

USE OF AFM FORCE SPECTROSCOPY FOR ASSESSMENT OF POLYMER RESPONSE TO CONDITIONS SIMILAR TO THE WOUND, DURING HEALING

UPORABA AFM-SPEKTROSKOPIJE SIL ZA SPREMLJANJE ODZIVA POLIMERNIH MOLEKUL NA V RANI PODOBNA OKOLJA MED CELJENJEM

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Force spectroscopy is a very promising technique for the evaluation of interactions within different environments. Knowledge about them is especially important during the design and preparation of those modern wound dressings in contact with a changing wound-environment over a prolonged time. Such exposure can cause a drastic decrease in the material's mechanical performance, and can lead to degradation, thus lowering the success of any healing process. Our study tries to establish a model system, which would enable us to assess the applicability of the mentioned technique for the evaluation of any interaction changes between polymer molecules and a chosen surface, after exposure to different environments. Our proposed experimental setup consists of two representative polymers, a model silicon surface, and two solutions of various pHs and ionic strengths, respectively. Within the chosen range of parameters, we are confident that we can prove the usefulness of force spectroscopy for further research into polymer suitability, for the development of novel wound dressings.

Keywords: force spectroscopy, AFM, wound dressings, polymer materials, model system

Spektroskopija sil je zelo obetavna tehnika za uporabo pri določanju interakcij v številnih različnih okoljih. Poznanje le-teh je še posebej pomembno pri načrtovanju in pripravi novodobnih obližev, ki morajo biti v stiku z rano dalj časa in so tako izpostavljeni spreminjajočemu se okolju. To lahko na samem materialu povzroči drastične spremembe in vodi v otežen potek celjenja. V tem delu smo želeli pripraviti modelni sistem, s katerim bi preverjali možnost uporabe omenjene tehnike za ugotavljanje sprememb interakcij med polimernimi molekulami ter izbrano površino po izpostavitvi različnim okoljem. Eksperimentalni sistem je vseboval dva vzorčna polimera, modelno silicijevo površino ter po dve raztopini z različnimi pH in ionskimi močmi. S takim naborom parametrov smo zajeli dovolj možnosti za ugotovitev uporabnosti tehnike za nadaljnje raziskave primernosti polimernih materialov za nove obliže.

Ključne besede: spektroskopija sil, mikroskopija na atomsko silo, obliži, polimerni materiali, modelni sistem

1 INTRODUCTION

Using atomic force microscopy (AFM), a tip attached to a flexible cantilever will move across the sample surface to measure surface morphology on the atomic scale. The force between the tip and the sample is measured during scanning, by monitoring the deflection of the cantilever.¹ This force is a function of tip sample separation and the material properties of the tip and the sample. Further interactions arising between tip and sample, can be used to investigate other characteristics of the sample, the tip, or the medium inbetween.² Such measurements are usually known as force measurements. The basics of a AFM force measurement is as follows: the tip attached to a cantilever spring is moved towards the sample in a normal direction, during this movement, the vertical position of the tip and the deflection of the cantilever are recorded and converted to force-versus-distance curves, briefly called force curves.²

In addition to evaluating interaction forces between the tip and model surfaces, AFM can also produce two-dimensional chemical affinity maps by modifying the cantilever tip with specific molecules. In this way, it is possible to characterize differently responding regions on the material's surface, resulting in a better understanding and, consequently, application of the examined materials.³⁻⁶

Mapping chemical functional groups and examining their interactions with different materials is of significant importance for problems ranging from lubrication and adhesion, to the recognition of biological systems wide spectrum of fields.⁷ A chemical force microscope has been extensively used to monitor changes in the interactions between different functional groups and surfaces, whilst changing measurement conditions such as pH and ionic strength.^{8,9} This technique is particularly useful when applied in combination with electron spectroscopy techniques for the determination of surface functional

groups, such as X-ray photoelectron spectroscopy (XPS).^{10–28}

Additionally, some research has already been performed in the field of monitoring the variations of pull-off forces between differently functionalized tips and surfaces.²⁹ This method's main advantage is that it almost always allows for measurement under those conditions present in the system in which the sample will be later used.

The ultimate use of AFM is for single-molecule recognition, which can be achieved by applying force spectroscopy. This technique was introduced many years ago, but has only recently achieved most of its scientific acclaim and is now one of the more used AFM modes. Clausen-Schaumann et al. were the first to introduce some of the possibilities, which enabled all the other researchers to gain a thorough understanding of single-molecule recognition measurements using AFM.³⁰ Additional weight to the measurements was introduced by Kienberger et al., whilst introducing additional blocking experiments to prove the specificity and effectiveness of the measurements.³¹

Within the field of polymer sciences, AFM has been used to quantify the entropic elasticity of single polymer chains,³² the elastic moduli of nanowires,³³ single polymer chain elongation³⁴, molecular stiffness of hyperbranched macromolecules,³⁵ friction of single polymers on surfaces,³⁶ influence of temperature on the stability of single chain conformation,³⁷ and surface glass transition temperature.³⁸ It has also been used to perform stretching experiments on single carboxymethylated amylase,³⁹ and to differentiate between sugar isomers.⁴⁰

This work focuses on the AFM as a polymer characterization method. It attempts to simulate those conditions arising from changes in the solvent, the pH and ionic strength, and possibility to use AFM force spectroscopy to assess their influence on polymer behaviour in such media. Such information would be really crucial in the preparation of wound-dressings, which are mostly polymer-based and are exposed to drastically-changing environments in the wound during the healing process.

2 EXPERIMENTAL

2.1 Preparation of functionalized AFM tips

Firstly AFM tips were decorated in order to show the possibility of using them for force spectroscopy measurements. Two different cellulose derivatives (amylose-AM and carboxymethyl cellulose-CMC) were chosen for adsorption onto the tip.

2.1.1 Adsorption of polymers to the tip surface

In order to achieve adsorption onto the tips, solutions with 10 mmol concentrations were used for both polymers (AM and CMC). Pure tips, out of the box, (NP-S10 contact-mode tips, Veeco) were gently dipped into the solution using freshly-cleaned tweezers (they were sonicated in acetone for 30 min and dried using clean nitrogen), then left in the solution for 2 h. Afterwards tips were removed from the solution and washed carefully 6 times using two different solvents (water and chloroform – CF), alternately.

All used solvents and other chemicals (when not stated otherwise) were of analytical grade (high purity) and bought from Sigma-Aldrich.

The preparation procedure is schematically depicted in **Figure 1**.

2.2 Methods

2.2.1 AFM

We performed force spectroscopy measurements using Agilent 5500 AFM. All measurements were done in liquid medium (milli-Q water) to avoid capillary forces, and to simulate different conditions. We measured 100 curves over each time-scale at different points on the sample's surface and eliminated the outliers. In order to achieve better statistics and prove the measurements were performed correctly, some of the measurements were repeated and included in the final results. In all cases an atomically flat silicon surface was used to avoid any discrepancies due to specific surface characteristics or different roughness at different spots where the force spectroscopy was performed. Two sets of measurements were performed for each medium type. Two different pHs and two different ionic strengths

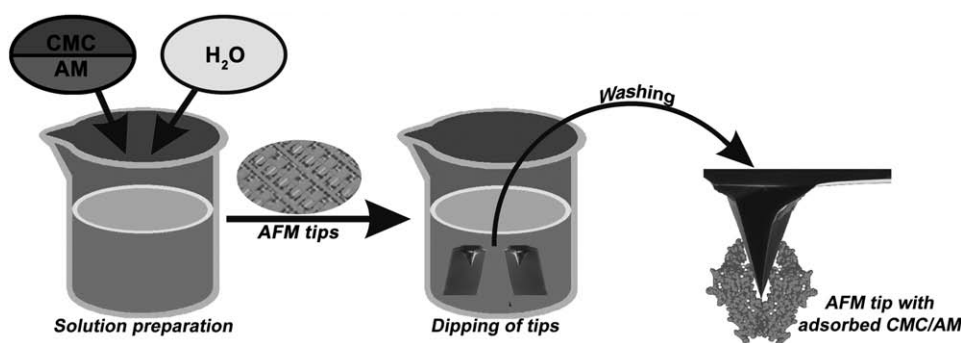


Figure 1: Preparation scheme for the attachment of polymer molecules to the AFM tip.

Slika 1: Shema pripenjanja polimernih molekul na AFM-konico

were used, in order to achieve a representative range of measurement conditions. In order to achieve desired pHs, citric acid (CA) was used as the sole reagent to lower the pH, whilst KCl was used to alter the ionic strengths. pHs were 3, 6, and ionic strengths were 5 mmol, 100 mmol, respectively.

Although measurements were also performed during other than the mentioned time-scales, only determined force using two especially representative durations were used for further discussion. The first of them was used to simulate the interactions involved in the first contact of the polymer with the environment and the second, simulating conditions after the polymer's prolonged exposure to the wound-environment.

3 RESULTS

3.1 AFM measurements

These measurements were performed to show their possible use and assessment for the best scenario, predict the behaviour of those polymer materials used for the preparation of several modern wound dressings, after their exposure to any changing environment within the healing-wound.

In our attempt to prepare the right methodology to tackle such a tough and complex problem, an attempt was made to keep the experimental setup as simple as

possible. This would allow us to apply such methodology to more complex and by far more realistic systems.

3.1.1 pH variation

Force spectroscopy was performed during the first set of measurements whilst varying the pH. As mentioned in the Experimental section, two different polymers were used for the measurements, which are, in rough approximation, representatives of possible polymers for wound-dressing preparation. One of them is the simple amylose, undissociable under the measured conditions but can, as all other cellulose derivatives, form intra- and inter-molecular H-bonds. The second is carboxymethyl cellulose which is, on the other hand, able to dissociate in alkaline solution due to its additional carboxyl groups. Two different measurement durations were used additionally one to show the forces and ranges involved upon first contact with the media, and the second to show how this changes after prolonged exposure. **Figure 2** shows the measured data for this set of force spectroscopy.

The results show that, with prolonged exposure, interactions increased for both polymers. Also it was observed that polymer nature plays a crucial role when the pH changes, as in this study. Lowering pH causes a decrease in the number of dissociated functional groups in CMC molecules, which clearly lowers the interaction. This effect is less pronounced with AM, which does not possess such groups.

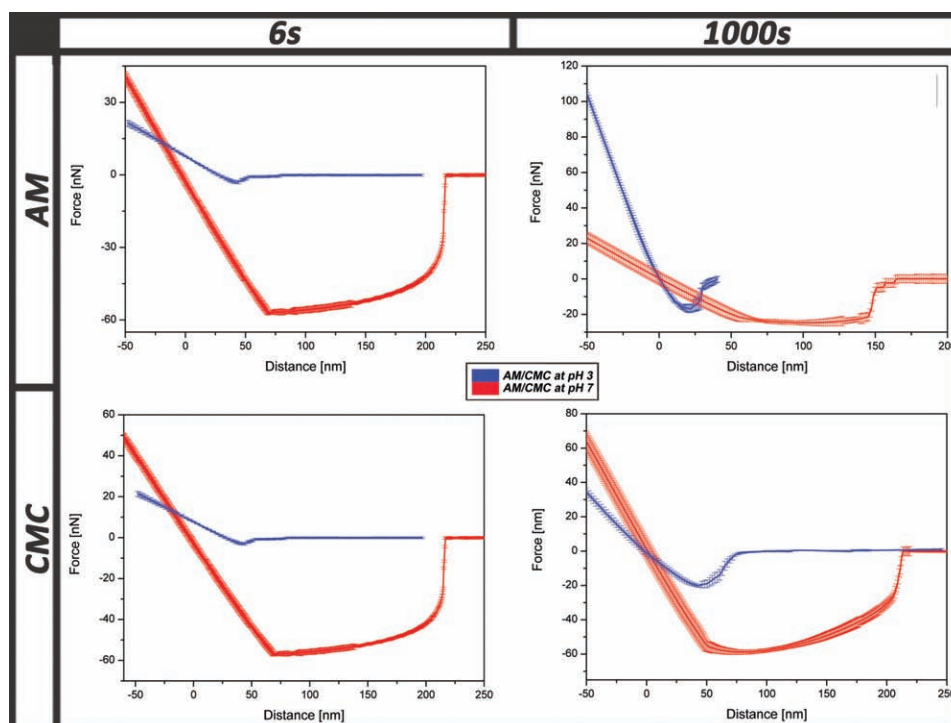


Figure 2: Force spectroscopy results for the measurements in two solutions with different pHs. TOP: retract force curves for amylose at two different measurement durations with two pHs, BOTTOM: retract force curves for carboxymethyl cellulose at two different measurement durations with two pHs.

Slika 2: Rezultati spektroskopije sil za meritve v raztopinah z dvema različnima pH. ZGORAJ: krivulje oddaljevanja pri uporabi amiloze; meritve so bile izvedene na dveh časovnih skalah in v dveh raztopinah različnih pH, SPODAJ: krivulje oddaljevanja za meritve pri uporabi karboksimetil celuloze; meritve so bile izvedene na dveh časovnih skalah in v dveh raztopinah različnih pH.

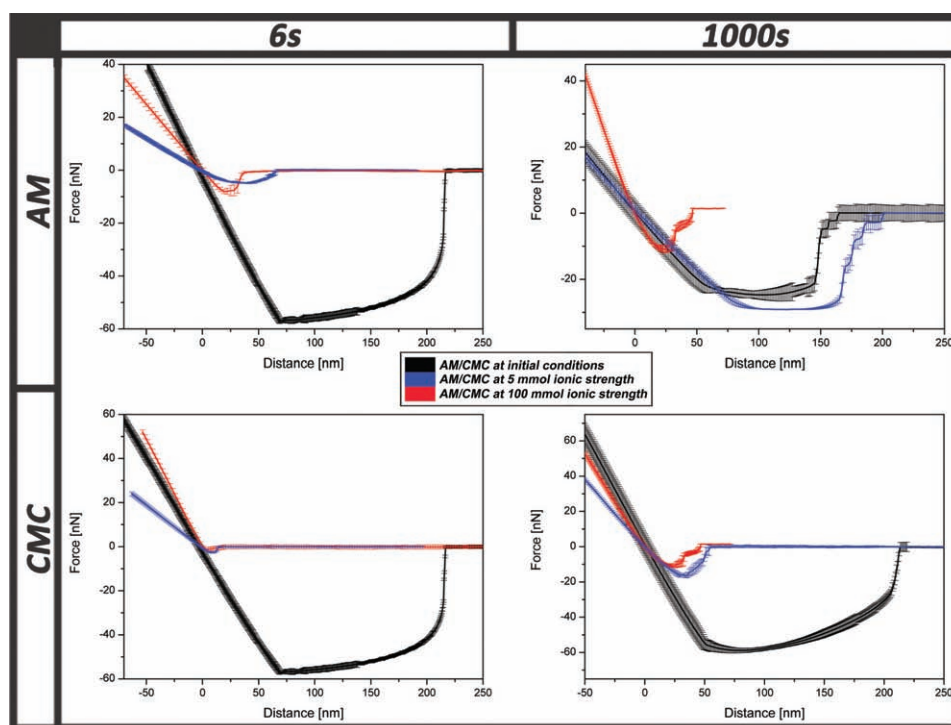


Figure 3: Force spectroscopy results for the measurements in two solutions with different ionic strengths. TOP: retract force curves for amylose at two different measurement durations in two solutions with different ionic strengths, BOTTOM: retract force curves for carboxymethyl cellulose at two different measurement durations in two solutions with different ionic strengths.

Slika 3: Rezultati spektroskopije sil za meritve v raztopinah z različnima ionskima močema. ZGORAJ: krivulje oddaljevanja za meritve pri uporabi amiloze; meritve so bile izvedene na dveh časovnih skalah in v dveh raztopinah različnih ionskih moči. SPODAJ: krivulje oddaljevanja pri uporabi karboksimetil celuloze; meritve so bile izvedene na dveh časovnih skalah in v dveh raztopinah različnih ionskih moči.

3.1.2 Ionic strength variation

In the second set of measurements, an attempt was made to assess the influence of another important and changing parameter during healing, namely the ionic strength. Again the same two polymers were used and the time was varied in order to gain some insight into the effect of prolonged polymer exposure to the changed environment over time. The results for this set of measurement are shown in **Figure 3**.

The results show that ionic strength changes influence the modified cellulose derivate, CMC more rigorously. Its carboxyl groups are clearly more sensitive to these changes, which lead to lower interactions. This effect loses some of its magnitude, with increased exposure, but the interactions stay lower when compared to AM, which does not seem to be influenced by any change in ionic strength.

4 DISCUSSION

Force spectroscopy, when used correctly, is a perfect method for assessing any interactions over a wide range of environmental conditions, especially in liquid media, which allows for the exclusion of capillary forces capable of hiding smaller interaction contributions. When using them for the first time in any research area or a new system, it is crucial that proof is found that the prepared experimental setup works and yields correct

results. For this, our experiments had to be kept as simple as possible to avoid any unexplainable discrepancies from the expected results. This presented study tried to do precisely that. By simplifying the setup to only two changing-parameters separately, it was possible to show that our proposed technique could serve as a good platform for assessing any changing wound-environment during healing.

The experiments were performed over two sets of experiments. The first used two different pHs to follow any changes in the interaction force and range between the polymer molecule and the model surface. After comparing the two used polymers, it can be said that the main difference in behaviour of CMC and AM arises due to the acidic COOH group on the glucose rings. This caused the appearance of negative charges on the chain. Repulsion of the negative charges stretched the polymer chain. When using the solution with pH 3, the H^+ concentration was sufficiently high to prevent deprotonation of COOH groups, and the formation of charges. These enabled dense packing of CMC. Due to this effect, CMC experienced lower forces (less functional groups to interact with the surface), when put into the solution of an acidic pH, This effect is far less significant for AM interactions with the model surface, because it has no such groups in its structure. Prolonged exposure times lead to an increase in interaction for the used pHs.

A similar structure-related explanation can be used to explain the results of the second experimental set, in which force spectroscopy was performed in two solutions with different ionic strengths. Again it started with the acidic carboxyl groups of CMC, which gave rise to negative charges on the CMC polymer chain. The stretching of a negatively-charged molecule is inevitable due to electrostatic repulsion. But increased ionic strength shields charged and, hence, decreased electrostatic repulsion, leading to closer packing of glucose groups. The influence of increasing ionic strength on amylose was, as expected, less drastic as no charges appeared on the amylose chain. The results to a great extent fit such an explanation. Lastly it could again be seen that an increase in interactions took place during the exposure to the medium.

5 CONCLUSIONS

We were able to design a simple test for an evaluation regarding the possible use of force spectroscopy in the assessment of polymer behaviour upon exposure to different liquid environments. Such knowledge would be of great use, as polymers are the main constituents of most modern wound-dressings, which have to stay intact upon contact with the changing environment within a healing wound. Force spectroscopy proved to be the right method for following such changes in our model system consisting of two polymers and a model surface. The results proved that such an approach is very promising for future testing in order to find the correct combination of materials for wound-dressing design regarding specific wounds.

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