

# FUNCTIONALIZATION OF AFM TIPS FOR USE IN FORCE SPECTROSCOPY BETWEEN POLYMERS AND MODEL SURFACES

## FUNKCIONALIZACIJA AFM-KONIC ZA UPORABO V SPEKTROSKOPIJI SIL MED POLIMERI IN MODELNIMI POVRŠINAMI

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The following work presents the use of two different methods for the attachment of different functional groups onto the AFM tip surface. Such functionalized tips then allow for further binding of molecules with different origins and natures, thus allowing for use when measuring forces, and the extent of interactions appearing between two model surfaces and in real systems. Force spectroscopy, in combination with chemical force microscopy (CFM), as used in this study, exhibits great potential for chemical sensing in the field of polymer sciences. In modern wound treatment, it is very important to know the type and ranges of interactions between different polymer materials, which are mostly crucial components of the dressings. Precise measurement of these interactions would help to choose those materials that fit together without the use of additional chemical modifications on their surfaces. Such modifications are often the cause of unpredictable complications during the course of wound healing. This same method could also be used for interaction evaluation between chosen polymer materials with biological macromolecules, which appear within the wound during the healing process. Such in vitro testing could be of great help when optimal wound dressing materials need to be chosen in order to alleviate a patient's suffering after application. Scanning electron and atomic force microscopies were used in order to prove the effectiveness and applicability of the used functionalization procedures.

Keywords: atomic force microscopy, chemical force microscopy, force spectroscopy, functionalization of AFM tips

V prispevku predstavljamo uporabo dveh različnih metod za pripravo AFM-konic z različnimi funkcionalnimi skupinami. Takšna funkcionalizacija je primerna za nadaljnje pripenjanje različnih molekul, kar je osnova za uporabo pri določanju moči in obsega interakcij med modelnimi površinami in v realnih sistemih. Spektroskopija sil v kombinaciji z mikroskopijo na kemijsko silo, ki je bila uporabljena v tem delu, ima velik potencial na področju kemijskega zaznavanja v polimerni znanosti. Pri pripravi večplastnih polimernih materialov, ki se uporabljajo pri moderni obravnavi kroničnih ran, nam natančno določanje interakcij lahko pomaga pri njihovi izbiri. Na podlagi takih meritev bi lahko izbrali materiale, ki med seboj najbolje interagirajo, in ne bi bilo treba vpeljati nobene kemijske modifikacije, ki bi lahko negativno vplivala na potek celjenja ran. Prav tako bi bilo mogoče metodo uporabiti za določanje interakcij izbranih polimernih materialov z biološkimi molekulami v rani, s čimer bi in vitro določili optimalen material za plast obliža, ki je v stiku z rano. V delu smo uspešnost in uporabnost funkcionalizacije preverjali z elektronsko mikroskopijo in mikroskopijo na atomsko silo.

Ključne besede: mikroskop na atomsko silo, mikroskop na kemijsko silo, spektroskopija sil, funkcionalizacija konic

## 1 INTRODUCTION

Polymers have found their way into all fields of science and industry over the last decades. Their potential applications range from binders in batteries<sup>1</sup> to composite materials in drug delivery.<sup>2</sup> Whilst the range of possible combinations between different monomers is endless, polymers found or based on natural polymers have recently become the subject of thorough research, once again.<sup>3,4</sup> Synthetic changes to their native structure make them even more propelling; especially cellulose derivatives exhibit a lot of potential for satisfying most industrial needs.<sup>3</sup>

Although AFM has been used for more than three decades; we are still experiencing some breakthroughs for its use in several areas of science and industry. This

AFM method is particularly useful for studying those modifications obtained on surfaces of polymer materials treated with reactive non-equilibrium gaseous plasma.<sup>5–18</sup>

Namely, it has been shown that even a mild plasma treatment results in important changes in surface morphology. Such changes may eventually lead to a nano-textured surface and thus to an extremely high hydrophilicity for otherwise moderately hydrophobic polymer materials.<sup>19–32</sup>

From its origins in 1986,<sup>33</sup> this technique has evolved from a shaky method to the ultimate tool on the nanoscale.<sup>34</sup> The first years of AFM use were dedicated to pushing the resolution boundary, to some unimaginable extent,<sup>35</sup> during the last decade research has been dedicated more to force measurements,<sup>36</sup> the identifi-

cation and characterization of processes at the molecular level<sup>37</sup> and mainly by the use of force spectroscopy and chemical force microscopy (CFM).

The latter is, at the moment, the most propelling within the field of polymer examination on the nano-scale.<sup>38,39</sup> CFM provides the possibility of gaining knowledge about those interactions appearing between polymer molecules or polymers, and different surfaces.<sup>40</sup> This additional information allows for the prediction of final material characteristics based on the examined polymers, even before their finalization. Quantitative assessment of the involved forces and their extent makes it easier to choose the correct polymers for achieving desired interactions between those materials used in several different interest fields (adhesion, adsorption, repelling etc.). Multilayer polymeric materials are, at the moment, first choice materials for the preparation of modern wound dressings. When sticking together layers of different polymeric origins, their interaction gains importance regarding the behavior of the final product. Methods enabling us to monitor this would greatly improve any success when choosing the right material and consequentially, avoid any chemical modification. Wound healing is a precisely regulated process, which can be interrupted or even worsened by even small interventions, thus making derivatives of previously proven biocompatible polymers dangerous and sometimes even toxic. The use of CFM for this aspect could decrease the need for chemical modification and thus improve the success of those polymers used for wound dressings.

Successful research using AFM is impossible without the proper tools, so choosing right tips is crucial in this regard. Many commercial tips are available at the moment, but only some exhibit characteristics that allow for easy and repeatable functionalization. Whilst the functionalization of tips may seem quite simple during the first iteration, it quickly becomes clear, that a lot of chemical skills are needed to bind the right species to the right place.<sup>41</sup> As a careful researcher always desires to know as much as possible about each preparation step, a lot of statistical evaluation is needed in order to prove and evaluate the success of any attachment<sup>42</sup>. During the course of our research, we found that silicon-based tips are the most promising in regard to ease of chemical modification. Their biggest advantage proved to be the commercial availability of several different silica precursors, which are highly suitable for decorating the AFM tips with the desired functional groups. Although the literature shows some direction towards the attachment of  $\text{NH}_2$  groups on the surface,<sup>43</sup> we attempted to widen the field of possible attachments by introducing other functional groups, such as the SH groups or certain hydrophobic ones like  $\text{CH}_3$ . Gold-coated tips were also used additionally in order to prove their applicability for functionalization purposes.

The purpose of our study was to evaluate, test, and alter available techniques for AFM tip functionalization,

thus enabling its later use for the binding of test molecules. Different types of silica precursors were used to prepare those tips with the desired functional groups, which are known to allow further functionalization at mild conditions. These are often necessary when soft molecules, such as polymers or biological macromolecules, are the test subjects.

## 2 EXPERIMENTAL

### 2.1 Preparation of functionalized AFM tips

#### 2.1.1 Silicon-based tips

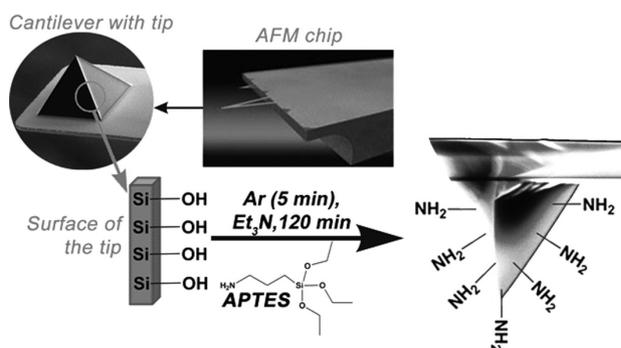
**Table 1** shows the chemical formulae and short names for the used silica precursors in this study. A known procedure was used for the attachment of  $\text{NH}_2$  groups to the surface, which is described elsewhere.<sup>43</sup> Depending on the characteristics of the used precursors, the initial procedure was modified to suit the attachment of desired functional groups. The used parameters are written along with their chemical structures and names, in **Table 1**.

##### 2.1.1.1 Attachment of $\text{NH}_2$ groups

Firstly APTES (30  $\mu\text{L}$ ) triethylamine-TEA (10  $\mu\text{L}$ ) was poured into separate Eppendorf tubes of 1 ml. These tubes were then placed inside an exiccator, which was thoroughly cleaned with acetone and ethanol, respectively and blown through using argon gas. Fresh tips (NP-S10 contact-mode tips, Veeco) were placed beside the two tubes on a flat surface and then the exiccator was again mildly blown through with argon gas and sealed. The precursors and tips were left inside the exiccator for two hours. **Figure 1** shows the scheme of the procedure.

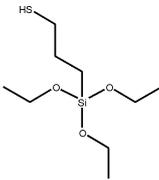
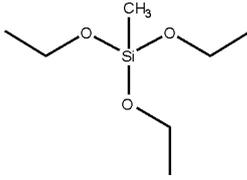
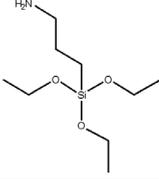
##### 2.1.1.2 Attachment of SH and $\text{CH}_3$ groups

This procedure was similar to the previous one but with slight changes regarding the used volumes and times of tip exposure to the reagents in the exiccator. Molecular weight and relative density were the only parameters taken into account when adjusting the used volume because the structural differences between the three used silica precursors appear on the same fragment of the molecule (whilst all other parts remained the



**Figure 1:** Preparation scheme for the amino functionalized AFM tips  
**Slika 1:** Shema priprave AFM-konic s amino skupinami

**Table 1:** Used silica precursors and their physic–chemical characteristics**Tabela 1:** Uporabljeni silikatni prekurzorji in njihove fizikalno-kemijske lastnosti

| SILICA PRECURSOR                  |   | STRUCTURAL FORMULA  |
|-----------------------------------|---|---|
| Name                              | MPTES [3-mercaptopropyltriethoxysilane] |  |
| $\rho/(\text{g}/\text{cm}^3)$     | 0.98                                    |   |
| $M_r/(\text{g}/\text{mol})$       | 238.42                                  |   |
| $T_{\text{tal.}}/^\circ\text{C}$  | /                                       |   |
| $T_{\text{vrel.}}/^\circ\text{C}$ | 210                                     |   |
| Vapour pressure $p/\text{hPa}$    | 6.66 (at 20 °C)                         |   |
| Name                              | MTES [methyl–triethoxysilane]           |  |
| $\rho/(\text{g}/\text{cm}^3)$     | 0.895                                   |   |
| $M_r/(\text{g}/\text{mol})$       | 178.30                                  |   |
| $T_{\text{tal.}}/^\circ\text{C}$  | –40                                     |   |
| $T_{\text{vrel.}}/^\circ\text{C}$ | 142                                     |   |
| Vapour pressure $p/\text{hPa}$    | 6,40 (at 20 °C)                         |   |
| Name                              | APTES [(3-aminopropyl)triethoxysilane]  |  |
| $\rho/(\text{g}/\text{cm}^3)$     | 0.949                                   |   |
| $M_r$                             | 221.37                                  |   |
| $T_{\text{tal.}}/^\circ\text{C}$  | /                                       |   |
| $T_{\text{vrel.}}/^\circ\text{C}$ | 122–123                                 |   |
| Vapour pressure $p/\text{hPa}$    | 13,30 (at 20 °C)                        |   |

same). Vapour pressure had, in our case, the biggest impact on the relative rate of functional group attachment to the surface and was therefore used to adjust the time of tips exposure in order to achieve the required coverage. The used volumes and times for the attachment of SH and CH<sub>3</sub> groups were 32  $\mu\text{L}$ , 25  $\mu\text{L}$  and 4 h, 4 h, respectively.

### 2.1.2 Gold-coated tips

Tips without coating on the reflective side were chosen for this purpose, in order to avoid any possible loss of resolution. Functionalization of the tips reflective side could cause laser refraction, resulting in signal decrease. Fresh gold-coated tips (ATEC-CONTAu-10, Nanosensors) were dipped into a mixture of MPTES (20  $\mu\text{L}$ ) and EtOH (2 mL). They remained in the solution for 1 h in order to achieve the desired surface coverage.<sup>44</sup> Immediately after removal from the mixture, tips were washed over a few stages, by combined dipping into water (millie Q) and chloroform (CF), respectively and

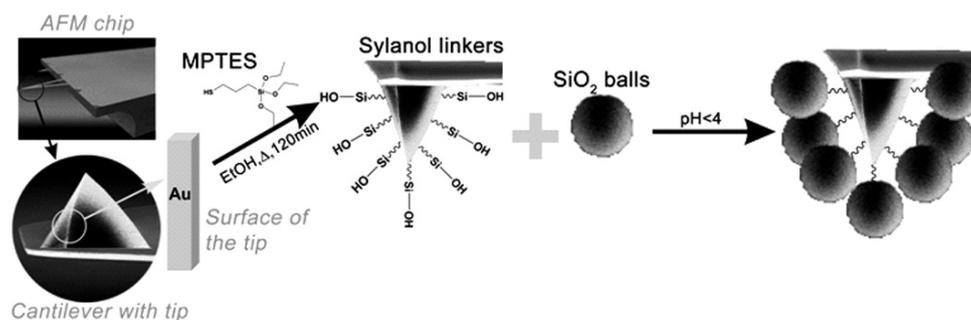
then dried using high grade nitrogen (5.0.). **Figure 2** shows a schematic depiction of this procedure.

## 2.2 Attachment of polymers to the tip surface

Two approaches were used to show that the functionalized tips are able to bind additional species to their decorated surface. The first enabled us to adsorb two different cellulose derivatives (amylose – AM and carboxymethyl cellulose – CMC), and the second was used to covalently bind one of them (CMC) to the tip for further use. Additionally silicon spheres were bound to the surface of the functionalized tips (as shown in **Figure 2**), which is proven to be very useful for examining the elasticity of films, biological structures, such as cells and macromolecules.<sup>45</sup>

### 2.2.1 Adsorption

Solutions with 10 mmol concentrations were used for both polymers in order to achieve adsorption on the tips. Pure tips, out of the box, were gently dipped into the

**Figure 2:** Preparation scheme for gold-coated AFM tips**Slika 2:** Shema funkcionalizacije z zlatom obloženih AFM-konic

solution using freshly cleaned tweezers (they were sonicated in acetone for 30 min and dried using clean nitrogen), and left there for 2 h. Afterwards, tips were removed from the solution and carefully washed 6 times using two different solvents (water and chloroform – CF), alternately.

### 2.2.2 Covalent binding

The amino decorated tips, prepared as mentioned previously, were used for the covalent attachment. CMC was attached to amino-functionalized tips, via amide bond, using *N,N'*-carbonyldiimidazole (N-CDI) (CDI method). In this way, the carboxyl group of CMC was activated, which was then prone to binding to the amino species of the tips, under mild conditions. The reaction took place in a small beaker containing the solution of the activated CMC in water. Amino-functionalized tips were placed inside the beaker, and left there for 24 h. Tips were washed and dried in the same way as previously mentioned, within the section for the adsorption of polymers.

### 2.2.3 Attachment of silicon balls

The same procedure, as shown in **Figure 3** was used for the attachment of silicon balls. First the gold-coated tips were functionalized using MPTES, as mentioned in the section Gold-coated tips. These were then gently dipped in an extremely diluted water suspension of silicon balls with a pH below 4, which was adjusted using citric acid, and left there for 2 h to allow for attachment of the balls. Silicon balls were obtained during the same preparation, procedure as stated elsewhere.<sup>46</sup>

All the used solvents and other chemicals (unless stated otherwise) were of analytical grade (high purity), and bought from Sigma-Aldrich.

## 2.3 Methods

### 2.3.1 Scanning electron microscopy

Dried AFM tips for scanning electron microscopy were pressed on a double-sided adhesive carbon tape (SPI Supplies, USA). Tips were imaged using field emission scanning electron microscopy (FE-SEM, Supra 35 VP, Carl Zeiss, Germany) operated at 1 keV.

### 2.3.2 AFM

Force spectroscopy measurements were performed using an Agilent 5500 AFM. All measurements were done in liquid medium (milli-Q water) to avoid capillary forces. 100 curves were measured during each time scale at different points on the sample's surface, and the outliers eliminated. Some of the measurements were repeated and the measurements included in the final results in order to achieve better statistics and prove the measurements were performed correctly. In all cases, a model atomically flat silicon surface was used to avoid any discrepancies due to specific surface characteristics

or various roughness at different spots where force spectroscopy was performed.

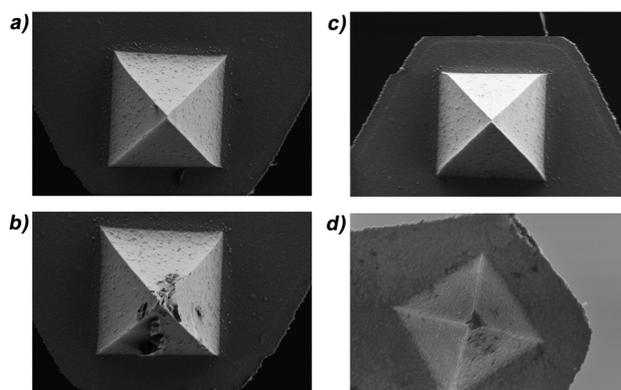
## 3 RESULTS

### 3.1 SEM imaging of the functionalized tips

Scanning electron microscopy showed that almost all functionalization attempts were successful. No clear differences could be seen between the images of those functionalized tips with different functional groups. Images also showed that the functionalization was mostly confined to the actual end of the tip and not to the surrounding chip. **Figure 3** shows representative images of the functionalized tips.

### 3.2 AFM measurements

These measurements were performed to show the applicability of the proposed functionalization procedures in further investigations regarding interacting polymers species to be used in materials preparation or drug delivery. All the measured force curves were analyzed and those maximal forces that the functionalized tips experienced were compared for chosen tips. Addition-



**Figure 3:** SEM micrographs of functionalized AFM tips: a) decorated with amino groups, b) decorated with thiol groups, c) with adsorbed amylose and d) with adsorbed carboxymethyl cellulose

**Slika 3:** Slike funkcionaliziranih AFM-konic, pridobljene z uporabo vrstične elektronske mikroskopije: a) s pripetimi amino skupinami, b) s pripetimi tiolnimi skupinami, c) z adsorbirano amilozo in d) z adsorbirano karboksimetil celulozo

**Table 2:** Results of force spectroscopy measurements

**Tabela 2:** Rezultati spektroskopije sil

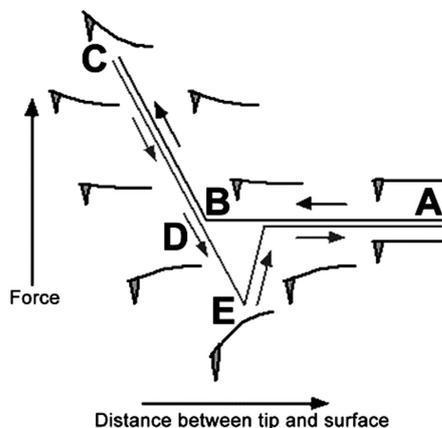
| Interaction type                             | Average force [nN] | Average distance [nm] |
|--|--------------------|-----------------------|
| Amylose (AM)                                 |                    |                       |
| Adsorption on pure tips                      | 50                 | 150                   |
| Adsorption on hydrophobic tips               | 80                 | 200                   |
| Carboxymethyl cellulose (CMC)                |                    |                       |
| Adsorption on pure tips                      | 27                 | 90                    |
| Covalent bond with amino functionalized tips | 103                | 300                   |

ally, those distances were analyzed, at which interaction with the surface disappeared. **Table 2** shows the representative data as measured and evaluated.

### 3.2.1 Force curve explanation

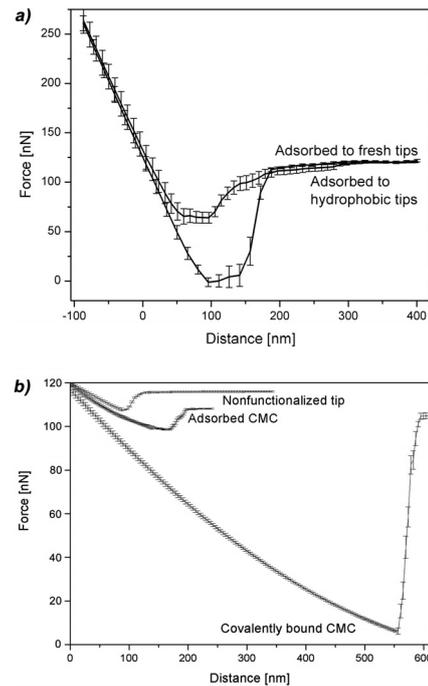
A typical force curve is shown in **Figure 4**. The explanation is as follows: when approaching the surface the cantilever is in an equilibrium position (A) and the curve is flat. As the tip approaches the surface (B), the cantilever is pushed up to the surface – being deflected upwards, which is seen as a sharp increase in the measured force. At (C) the approach half-cycle ends and the retract half-cycle begins. Once the tip starts retracting, the deflection starts to decrease and passes its equilibrium position at (D). As we start moving away from the surface the tip snaps in due to interaction with the surface, and the cantilever is deflected downwards (E). Once the tip-sample interactions are terminated due to increased distance, the tip snaps out, and returns to its equilibrium position.

**Figure 5** shows representative force curves, and their comparison when appropriate. It can be seen that the covalently bound CMC exhibits far greater forces and ranges when compared to the adsorbed species. Comparison of the response for the two used polymer species also reveals that the additional carboxyl groups in CMC contribute to a greater force and range when compared to AM. The final comparison shows the differences between the measured forces and ranges for the AM bound to two differently functionalized tips, namely the slightly more hydrophobic ones (additional  $\text{CH}_3$  groups on the surface) and the fresh tips without any functionalization. As the more hydrophobic nature of AM suggests, the interaction between AM and the slightly more hydrophobic tips (functionalized with additional  $\text{CH}_3$  groups) is greater, which is also reflected in the increased force involved in the interaction.



**Figure 4:** Example of a simple force curve. The upper is the approach curve, and the bottom the retract curve. More details regarding this characteristic feature can be found in the text

**Slika 4:** Primer krivulje pri spektroskopiji sil. Zgornja predstavlja krivuljo med približevanjem, medtem ko je spodnja krivulja oddaljavanja. Podrobnosti v zvezi s potekom meritve so zapisane v besedilu.



**Figure 5:** Representative force curves after tip functionalization. a) comparison between three different procedures for CMC attachment to the tip surface and b) comparison of force curves as measured using two types of amylose attachment. It can be clearly observed that the type of functionalization has a huge impact on any interaction between the tip and the model surface.

**Slika 5:** Vzorčni primeri spektroskopije sil. a) primerjava treh različnih načinov vezave CMC na površino AFM-konice in b) primerjava meritev sil med dvema načinoma pripetja amiloze. Krivulje jasno nakazujejo, da tip funkcionalizacije drastično vpliva na obseg interakcij med konico in modelno površino.

## 4 DISCUSSION

Chemical force microscopy can be a very powerful tool when used correctly. The most crucial part of achieving its purpose is the functionalization of the tips, thus enabling progressive sensing. Its use in polymer sciences is no exception. It seems that by employing some alterations to the known functionalization procedures, we are now able to attach different functional groups to the tip surface, thus providing numerous possibilities for the further bonding of a wide variety of different species. All used procedures proved to be successful for mainly decorating mostly the edge of tips, leaving the surroundings almost as clean as before the functionalization. In this way, there is no response by the AFM feedback system and none of the resolution is lost. SEM images confirm the latter by showing that the tip's surroundings remain almost unchanged, whilst the surface of the tip exhibits some new features when compared to the non-functionalized ones (**Figure 4**). The introduction of two different polymers to the tip's surface proves to be almost equally successful, as no increase in "chemical waste" can be examined on the surface surrounding the tip. Attached molecules seem to concentrate right at the end of the tip's pyramid, which is

therefore ready to interact with the model surface (**Figures 3c and d**).

AFM measurements revealed that, when using different bonding natures to bind the polymers to the tip, these changes alter the forces and distances at which they disappear. In the case of CMC, it can be seen that the forces involved are a couple of scales bigger when they are bound to the tip via a covalent bond, as compared to the preparation procedure, in which they are only adsorbed. Such knowledge could be of extreme importance when preparing novel polymer based materials; as such forces contribute to the materials final characteristics, such as adhesion properties, sensitivity to mechanical stress and others.

These measurements also reveal that the used preparation procedure is applicable for chemical sensing purposes. We have successfully showed that a different amount of force is exhibited when using different bond types and polymers. At the same time, we have discovered that these forces disappear at different distances when the tips are pulled away from the model's surface. This clear difference between measurements proves that our preparation procedure is applicable for chemical sensing, and can be further used during the characterization process of polymers and polymeric materials. The used method could be of great benefit to the public health because polymers are the most used materials in the preparation of wound dressings.

## 5 CONCLUSIONS

We have successfully prepared different functionalized AFM tips, which are not only capable of binding additional chemical species to their surfaces, but also allow for their use during chemical sensing. With further improvement and transfer to those systems found in real materials, these techniques could make a significant contribution to the characterization process of polymers in order to maximize the effectiveness of the preparation process, especially during the preparation of wound dressings.

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