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ELECTRICAL CHARACTERIZATION AND ELECTROGENIC CELL STIMULATION USING A CONDUCTIVE POLYMER COMPOSITE BASED ON PEDOT:PSS/PVA/EG

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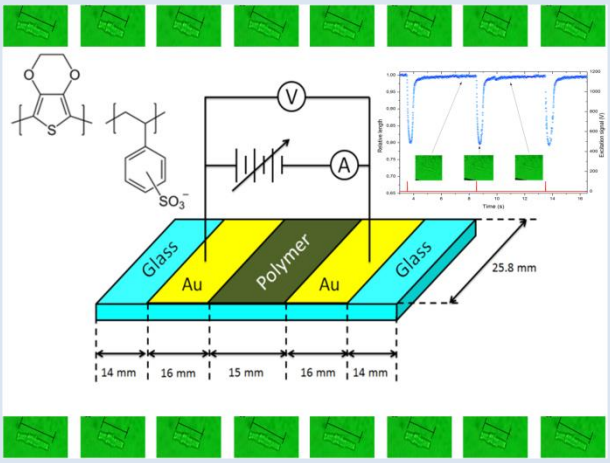

ABSTRACT

Modern microelectrode arrays are being developed with emergent materials and approaches that provide properties superior to conventional metallic and inorganic semiconductor based electrodes. Newer materials include conducting polymers, nanotubes and graphene-based films. Here we propose a composite mixture with controlled electrical properties based on the p-type conductive polymer poly(3,4-ethylenedioxythiophene) doped with poly(styrenesulfonate) (PEDOT:PSS). To improve the biocompatibility and conductivity of the material, we added a combination of the polar organic compound ethylene glycol (EG), the surfactant sodium dodecyl sulfate (SDS) and polyvinyl alcohol (PVA). Using these mixtures, we fabricated films to interface electrical stimuli with rodent skeletal muscle fibers and ventricular cardiomyocytes. We demonstrated strong contractile responses and excellent biocompatible cell coupling with polymer-based films.

Keywords: conductive polymers, biosensors, electrogenic cells.

CARACTERIZACIÓN ELÉCTRICA Y ESTIMULACIÓN DE CÉLULAS ELECTROGÉNICAS USANDO EL POLÍMERO CONDUCTOR PEDOT COMBINADO CON PEDOT:PSS/PVA/EG

RESUMEN

Arreglos de microelectrodos modernos están siendo desarrollados con materiales y enfoques emergentes que proporcionan propiedades superiores a los electrodos basados en semiconductores metálicos e inorgánicos convencionales. Estos nuevos materiales incluyen polímeros conductores, nanotubos de carbono, películas basadas en grafeno, etc. En este trabajo proponemos un compuesto con propiedades eléctricas controladas usando el polímero conductor tipo p Poli(3,4-etilenodioxitiofeno) dopado con poli(estirenosulfonato) (PEDOT: PSS). Con el fin de mejorar la biocompatibilidad y la conductividad del material añadimos una combinación del compuesto orgánico polar glicol etileno (EG), el dodecil sulfato de sodio tensoactivo (SDS) y el alcohol de polivinilo (PVA). Usando estas mezclas fabricamos películas para construir interfaces eléctricas y estimular dos tipos de células biológicas electrógenicas. Nuestros resultados muestran fuertes respuestas contráctiles de las fibras musculares esqueléticas y cardiomiocitos ventriculares de ratas, así como un excelente acoplamiento biocompatible de las células tras la excitación usando las películas basadas en polímeros.

Palabras Claves: polímeros conductores, biosensores, células electrogénicas

1. INTRODUCTION

Implantable tissue electrodes for delivery of precise stimulating signals, as well as for recording electrophysiological responses, have advanced clinical and fundamental research on heart, brain, cochlear, retina, and other tissues [1-3]. The advent of microelectrode arrays (MEAs) has produced fundamental advances in our understanding of electrophysiological function and microelectrode functionality, especially in brain and neuronal tissues [4,5].

The desire for less intrusive and damaging recording techniques and the advances in microelectronic devices during the last two decades have prompted a strong interest in merging biological elements with electronic devices by creating hybrid interfaces called *bioelectronic systems* [6,7].

The composite Poly(3,4-ethylenedioxythiophene) PEDOT – a conductive polymer, has been used for many technological applications in the burgeoning microelectronics industry, including sensing devices, touch-screen monitors, organic transistors, and photovoltaic applications [8]. Organic conductors, like PEDOT, have also been employed in sensor and actuators in biological systems [9]. There are advantages of PEDOT over metallic and inorganic semiconductor-based devices, the primary one being the inherent biocompatibility of organic materials with biological cells. Also, the mechanical plasticity and soft-matter nature of polymers allow them to be adaptable to different tissue morphologies without severely damaging cell structure and function. This aspect has been exploited in the design of drug delivery systems, chemical actuators, and organic artificial ion pumps for signal regulation in neurons [10].

Application properties of PEDOT have been improved by adding the polyelectrolyte poly(styrenesulfonate), PSS, producing a water-soluble polymer with high conductivity and very good film formation properties. In this work, we show that the addition of ethylene glycol (EG) to the PEDOT-PSS film surface can enhance transmission of stimuli to *in-vitro* cells. We report the electro-mechanical activity of rodent striated muscle cells stimulated by our polymer films.

2. EXPERIMENTAL PART

2.1 Preparation of chemical solutions

We used two commercial aqueous solutions of PEDOT-PSS to fabricate the films:

1. ORGACON HBS5^{TM} with the following characteristics: composition: % PEDOT:PSS = 1.1; Superficial Electrical Resistance (SER) < 100 ohm/sq. This product was purchased from ITO America Corporation: **(PEDOT 1)**.

2. PEDOT-PSS (Sigma-Aldrich) composition: PEDOT content = 0.5 wt. %, PSS content = 0.8 wt. %; concentration = 1.3 wt % dispersion in H_2O; conductivity 1 S/cm. **(PEDOT 2)**.

The organic compounds used to improve surface stability and films conductivity were ethylene glycol (EG): 25% (v/v) solution) and poly(vinyl alcohol) (PVA 140.00), Sigma Aldrich and Himedia, respectively. SDS and PEG were purchased from Merck.

Mixing procedures for the different PEDOT composites were as follows:

1. PEDOT:PSS + SDS (Sodium dodecyl sulfate): 1 g of SDS was added to 5 ml of PEDOT:PSS producing a 20 % m/v solution. The mixture was agitated magnetically in a reflux system for 24 h at 50 °C and 200 rpm.
2. PEDOT:PSS + PEG (Polyethylene glycol): 0.5 g of PEG was diluted in 1 ml of PEDOT:PSS, producing a 50 % m/v solution. The mixture was agitated magnetically in a reflux system for 3 h at 32.8 °C and 100 rpm.
3. PEDOT:PSS + EG (ethylene glycol) + PVA (polyvinyl alcohol): PVA was dissolved in EG in a ratio of 2:1 at 80 °C for 30 min and stirred with a magnetically driven paddle in a reflux system at 60 rpm. The final concentration of PVA in the formulation was 10% (w/v). The resulting PVA+EG solution was then mixed with one of the above described versions of PEDOT:PSS, and an aqueous solution of PEDOT-PSS:EG:PVA (2:2:1) was formed while being magnetically stirred at 100 rpm in a reflux system at 32 °C for 4 h.

2.2 Deposition of gold electrodes by thermal evaporation

Conventional Corning glass substrates of size 75 mm x 25.8 mm were inserted into a vacuum chamber where first chromium and then gold were successively heated until they evaporated and deposited on the non-protected sections of the glass, leaving two rectangular strips of well-structured gold electrodes. A very thin chromium layer was added in order to improve adherence of the gold to the glass. The geometric configuration of the electrodes and polymer area are detailed in Figure 1 below:

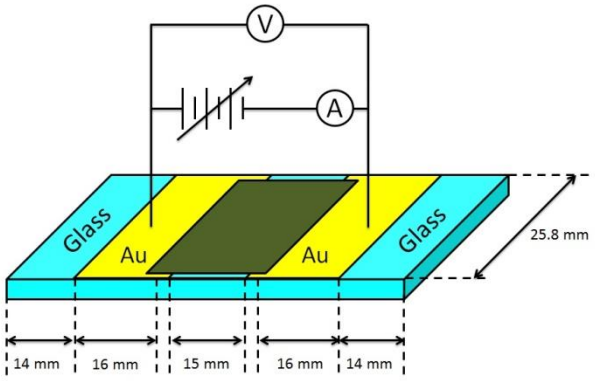

Figure 1. Geometric configuration of the gold electrodes and polymer film area and setup configuration for the electrical test on the film.

2.3 Glass surface cleaning and Spin Coating

Prior to PEDOT deposition, the glass slides with the gold electrodes were cleaned and dried by sonication in acetone and methanol for 10 min each. Then glass substrates were bathed in *Piranha* solution (H_2SO_4:H_2O_2= 3:1) for 15 min at 100 °C, rinsed in de-ionised water for 5 min and dried with pressured nitrogen gas. Finally, the cleaned substrates were heated in a conventional oven for 20 min at 85 ºC to remove moisture. This procedure removes any organic residues and makes the glass surfaces highly hydrophilic. The films of conducting polymer composite were formed on the substrates by spin coating: The PEDOT-PSS+EG+PVA solution was deposited in excess on the glass substrate and spun in a range of speeds from 1000 rpm to 5000 rpm for two minutes. This created a uniform transparent film over the area between the gold electrodes and in full contact with them. Finally, the film was dried in an oven for two hours at 60 °C. Our techniques of film formation and treatment followed standardized procedure [10].

The thickness of the resulting films was measured using a Sloan Dektak surface profile measurement system, model 900050. The procedure consisted of scratching the surface of the films with another glass of a defined width and creating a pattern of consecutive grooves then cleaning the scratched surface with alcohol. Using the profiler head the scratched surface was analyzed. We averaged the height of the film between several consecutives grooves. This average was reported as the sample average thickness used in the measurement of the conductivity of the films.

2.4 Conductivity measurement and frequency response

We used the standard two-probe method to measure the film surface electrical resistivity [11,12]. We used a Keithley 2400 source-meter interfaced with a computer and controlled with a LabView program. A voltage sweep was performed, and current flow between the contacts was measured. Copper threads were attached to the gold electrodes using indium solder. Film and gold electrode geometry were fixed and connected to the voltage source as shown in Fig. 1. Spin coating speed was used to control film width, and PEDOT/PVA/EG concentration and polarization voltage were other control parameters.

For the frequency response determination of the PEDOT films, we used a data acquisition card (PCI-2517 from Measuring Computing) and Matlab to generate harmonic signals of increasing frequency. A standard carbon resistor was added in series to the film to limit current flow.

It is important to mention that the measures reported in figures 3 to 5 were obtained from a single record. However, we performed these experiments many times for all the films and we observed the same behavior.

2.5 Electrogenic cell dissociation

We used two different dissociated cell types sourced from lab animals raised at our institute vivarium and followed the institute's bioethical research guidelines.

Adult Sprague-Dawley rats between 200-250 g were anaesthetized with sodium thiopental and operated to remove the hearts. The organs were immediately put into a Langendorff perfusion apparatus as described previously [13]. The hearts were perfused

by a combination of physiological solution and type 2 collagenase to dissociate the cells from the tissue. Finally, the ventricular cardiomyocytes were separated by centrifugation.

Mouse muscle fiber cells were dissociated from the *flexor digitorum brevis* (FDB) from our institutional strain, IVIC-NMRI. The mice were of 6 weeks old and weighed 31-36 g. The mice were killed by cervical dislocation, and the FDB muscles were extracted from the back legs surgically. Myocytes were enzymatically dissociated as described in the literature [14,15].

Single cell type isolation, (cells obtained by primary culture) was verified by optical inspection for each desired cell type, i. e., ventricular cardiomyocytes and peripheral skeletal fibers.

2.6 Electrophysiology experiments

The experimental setup to introduce a stimulus signal using the PEDOT films as delivery interfaces consists of two external stimulators (see Fig. 2 below). A current generator (Isostim A320) delivered square current pulses ranging in amplitude from 100 nA to 10 mA. A voltage generator (Grass SD9) generated square pulses of amplitude between 0.2 V and 100 V. They were connected in cascade, where the current stimulator is attached to the data acquisition system (Digidata, Molecular Devices) for control and synchronization of response acquisition. The voltage generator introduced the stimulus to the cells via the PEDOT films. Pulse width was varied between 2 ms and 4 ms through a frequency range of 2 Hz to 5 Hz. The size of the pulses was set on 80 V for cardiomyocyte and 18 V for muscle fibers. These parameters were taken from the literature [14-18].

The cardiomyocites were confirmed to be quiescent in culture prior to external stimulation. The cell solution was contained within a well that was constructed with a small (1 cm diameter) O-ring attached with Vaseline (a water-impermeable petrolatum gel) to the top of the polymer film. There was no physical contact between the gold electrodes and the cell solution. The cell solutions contained approximately 80 cells in 250 µl of physiological solution with the following reactant concentrations: NaCl=140 mM, KCl=5.4 mM, $MgCl_2$=1 mM, NaH_2PO_4=0.33mM, CaCl=1mM, glucose=10mM, Hepes=10mM.

Electrical activity of the cells was quantified with the set-up shown in Figure 2. Mechanical contractility was measured by recording a video of the cell motion using a CASIO Exilim EX-FC100 camera with capacity to record at 30 and 210 frames per second. Then image analysis was used to obtain the frequency and strength of contraction of the cells. An algorithm to calculate cell length in pixels was applied to each frame of the recording of the cell during the stimulation cycle. Cell length was then tracked in time using the temporal resolution of the frame rate set on the camera. With this information we created the temporal figures reported in Figures 6 and 7. It is important to mention that the measures reported in both figure 6 and 7 were obtained from a single record. However, we performed these experiments many times for both types of cells and we observed the same behavior.

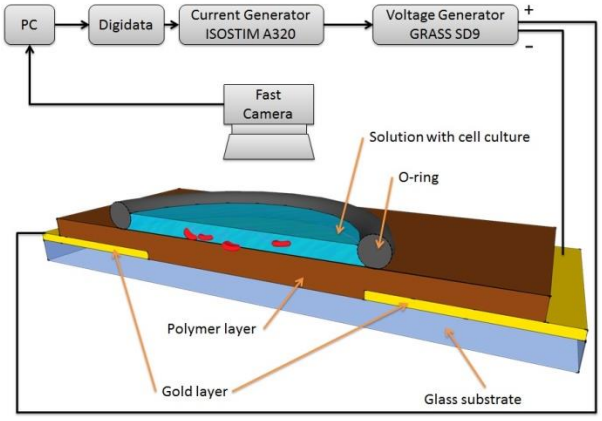

Figure 2. Experimental setup for excitation and measurement of the contractile response of the electrogenic cells (cardiomyocytes and muscle fibers). The excitation was delivered through the conducting polymer films only.

3. RESULTS AND DISCUSSION

3.1 Electrical characteristics

We measured the current-voltage (I-V) characteristic to assess the ability of the various films to sustain their conductivity and capacity to deliver current by varying the excitation voltage. Initially, PEDOT 2 was mixed in a ratio 5:1 with SDS. This surfactant has the ability to increase the attachment of the PEDOT on the glass surface as well as increase its intrinsic conductance. Using five different films widths from 130 nm to 390 nm, we obtained the average conductivity for this composite to be 380 +/- 50 S/m. Different widths were

obtained by varying the velocity of the spin coater.

It has been shown [19-22] that the addition of polar solvents helps to improve the electrical characteristics of conducting polymer films. In our experiments, we systematically added different combinations of solvents and evaluated the effects on the resulting films. Figure 3 shows the comparative effects the addition EG and SDS had in the I-V characteristics.

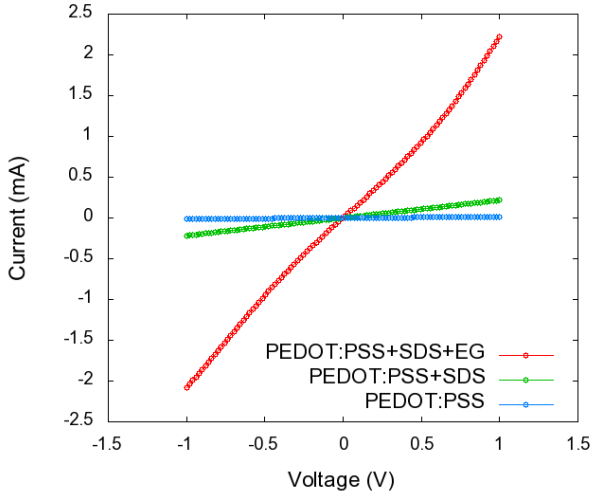

Figure 3. I-V characteristic of PEDOT 2 films with the addition of surfactant SDS and polar solvent EG at a single spinning speed (1000 RPM).

The mixture procedure of adding SDS and EG is detailed in Section 2.1. The addition of SDS increases the conductance of the films slightly; the addition of EG increases film conductance two-fold. Table 1 contains the computed values of the conductance for each case illustrated in Fig. 3.

Table 1. Relation between mixture and film conductance for the polymer mixtures of Fig. 3.

PEDOT:PSS + solvents	*Conductance (S)*	*Conductivity (S/m)*
PEDOT 2	0.13144200e-4 +/- 2e-13	28 +/- 6
PEDOT 2+SDS (20%)	2.16672000e-4 +/- 4e-12	330 +/- 50
PEDOT 2+SDS+EG (20%)	20.26980 e-4 +/- 1e-9	3400 +/- 600

Because the conductive polymer films are going to be used as excitation delivery media for biological cells, we were interested in testing the stability properties of the electrical characteristics when the films are subjected to high amplitude and changing stimulus voltages. Figure 4 shows how conductance stability is degraded with increasing driver voltage. The results for the PEDOT:PSS+SDS composite are shown in Figure 4(a), the PEDOT:PSS+PEG in Figure 4(b). The nearly constant conductance throughout the driver voltage range of the polyethylene glycol composite, compared to the SDS composite, is clearly demonstrated.

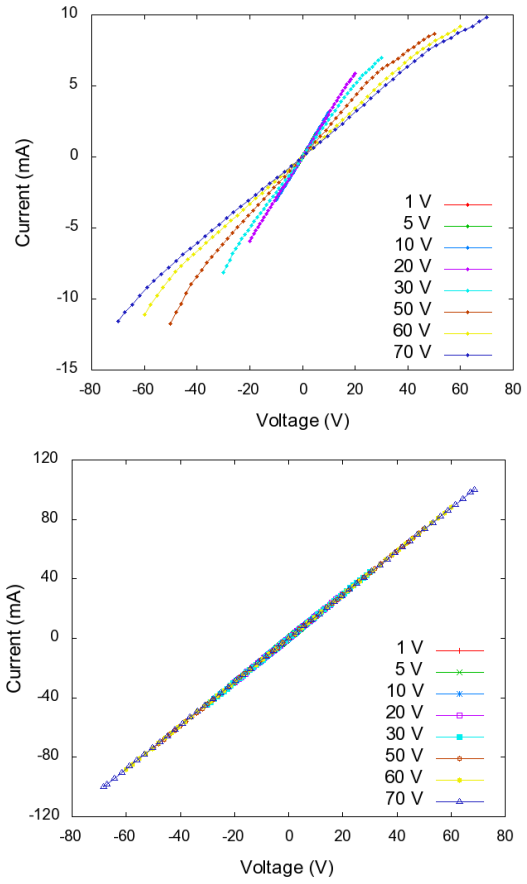

Figure 4. (a) Degradation of the conductance of the film made with PEDOT:PSS+SDS when the polarization voltage is highly increased. (b) Conductance stability for the film made with PEDOT:PSS+PEG and increasing polarization voltage.

Because of its good adhesion properties, the hydrogel poly(vinyl alcohol) is highly resistant to solvents, oil, and grease [23-25]. Figure 5 shows conductance as a function of PVA concentration and EG concentration. We used PVA to strengthen the adhesive properties of the films and added EG to

counter the strong insulating property of PVA.

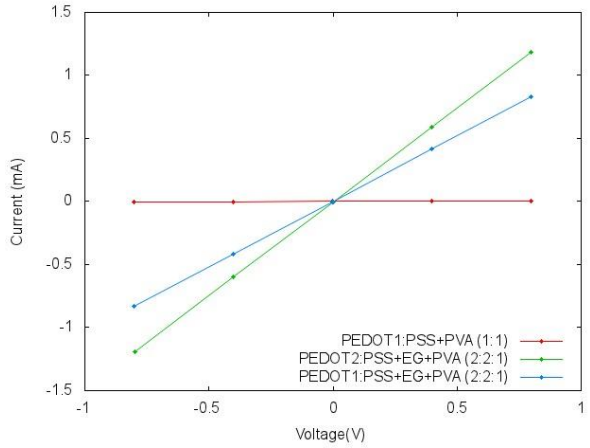

Figure 5: I-V curves of the polymer films showing improved conductance by adding EG and PVA to PEDOT 1 (red) and PEDOT 2 (green).

Table 2. Relation between mixture and film conductance for the polymer mixtures of Fig. 5.

PEDOT:PSS + solvents	*Conductance (S)*	*Conductivity (S/m)*
PEDOT 1+ PVA	0.553e-8 +/- 2e-11	4.60e-3 +/- 2e-5
PEDOT 2 + EG + PVA	1.04273e-3 +/- 4e-8	63.8 +/- 0.3
PEDOT 1 + EG + PVA	1.48486e-3 +/- 2e-8	432 +/ -3

The improvement of the conductivity on PEDOT films by the introduction of EG and PEG has been reported in [19]. This combination makes the composite water-insoluble, and it is suggested that an increase in inter-chain interaction and chain transformation from the benzoid to the quinoid structure occur following EG and PEG addition. The transformation of the resonant structure suggests a conformational change of the PEDOT chains from the coil structure into expanded-coil or linear structure. In the reference [19], the authors propose that the driving force for the conformational changes in the PEDOT chains is the interaction between the dipole of one polar group of the organic compounds and the dipoles or the charges on the PEDOT chains that produce the enhanced conductivity.

The last electrical characterization performed was the frequency response of the films. It is important, especially with the aim of building more complex devices, to know the spectral characteristics of film-based conductive polymers. We subjected several films combined with common carbon electrical resistors to a frequency-varying source of constant-amplitude sinusoidal signals. We obtained a constant response of the polymer films to the change of frequency in the indicated range. This response is typical for linear ohm's-law resistors, hence our films can be modeled by this basic electrical device. Frequency responses were evaluated up to 1 kHz since the typical response and activation times of biological cells do not exceed that frequency [26,27].

3.2 Physiological measurements

To perform this experiment we deposited a fixed amount of physiological solution with cells into the O-ring and left the cells to accommodate and adjust to their new environment for 6 hours before the measurements began. In Figure 6 we show that the transmission of the excitation pulse by the film PEDOT:PSS+EG+PVA with concentration as shown in Figure 5, was delivered successfully achieving a strong response in the excitable cardiac cell. The figure plots the relation between the relative lengths of the cell during maximum contraction (insert picture). This contractile motion (blue curve) is in full synchrony with the excitation signal (red) delivered exclusively through the conductive polymer film.

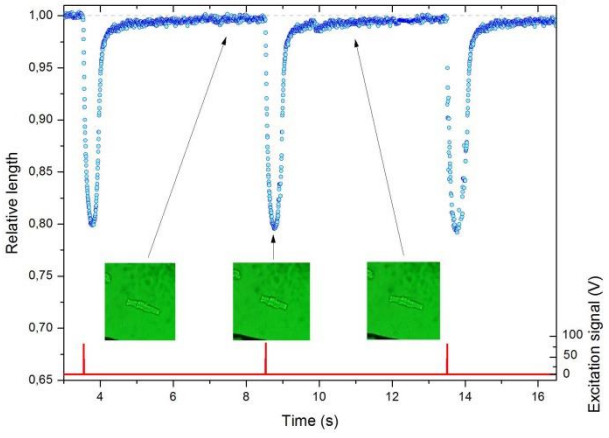

Figure 6. Cardiomyocyte contraction in response to PEDOT+EG+PVA film excitation. The excitation voltage (red spikes) used for cardiomyocyte was 80 V in amplitude and 4 ms in width.

Next in Figure 7, we show the same experiment but changing the excitable cells only. This time we used

muscle fibers FBD and obtain a very similar response as for the cardiomyocytes. The insert pictures show different instants while the cell are performing a full contraction motion, and their contractility motion (blue curve) measured as a relative size change, is again in full synchrony with the excitation signal (red) being delivered only through the conducting polymer.

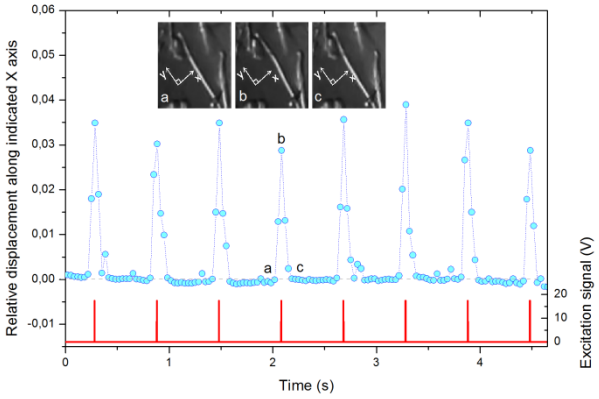

Figure 7. Skeletal muscle fiber contraction in response to PEDOT+EG+PVA film excitation. The excitation voltage (red spikes) used for muscle fibre was 18 V in amplitude and 4 ms in width.

4. CONCLUSIONS

We have shown that the conducting polymer PEDOT can be combined with a diverse mixture of composites in order to improve its conducting and bio-sensing-actuation capacities. We showed the specific construction of simple films for biosensing applications and their use in real biological experiments. In particular, we showed that the addition of the polar organic compound EG, polyvinyl alcohol, and PEG greatly improve film conductance in a biological environment.

It is important to note here that our films were reusable. A single stimulation film was reused in two or three experiments before showing signs of degradation in consistency and homogeneity. Only the PEDOT:PSS+EG+PVA compound was used in the biological experiment. Room for improvement in biosensor technology remains regarding longer duration times in harsh biological environments. Our demonstration of a PEDOT film biosensor/actuator with excellent conductance is a good base to build upon for future applications, including new drug testing in diseased-cell environments.

In order to have a more accurate representation of the contractility signals it is necessary to perform a greater number of experiments than those reported in figures 6 and 7 above. However we can attest that the phenomenon is very robust and repeatable as we observed in other experiments that are not reported here. The same can be said of the conductivity measures reported in figures 3 to 5, where the plots represent a single film measurement, however again we did many more conductivity tests that were not included in this report.

5. ACKNOWLEDGEMENTS

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