DUAL-CONTROL MOLECULAR SWITCHES FOR BIOMEDICAL APPLICATIONS

Michale Zanotti1, Amy Gatlin1, Paul Molino2, Robert Byrnes1, Michael Higgins1, Klaus Wagner1, Sanjeev Gambhir2, Gordon Wallace3, David L. Office4 and Dermot Diamond5

1 CLARITY Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland.
2 Intelligent Polymer Research Institute, University of Wollongong, Wollongong 2522, Australia.
3 Contact: Prof. Dermot Diamond (dermot.diamond@bci.uow.edu.au)

INTRODUCTION

Conducting polymers have attracted considerable research attention because of promising applications in biosensors, biomaterials, electronics, energy storage and conversion devices. Photo-responsive materials have great potential in biomedicine, particularly in the area of tissue regeneration and repair [1,2], as are conducting polymers, because they can provide a physical platform for the growth of several lines of living cells whose properties can be tuned by external stimulation and control [3]. The work explores the behaviour of hybrid conducting polymer/photo responsive materials, with particular emphasis for use in biomedical applications. 

Techniques like polypyrrole polymers (TTT-BSP, 1 and 2) synthesized and characterised in this work are examples of such hybrid materials, in that they can be switched between two or more states (each with their own distinct characteristics) using an external stimulus (photonic or electrochemical). These materials show particular propensity to functionalize surfaces. In our case ITO (glass and PET) and QCM crystals via electrochemical deposition. Photophysical switching shows its strongest effect when the monomers of these materials are dissolved in polar solvents the: the photochromic shift of BSPNO at (2) in fig 1(4)(e). Furthermore, irradiation of the electro-grown polymer with a 254nm light source for 15 minutes also showed evidence of photoinduceability. Post-synthesis electrochemical stimulation produced dramatic morphological and surface topological changes in the material, as evidenced by contact angle measurements and visualization with AFM. This study ultimately seeks to take advantage of these induced morphological changes of the polymer and to clarify the interactions between biomolecules like fibronectin and the TTT-BSP based materials, with the support of elegant, modern and appealing methodologies like protein-functionalized AFM tips and QCM.

Figure 1: (a) Structure of the monomers BSPOHacetoTTh (1), BSPNOacetoTTh (2) and their photonic effect in Acetonitrile. (b) UV-vis of BSPOHacetoTTh with the wavelength of Merooxazine formation highlighted (MC at 572nm) in Acetonitrile; (c) Thermal Relaxation of 0.1 from 15°C in Acetonitrile

PHOTOCHEMICAL PROPERTIES OF THE TTT – BSP MONOMERS

Fibronectin (fig 2a) is a high molecular weight (440kDa) extracellular matrix glycoprotein produced by the liver, important in the human for the following reasons:

1. It is a major component of the extracellular matrix (ECM).
2. It binds collagen, fibrin, proteoglycans based on heparin sulfate and heparin.
3. Fundamental for effective cell adhesion.
4. Important for cell growth.
5. Essential for cell migration.
6. Role in cell proliferation.
7. Important in wound healing.
8. Important in embryonic development.

Fig. 2b: Nanosilver PNP-DB tips (dx 0.5 nm) with gold reflective coating were functionalized with fibronectin (FN). The tips were initially cleaned in plasma cleaner for 5 minutes then placed into a 1% 3-EDSPA in toluene solution for 2 hours. The tips were then rinsed with toluene, then the PBS and excess fluid was drained off. The tips were then placed into a 10 mL PBS in PBS solution for 1 hour, then rinsed with PBS and the excess fluid drained off. The tips were then placed into a 10 mL PBS in PBS solution for 1 hour, then rinsed with PBS and stored in PBS in the fridge until required. Each tip was pre-calibrated before use in the experiments. The spring constant of the cantilever was calculated using the Sader method, which relies on the resonant frequency, quality factor and geometrical parameters of the cantilever. The sensitivity of such cantilever was measured in situ in PBS on a glass substrate. The protein adhesion measurements were carried out using force-distance curves of the functionalized tips onto the polymer surface in 10 nmol PBS solution. The force-distance curves were conducted over an approach range of 500 nm, at a rate of 0.5 Hz with a dwell time on the surface of 1 sec. Measurements were performed on an Asylum Research MFP-3D Atomic Force Microscope (AFM). OR.

Fig. 3: Surface study of the polymer. (a) p-BSPOHacetoTTh at reduced state; (b) p-BSPOHacetoTTh at oxidized state; (c) Topography of p-BSPOHaceto.

AFM ANALYSIS OF PROTEIN ADHESION: FIBRONECTIN VS BSPNOacetoTTh

Quality of adhesive forces between FN and the hybrid conducting polymer in exam. The results were reproducible and repeatable within this time period.

Fig. 5: (a) QCM test on p-BSPOHacetoTTh and (b) QCM test on p-BSPOHacetoTTh. These two experiment have been performed with this procedure: (a) was to interact with three different solutions of proteins flushed on the surface and added in this sequence: FN (concentration was 50µg/ml), Bovine Serum Albumin (concentration was 50µg/ml), FN specific Antibody (concentration was 1/150 active units), without any electrochemical stimulation for 4.2hrs. (b) was kept at -400mV for 8.2hrs (first 4hrs were required for standard equilibration of the system) and then allowed to interact with the three solutions of biomolecules with the same previous scheme flushed on the surface. The results obtained allowed remarkable differences from the frequencies point of view. For what concerned the dissipation is appreciable a different progression shape when the polymer is stimulated at negative potentials. This could be due to a different modality of the polymer at -400mV. Further study will investigate this aspect.

BIBLIOGRAPHY


This work is supported by Science Foundation Ireland under grant 07/CE/11147

UNIVERSITY COLLEGE DUBLIN • DUBLIN CITY UNIVERSITY • TYNDALL NATIONAL INSTITUTE