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) are effective tools to evaluate the effect of the environment in any solid surfaces. Furthermore, if they are used separately some information can be lost. They are helpful to characterize different materials, for instance, biofilm formed over polymers used for manufacturing contact lenses. Furthermore these techniques allow the evaluation of other solid materials such as: stainless steel and clay.

Although AFM is a very recent technique (4), it has quickly spreaded throughout many fields of science (7,8,9,12) due to its high versatility when used on different surfaces. The AFM can also record the force felt by the cantilever as the probe tip is brought close to a sample surface and then pulled away, as well as when it's indented into the sample. This operation mode can be used to measure the long range of attraction or repulsive forces between the probe tip and the sample surface, elucidating local chemical and mechanical properties, like adhesion and elasticity. The force curves (force x distance) typically show the deflection of the free end of the AFM cantilever as the fixed end of the cantilever is brought vertically towards and then away from the sample surface. Experimentally, this is done by applying a triangle-wave voltage pattern to the electrodes at the z-axis scanner. This makes the scanner to expand and then contract in the vertical direction, generating relative motion between the cantilever and sample. The deflection of the free end of the cantilever is measured and plotted through points as the z-axis scanner extends the cantilever towards the surface and then retracts it again. By controlling the amplitude and frequency of the triangle-wave voltage pattern, the researcher can vary the distance and speed that the AFM cantilever tip travels during the force measurement. AFM has become an essential microscopic technique, widely used in different sciences from physics to biology, although its use in the last one is rare. AFM allows samples to be observed without any surface treatment, being able to obtain complementary information in real situations without interferences or artifacts. Since the sample do not need to be electrically conductive, no metallic coating is required as it is in SEM. In the same way, no dehydration of the sample is necessary and so biofilm may be observed in its hydrated state (1,6). Using AFM, some technical trouble may arise when the surface is scanned beneath a sharp tip connected to a cantilever in a contact mode. It is common that surfaces gets scratched because the cantilever, sometimes, touches it in a contact mode, during the imaging

acquisition step. On the other hand using SEM, the pre-treatment of the samples is essential to allow a clear observation of surfaces in spite of causing some physical alteration. In the case of bacteria observation or any other microorganism, the vacuum obtained from the electron column of SEM can provoke morphological alterations. AFM has several major advantages over SEM but they are definitely complementary.

Materials and Methods

Each experiment used samples with different chemical composition and assayed in different aqueous solutions, sometimes with microorganisms. The samples followed identical procedure in order to be observed in SEM. This refers to the case for bacteria when fixation and dehydration are needed (5,11). When the samples were analyzed in AFM dehydratation process was not necessary. In the following section an explanation of each experimental procedure is presented.

a) Experiments in down flow anaerobic fluidized bed reactor: A 35 liters down flow anaerobic fluidized bed reactor was designed and constructed using polypropylene with dp = 4 mm as a biomass support material. The reactor was formed by two acrylic columns with 0.15 m diameter and 2.0 m height. Expansion was obtained by recycling the liquid phase at 35°C. The process started up using 10% v/v of sludge from a bench scale UASB reactor, fed with sucrose and similar nutrients. The experiments were run for about 65 days and inoculation was repeated every week to ensure the presence of methanogenic bacteria. The biofilm development was monitored using Scanning electron microscopy (SEM). Samples were taken out every 48-h and prepared for SEM fixing them in glutaraldehyde and then dried in ethanol increasing % serie, as described by Englert (5). The same procedure was applied to all samples evaluated by SEM. Some more important parameters for the biofilm formation were also evaluated such as volatile organic acids production, alkalinity, COD removal, redox potential, pH and gas composition. The synthetic substrate was a solution of sucrose and nutrients having COD varying from 770 to 1400 $mg.L^{-1}$ O_2. All analytic procedures were done according to Standard Methods (10).

b) Biofilm production in polymethylmethacrylate (PMMA) and silicone contact lenses.

In vitro experiments of ocular bacteria extracted from patients with cataract were evaluated. Biofilm production in polypropylene and silicone intraocular lenses with dp 15 and 10 mm respectively were immersed in laboratory during 48 hours. Bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, characterized in laboratory, were used *in vitro* experiments and all of them were incubated in specific broth media. Biofilm formation and adhesion bacteria were observed by SEM and AFM while dehydration was done as described elsewhere (5).

c) Electrochemical experiments with platinum and stainless steel in organic solutions.Platinum samples with ~2r=0.06 cm and stainless steel with an area of $0.0015cm^2$ were used as electrochemical coupons. Surface finishing treatment was obtained after grinding samples with sand paper from #280 to #600 and finally polished with alumina (4μm). All samples had electric contact through copper wire. Saturated Calomel Electrode was the reference electrode and platinum wire was the counterelectrode. The potentiodynamic results were obtained using a potentiostat G&G model 275A at quiescent conditions. The experiments were triplicate and started up at -1V until +1V (50 mV/s rate) and the results were analyzed with an appropriate software. Electrolytic solutions used were: 0.25% NaCl as blank solution, 0.25% NaCl + 200 ppm glucoronic acid, 0.25% NaCl + 200 polygalacturonic acid; 0.25% NaCl + 200 glucose, 0.25% NaCl + 200 ppm d-galactose; 0.25% NaCl + 200 ppm albumin and 0.25% NaCl + 200 glucosamine. The planktonic EPS was obtained in the same way as previously published (6) containing 0.25% NaCl. Measurements of electrolytic conductivity and pH are shown in Table 1.

Table 1 Conductivity and pH of used solutions.

Concentration (ppm)	Conductivity (μS/cm)	pH
A 0.25% NaCl	5510	7.0
A+200 glucoronic acid	5600	3.2
A+200polygalacturonic acid	4230	3.7
A +200 glucose	4850	7.0
A+200 albumin	4580	6.3
A+ 200 glucosamine	3770	5.4
A+ 200 (D+) galactose	4300	5.3
A+ EPS	4200	6.6

d) Metallic ion extraction.

Clays were treated in order to evaluate the copper ion uptaking from synthetic aqueous solution with organic compounds such as: Ethylenediamine – ET - ($C_2H_8N_2$) and 1-1 Ortophenanthroline – OP - ($C_{12}H_8N_2.H_20$). The solid adsorbent obtained were named ETIL and FENAN clays respectively, forms piled organically. Contact mode AFM version was used to measure forces between surfaces with a silicon nitride tip without coating material. The goal of this part of the work is to measure relative force-distance in order to compare the same substrate with two organic extractors used to catch metallic ions.

Samples clays were prepared according to the following process. Bentonite named BRASGEL, is an industrial Brazilian Na^{1+} bentonite, from Campina Grande-Paraiba, Brazil. The preparation of piled bentonites (PB) was done in two steps. First: by suspending Na^{1+} bentonites in deionized water in a 1:60 clay:water ratios, containing 3 mEq of the replacing cation (Ca^{2+} in the chloride form) per gram of clay; the solid materials obtained were named BENTOCAL. The second step was carried out intercalating some organic compounds in the BENTOCAL clays adding the reagent at the concentration of 1.5 mEq per gram of clay.

Discussion

Polypropylene spheres used as a support in down fluidized bed reactor showed a biofilm formation and adhesion bacteria (Figure 1). Imaging with SEM detected the biofilm growth and the attached bacteria. However the biofilm shrinking due to dehydration process may cause a misinterpretation of results. When using AFM this inconvenience does not happen. Polypropylene spheres

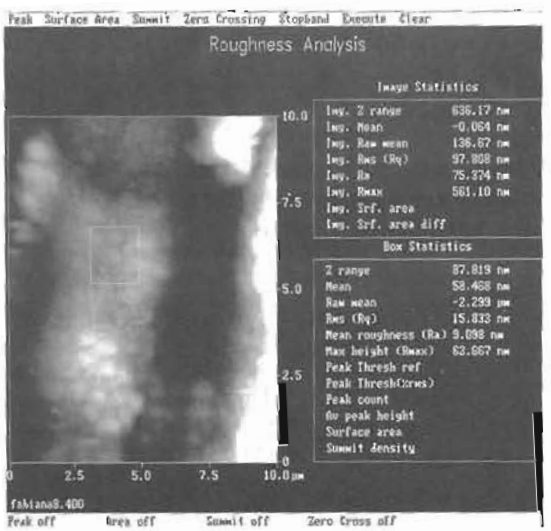

Figure 1.Spheres Polypropilene with bacteria adhered

usually have high roughness due to their manufacturing process and it probably aids the bacteria adhesion. The biofilm production reinforce the bacteria settlement and so the extraction of undesired compounds. It is also possible that a decrease in the roughness value, due to biofilm, could aid other microorganisms to settle. From the electrochemical viewpoint, it is necessary to consider that the adsorption of organic species interfere in the metallic dissolution mechanisms. It implicates directly on the corrosion mechanisms because the organic film may interfere in the ions movement catching them on the metallic surface. As a result of this, there is a direct interference in the rate corrosion values. Compounds as albumin, sucrose, glucose, and others, may be produced in microorganism's culture. It is already known that a bad cleaning of stainless steel surfaces is crucial to bacteria adhesion, particularly in food industry (3) and this could be due to the organic compounds which helps bacteria to adhere on surfaces. AFM helps to observe the first stages

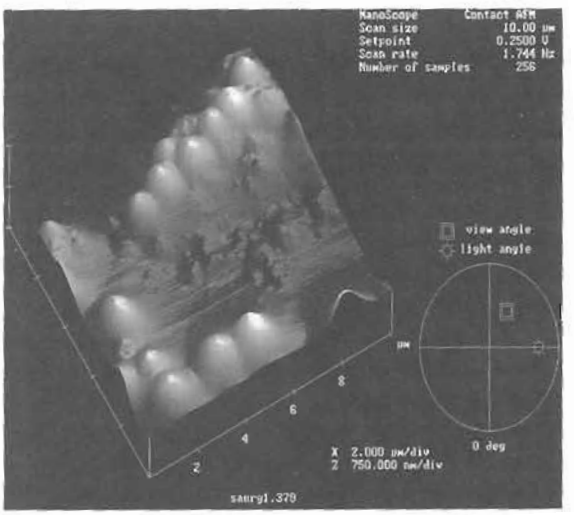

Figure 2. Polymethylmetacrilate surfaces with biofilm and *Staphylococcus aureus*.

of the biofilm formation after the assetlement of an organic film. In the case of bacteria adhered on polypropylene and silicone ocular lenses, it quickly grows. Since lenses have a small size, it is easier to adapt

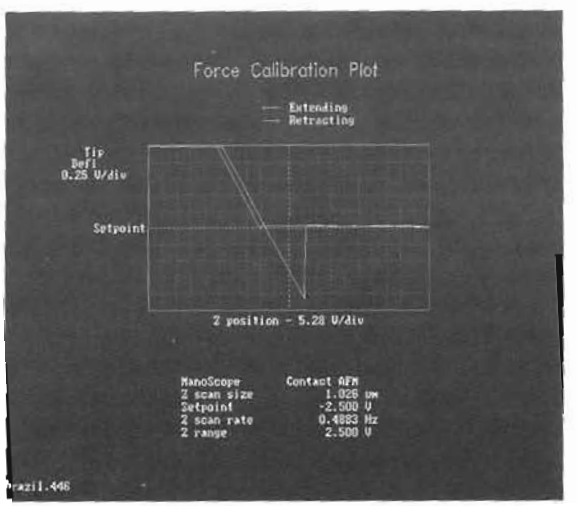

Figure 3. Force calibration curve over clay surface.

them in order to be observed in AFM, and if any infection occurs it may be detected quickly (Figures 2 and 4). The force version mode in AFM may help to discuss some mechanistic effect on clays. In the case of using different organic extractors the remotion of metallic ions change.

Figure 3 shows the typical graph Force x distance, in which can be seen the trace-retrace movement of microprobe over a particular sample. In this specific case the graphic clearly shows the relationship between the set point and the deflection of the cantilever. As the set point defines the value of the signal deflection maintained by the feedback loop, the force curve can be used to calculate the nominal contact force of the tip on the bentonite sample. The force curve represents

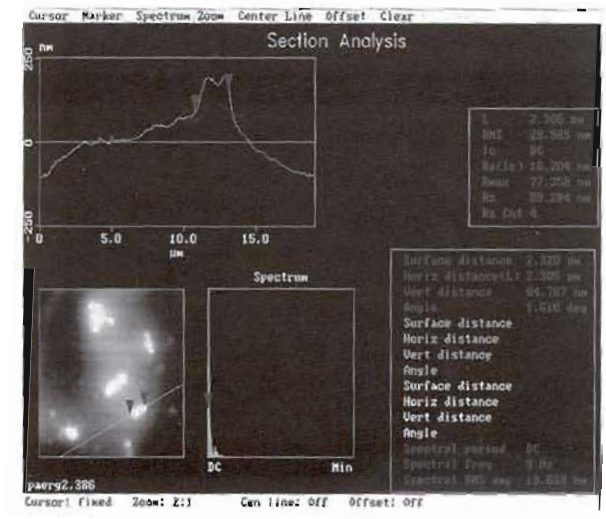

Figure 4 Bacterial distribution on silicon surface after organic extraction

the deflection signal for each complete trace-retrace cycle of the piezo. In point *a*, the cantilever is not deflected, but due to attractive forces between the tip and the bentonite surface, the tip sticks to the sample, and the cantilever is pulled down as the piezo continues to retract. Eventually, the spring force of the bent cantilever overcomes the attractive forces, and the cantilever quickly returns to its non-deflected, non-contact position. This is represented by point *c* on the figure. In point *b*, the spring force of the cantilever equals the attractive forces between the tip and the BRASGEL's surface. The examination of the graphic proved to be useful in determining the superficial characteristics and this curve represents a typical large adhesion curve and a hard surface. Some other force calibration plots, with the same behaviour that the BRASGEL curve, were made for another kind of bentonite samples and the resulting contact force was calculated. The values calculated are the followings: Bare bentonite (BRASGEL) showed a superficial contact force about 11.1 nN but after homoionic studies this value increased to 39.7 nN. It is important to point out that after organic compound intercalation, the resulting contact force showed a significant reduction, and different values between the OP and ET compounds were found, about 10 and 5.3 nN respectively. The results in more details are presented elsewhere (4). As a final conclusion AFM techniques show to be a useful tool to explain interactions between solid surfaces. If it is compared with SEM technique it is impossible to disassociate one from the other. Both techniques are complementary tools to help researchers when high resolution it is necessary to imaging detail in great magnifications. In this work the size of bacteria, biofilm formation, organic adsorption tendency and the force calculations helped to determine the probable beginning of infections over ocular lenses. The use of polymer to grow biofilm and microorganisms aids to remove undesired materials from aqueous solutions. It was observed that different organic compounds may influence mechanism of corrosion. Finally, the calculus of force may indicate the possibility to know the tendency of different compounds to increase the extraction of metallic ions.

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