2009

Electrochemical AFM : understanding the electromaterial-cellular interface

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Abstract

Organic conducting polymers are emerging as an exciting new class of biomaterial that can be used to enhance and control the growth of mammalian cells for tissue regeneration and engineering applications

Keywords

electromaterial, understanding, afm, electrochemical, interface, cellular

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Electrochemical AFM

Understanding the Electromaterial-Cellular Interface

Organic conducting polymers are emerging as an exciting new class of biomaterial that can be used to enhance and control the growth of mammalian cells for tissue regeneration and engineering applications [1].

Keywords:

Electrochemical-Atomic Force Microscopy, (EC-AFM), Organic conducting polymers, polypyrrole, mechanical actuators, biomaterials

Image insert: Dr. Scott McGovern
f.l.t.r. Dr. Michael Higgins, Prof. Gordon Wallace, Ms. Amy Gelmi

Conducting Polymers as Stimuli Responsive Substrates

In contrast to traditional biomaterials, conducting polymers are attractive for their ability to not only provide a biocompatible substrate but to also deliver additional multiple stimuli to the cells through electrical stimulation of the polymer. For example, when a potential is applied to a conducting polymer in electrolyte, the polymer chains can rapidly switch between oxidized (loss of an electron) and reduced (gain of electron) states causing incorporated dopant ions in the polymer to move in and out of the polymer to compensate for a charge imbalance. In terms of medical applications, the dopant ion can be a bioactive species for the controlled release of drugs, while solvent accompanying the movement of dopant ions results in the expansion and contraction (i.e. mechanical actuation) of the polymer [2].

This remarkable ability to transmit simultaneous chemical and mechanical stimuli occurs along with numerous other dynamic property changes at the polymer interface (These include changes in the roughness, elasticity, adhesion, wettability, conductivity), all of which have the potential to cooperatively address the complexity of multiple cell growth processes to facilitate tissue regeneration.

Understanding the Dynamic Interfacial Properties of Conducting Polymers

In many of the potential conducting polymer applications for tissue engineering and other medical devices (e.g. neural probe coatings), an understanding of

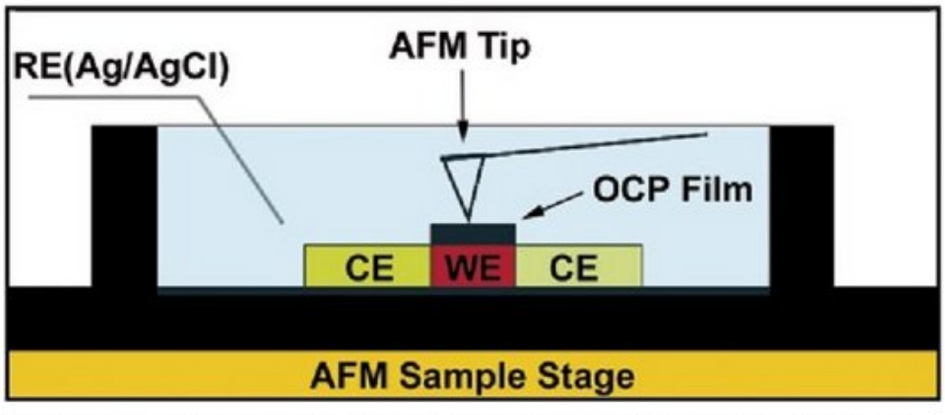

Fig. 1: Schematic diagram of EC-AFM design. Organic conducting polymer (OCP). Counter electrode (CE). Working electrode (WE). Reference electrode (RE).

their dynamic interfacial properties as a function of electrochemical stimulation is viewed as an important stage in designing optimal devices based on conducting polymers. Characterizing lateral variations in the properties at the nanoscale level will also be an important factor when considering their possible influence on cellular-substrate interactions, which can occur over similar lengthscales. It is likely that modulating the nanoscale properties and surface interactions of conducting polymers will provide finer control over cell growth and allow the design of materials for cell-type specific applications. To address this, we have implemented Electrochemical-Atomic Force Microscopy (EC-AFM) to probe the nanoscale properties of conducting polymer substrates during electrochemical stimulation [3]. Previous studies have used EC-AFM to study the degradation, growth processes, and actuation of conducting polymer films [4, 5], though it has been used to a lesser extent in the general context of conducting polymers for tissue engineering applications; for example, probing the nanoscale properties that living cells may encounter at the polymer interface.

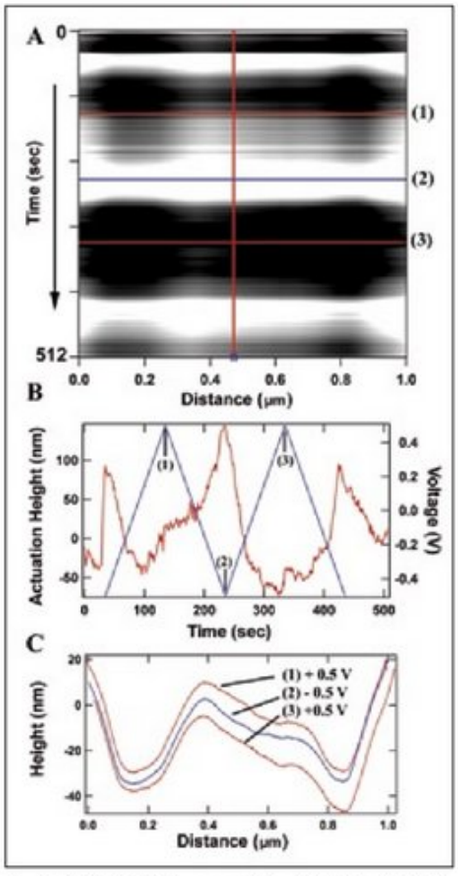

Fig. 2: (A) EC-AFM image of Ppy/PSS film in 0.05 M NaPSS electrolyte. The image was taken with the slow scan x-axis disabled and represents an image formed from the AFM tip scanning multiple times over the same topographic line. (B) Actuation height profile (red trace) taken through the vertical line in (A) and corresponding voltage signal (blue trace) of the applied CV. (C) Topographic line profiles taken through the corresponding numbered horizontal lines in (A). The applied potential and time during the CV when each trace was recorded is indicated with corresponding numbers in (B).

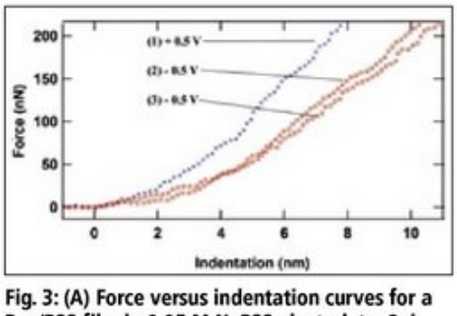

Fig. 3: (A) Force versus indentation curves for a Ppy/PSS film in 0.05 M NaPSS electrolyte. Oxidized state (blue hatch). Reduced state (red squares).

EC-AFM to Probe Nanoscale Surface Properties

Figure 1 shows a schematic diagram of an EC-AFM design for the measurements used in this study. The design consists of a 2-D electrochemical cell supported on a substrate with a counter electrode surrounding a conducting polymer coated working electrode grown galvanostatically at a current density of 1.3 mA/cm^2 for 10 min, which is positioned under the AFM tip. A silver/silver chloride wire can also be positioned near the working electrode to record the reference voltage. For EC-AFM measurements, Figure 2A shows an AFM image of a polystyrene sulfonate doped polypyrrole (Ppy/PSS) film in 0.05M sodium polystyrene sulfonate (NaPSS) electrolyte during the application of a cyclic voltammetry (CV) potential swept from -0.5 V to +0.5 V for two cycles at 0.005 Hz. During electrical stimulation of the polymer film, with the onset ≈ 35 sec, the images showed a cyclic increase (light bands) and decrease in height (dark bands), indicating the dynamic actuation of the Ppy/PSS film. A height profile of this actuation taken through the vertical line in the image is plotted versus time (red trace) in Figure 2B and overlaid with the applied voltage signal (blue trace). Figure 2B shows that the film expanded during reduction (-0.5 V) and contracted during oxidation (+0.5 V), with an actuation height of ≈ 175 nm for the two cycles. It is noted that the initial smaller peak on the left was due to the immediate expansion of the film when the CV was initiated at a reduction potential of – 0.5 V, while the subsequent two peaks correspond to the two fully completed CV cycles. In particular, the out-of-phase response between the actuation and voltage signals indicated that the increasing negative charge on the polymer backbone during reduction was balanced by the incorporation of Na^+ ions from the electrolyte, as opposed to the ejection of the large, immobile PSS dopant ions in the polymer. This cation driven transport in and out of the polymer, along with accompanying solvent, results in the reversible swelling and deswelling of the film. In terms of biomedical engineering, this process has the potential for applying a mechanical stimulus to promote cell growth and differentiation.

Figure 2C shows line profiles of the actual film topography taken through the three horizontal lines in Figure 2A, which were recorded at the maximum oxidation and reduction potentials indicated by corresponding numbers on the voltage signal in Figure 2B. Figure 2C revealed that with this chemistry there were no significant changes in the film morphology or roughness during oxidation and reduction of the polymer; however previous studies have shown that switching between oxidation and reduction states of conducting polymers with different dopants can induce roughness changes [6] that are most likely due to the uptake and release of solvent, or conformational changes in the polymer backbone.

In addition to morphology and mechanical actuation, the compressive stiffness of the polymer films can also be determined as a function of electrical stimulation. Figure 3 shows representative force versus indentation curves calculated from force measurements taken on a Ppy/PSS film in 0.05M NaPSS electrolyte during the application of a CV potential swept from –0.5 V to +0.5 V at 0.01 Hz. The curves revealed that the indentation of the film increased, or its effective stiffness decreased, when the polymer was in the reduced state (red curves) compared to the oxidized state (blue curve). Hertzian model fits to the curves in Figure 3 (data not shown) provide a relative estimation of the Young's modulus, with values of 1.0 GPa obtained for the oxidized state (blue curve 1), and 0.67 GPa (red curve 2) and 0.63 GPa (red curve 3) obtained for the reduced state. These values indicated a 330-350 MPa shift in the compressive modulus during redox switching of the polymer. The curves were recorded within one cycle of the CV, i.e. reduced state (curve 3) → oxidized state (curve 1) → reduced state (curve 2), hence the data further indicated that these quantitative redox

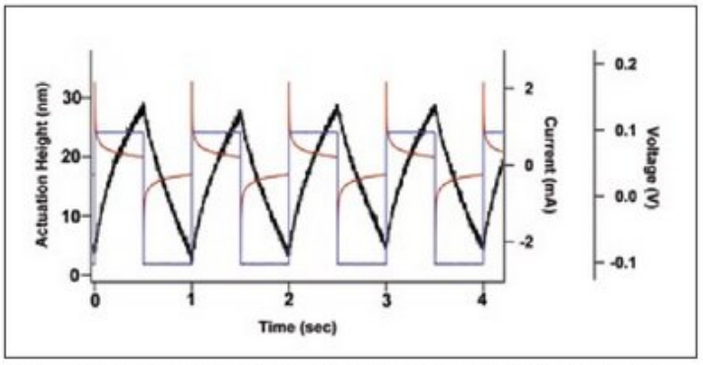

Fig. 4: (A) Actuation height signal (black) for a Ppy/HA film in 0.1M NaCl using an applied biphasic ± 0.1 V pulse stimulation. Voltage signal (blue). Current signal (red).

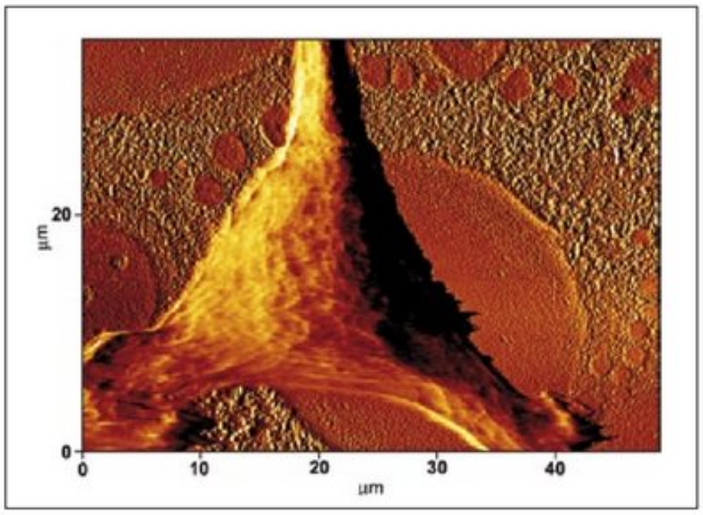

Fig. 5: AFM image of a living C2C12 cell on vapour phase deposited Ppy in phosphate buffer saline (PBS) solution.

changes in the material properties were fully reversible.

We have also extended these EC-AFM measurements to biocomposite conducting polymers and electrical stimulation protocol that are more relevant to the biological setting. For example, the mechanical actuation of hyaluronic acid doped Ppy (Ppy/HA) films (grown galvanostically at a current density of 0.25 mA/cm² for 10 min) was assessed using a biphasic square wave potential with stimulation frequency of 1 Hz. In contrast to the actuation measurements described above, the potential applied in these experiments is more representative of clinical protocols used for electrical stimulation of nerve and muscle tissue both in terms of the potential waveform, amplitude and higher frequency. In addition, the hyaluronic acid incorporated into these films is a major component of the extracellular matrix contributing to cell growth, and expected to enhance the biocompatible properties of the conducting polymer. To assess how these biocomposite Ppy/HA films actuate under more applied conditions, a biphasic pulse of +0.1 V at 1 Hz was applied to a film in 0.1M NaCl electrolyte. Figure 4 shows the applied voltage (blue trace) and current signals (red trace), and the corresponding actuation response (black trace) of the film, which was recorded by measuring the z-feedback height of the AFM tip positioned on the surface. In contrast to the Ppy/PSS films, it was observed that the Ppy/HA films expand and contract during oxidation and reduction, respectively, with an amplitude of 25 nm. Under this low voltage stimulation, the Ppy polymer is unable to be reduced and because the large hyaluronic acid dopant is mostly immobile (i.e., similar to PSS-), Figure 4 suggests that the actuation was driven by the movement of solvated Cl- ions during the charging/discharging of the conducting polymer surface. However, further work is required to elucidate the exact mechanism. Importantly, these results highlight that the biocomposite films are capable of providing a mechanical stimulus using biphasic stimulation at higher frequencies and lower potentials (i.e. ± 100 mV), with the latter being an important parameter for the safe stimulation of living cells and tissue.

An eventual goal is to use EC-AFM for directly observing cellular interactions at the conducting polymer interface during electrical stimulation. An example of this EC-AFM application is highlighted in Figure 5, which shows an AFM image of a living C2C12 (muscle) cell on a vapor phase deposited Ppy film positioned within an EC-AFM. Figure 5 highlights the ability to observe the cell morphology and its adhesion to the surface with respect to the underlying nanostructure of the polymer film. In addition to observing structural changes of the cells, further AFM studies could also be used to probe cellular forces in response to electrical stimulation, for example, changes in the mechanical properties of the cells.

Conclusion

In conclusion, EC-AFM is an exciting technique for studying the dynamic, nanoscale surface properties of organic conducting polymers. In particular, the nanoscale properties observed in this study may well be modulated using electrical stimulation to enhance and control cell growth. If the nanoscale properties and their influence on cellular interactions at the polymer interface as a function of electrical stimulation can be identified, then more optimal conducting polymer materials could be designed. This could lead to envisaged applications such as coatings for neural probes, implant for drug release, and scaffolds for muscle and spinal cord repair.

Acknowledgements

The Australian Research Council (ARC) for funding support. This research was supported by the ARC Federation Fellowship Program (G.G.W.).

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