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Application of Bio-Nanocomposite Photoelectric Material for the Therapeutic of Cardiac Disorder: an Integration of Bio-hybrid System

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Abstract

For the treatment of fibrillation i.e. a cardiac disorder, wherein electrical currents are used as therapy. This disorder manifests chaotic and accelerated heart rhythm, preventing the pumping of blood to the rest the organism. In considering the remedy of this heart disease, in this study we devised therapeutics for the same through bio-nanocompositephotoelectric patches; by the application of direct immobilization of bacterial photosynthetic reaction center (RC) proteins on nanocrystalline TiO_2matrix.

Key words : Cardiac therapeutics, nanocomposite, biomaterial, photoelectric.

1. Introduction

As technology has advanced, the capabilities and sensitivity of cellular studies have greatly improved. Such that this improved Techniques[1-3] allow examination of single cell electrical characteristics eitherin vivo(within the body) orin vitro(outside of the body) via glass micropipette or wire microelectrodes [4-6]. More recent advances allow for populations of cells to beexamined simultaneously [7-11] thereby permitting researchers to gain better insight into the functionality andinterconnectivity of cellular networks. These same technologies also offer theopportunity to utilize living systems in different sensor [12,15] or actuator [16, 17] applications. The use of living cells as sensor elements provides the opportunity for high sensitivity to a broad range of biologically active substances which affect the electrochemical response of a cell. These biologically relevant signals can be directly measured using microelectrodes which provide a stable, non-invasive interface for monitoring populations of cells. The potential uses for such cell based sensors and actuators include environmental monitoring (chemical/biological warfare agents, groundwater contamination, etc.), pharmaceutical screening, drug discovery and basic neuroscience.

It is well known that some cardiac disorders are treated by using external electrical discharges as therapy. Within heart cells, calcium plays a major role in orchestrating normal heart pump function. However, in ventricular fibrillation or diastolic failure the calcium signalling process is slowed; calcium levels rise to the peak needed for the squeezing action of the heart but don't then drop quickly enough for an efficient relaxation period. In this case, the patient needs an electrical discharge of defibrillation to correct the heart rhythm [18, 19]. Thus, at this instance a photosensitive device is required which can release such electrical dischargesat controlled rate. Since bacterial photosynthetic reaction center (RC) was reported as a functional protein isolated from purple bacteria *Rb. Sphaeroides* with very high quantum efficiency (ca. 100%) of the photo-induced charge separation in the red to NIR region [20, 21], it greatly thrust our research to construct a Photoelectric patches through modification of RC onto a specific matrix. With the aim at exploit the

photoelectric responses of RC-based photoelectric devices, it is conceivable to make RC serve to photosensitize the semiconductors that have feeble light absorption at relative long wavelength region for their wide band gaps. The photoelectric properties of RC/TiO_2 composite film showed high efficient NIR light-harvesting capability, which made it possible to develop novel bio photo-actuated device at molecular level. To ascertain RC/TiO_2as photoinduced molecular pacemaker,experiments regarding measurements of the photoelectrical characteristics of RC/TiO_2 system are examined.

2. Experimental Details: Preparation & Measurement of the RC/TiO_2 Bio-Nanocomposite Material

By using anodic oxidative hydrolysis of $TiCl_3$ Nanocrystalline TiO_2 was prepared [22]. RC prepared from the photosynthetic bacterium *Rb. Sphaeroidesstrain RS601* was separated and purified (the detailed process is protected as it is filed for patent). Later, protein immobilization was attained by immersing the TiO_2 matrix on a film in the RC solution (at 4°C) for 2 ~ 3 days. Here, RC solution was consist of 0.5 ~ 4μM RC and the pH 8.0 Tris-HCl buffer which furthermore contain 50 mMTris amino-methane and 50 mMKCl. Here, TiO_2 film (amorphous form) was prepared by the anodic electrodeposition. Then, it was annealed at 450° C. Finally, the porosity derived from the intercrystalline voids of the TiO_2 film results in a relatively high surface area.

NIR-visible absorption spectra and fluorescence emission spectra were obtained by using SM-240 CCD spectrophotometer and a SM-300 luminescence spectrometer at room temperature. All experimental solutions were deoxygenated by bubbling nitrogen through them before measurements. Photocurrent tests were carried out in a shielding box by illuminating the working electrode with a 20 W in candescent lamp. Incident light intensity (I_{inc}) on the external surface of the cell was 5 mW cm^{-2} measured with a CCD detector (SM-240). A filter (λ> 600 nm) was applied or not and the intensity of the output through the filter was measured to be 0.1 mW cm^{-2}. The photoelectric signals were recorded by a CHI-660A electrochemical workstation (CHI Instrument Co., USA). The electrode potential was set at the open-circuit voltage (~ - 0.15 V for the bare TiO_2 film and ~ -0.1 V for the RC/TiO_2 film) before measuring the short-circuit photocurrent (I_{sc}). The back-vground dark current was less than 10 nA cm^{-2}.

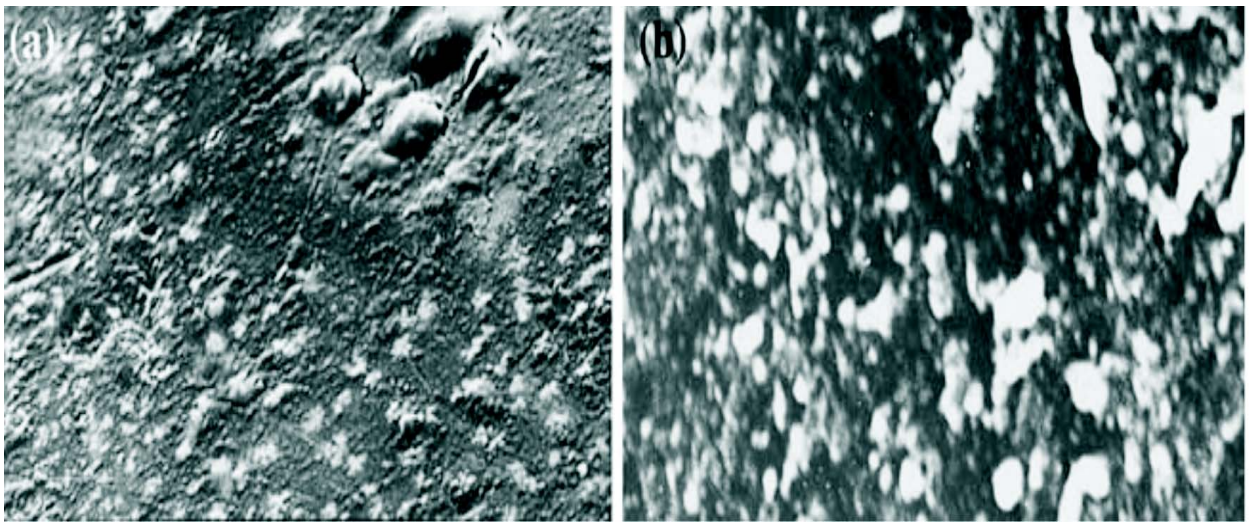

Fig.1. Scanning electron micrograph of a 500-nm-thick film consisting of the nanocrystalline TiO_2 particles with grain size ranging from 30-70nm. (a) processedRC/$TiO_2$with immobilized Rhodobactersphaeroides *RS601* without attachment to any cell, (b) Processed RC/TiO_2 with attachment to cardiac cells at the surface

Table 1
Free ionic concentrations and equilibrium potentials for mammalian skeletal muscle [23]

Ion	Extracellular Concentration (mM)	Intracellular Concentration (nM)	Free ionic concentration	Equilibrium potential(at 37°C) in mV
Na^+	145	12	12	+67
K^+	4	155	0.026	-98
Ca^{2+}	1.5	10^{-7} M	15,000	+129
Cl^-	123	4.2	29	-90

3. Results and Discussion

The cardiac action potential involves the combined action of at least four major classes of biologically active ions. It is certain from the Table 1 that in all cases, both potassium and chloride drive the intra cellular potential negative with respect to the extra cellular, while sodium and calcium attempt to drive it positive. This holds true for most cell types even though the equilibrium potentials are different.

The sodium channels open and allow a large influx of Na^+, causing a rapid depolarization and overshoot (the transmembrane potential becomes positive). After a brief period, these channels close and remain closed until the action potential is over. This is a phenomenon known as inactivation and occurs with almost all gated ion channels. It sets a limiton the duration of different portions of the action potential, as well as the overall rate at which a cell may fire. As the Na^+ influx drives the transmembrane potential more positive, the threshold for opening

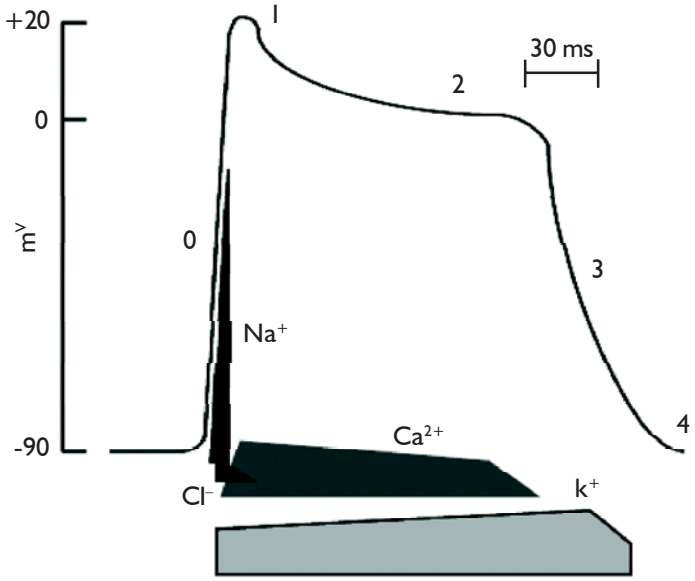

Fig. 2. Phases of a human cardiac action potential. The relative conductance of each of the four major ionic channels are also shown, where the area of the shaded regions is proportional to the conductance of that channel. In phase 0, a large influx of Na^+ ions causes the rapid depolarization and overshoot of the membrane. As the Na^+ channels close and Cl^- channels open, there is an initial rapid repolarization (phase 1). Phase 2 is known as the plateau and is due to the slower, prolonged opening of Ca^{2+} channels. This state causes fibrillation & if stays prolong, then may cause diastolic failure. The final repolarization (phase 3) is due to closure of the Ca^{2+} channels while the K^+ channels remain open (allowing potassium efflux). The resting potential is restored in phase 4 [24]

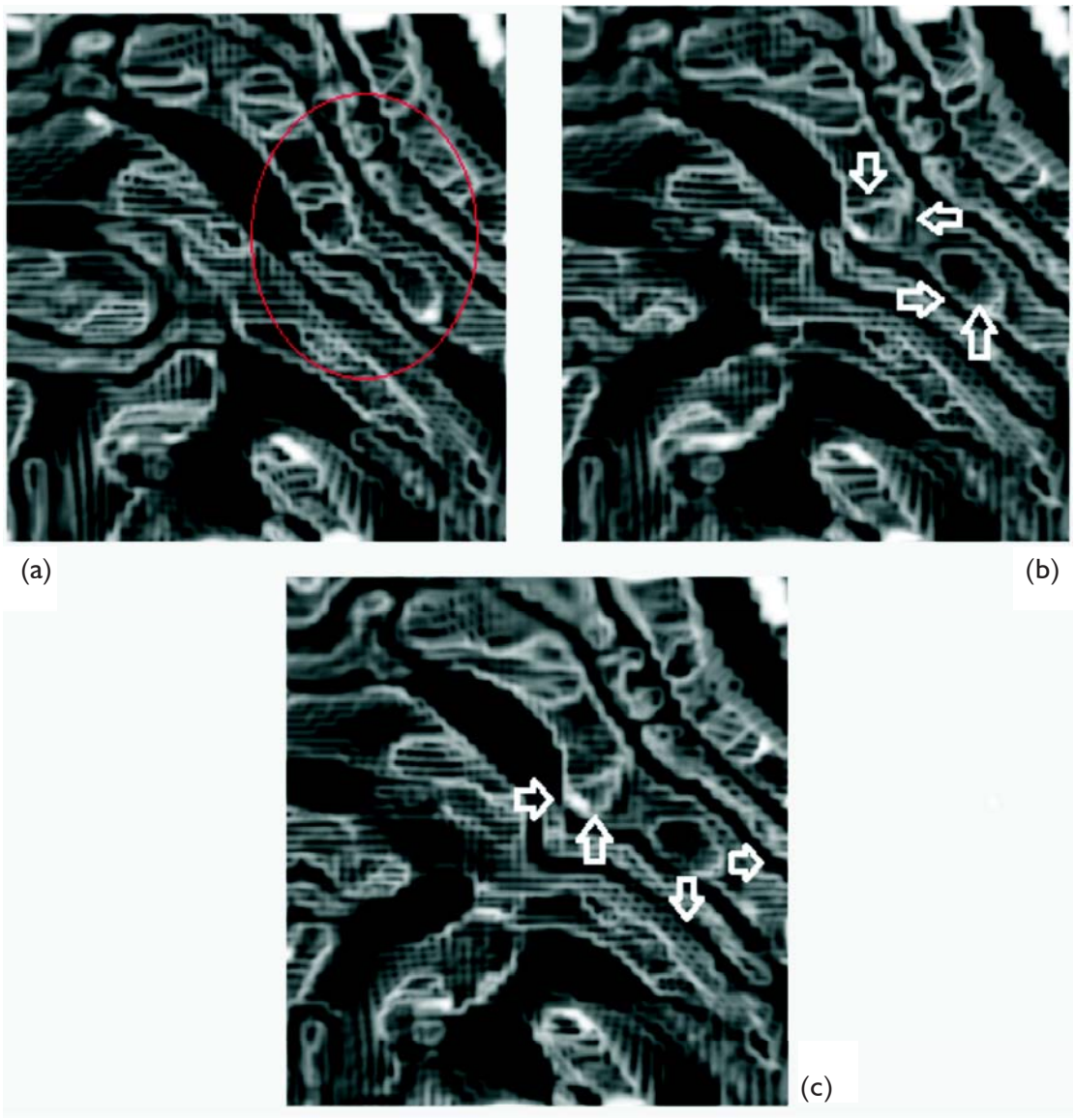

Fig. 3. 3D reconstruction of fluorescence images for Localization of RC/TiO_2patches in cardiaccells. Optical sections of the cells representing the different phases of the cardiac action potential in cell polarization cycle: (a) Circle bounded region(most noticeable area) represents Phase 2 state of fibrillation from figure.4 which is due to the slower, prolonged opening of Ca^{2+} channels(b) The white arrow points for initiating repolarization (phase 3) due to conjugation between cells &RC/TiO_2photoelectricpatches, and (c) unlaxing nearing completion(phase 4) as the resting potential is restored. No scale bar is presented for panel, as the 3D reconstruction process can fractionally distorts the image parameters, and therefore, a scale bar might prove misleading in terms of the dimensions of the 3D reconstruction structure

of Cl^- channels is reached. These channels remain open briefly allowing a small influx of chloride ions which cause a rapid (and small) repolarization. At the same time, calcium and potassium channels are opened and remain open for an extended time. Thus, there is an influx of Ca^{2+} and efflux of K^+ which tend to balance each other resulting in the plateau phase of the action potential. The calcium channels closes before the potassium channels, resulting in the repolarization of the membrane. Eventually the

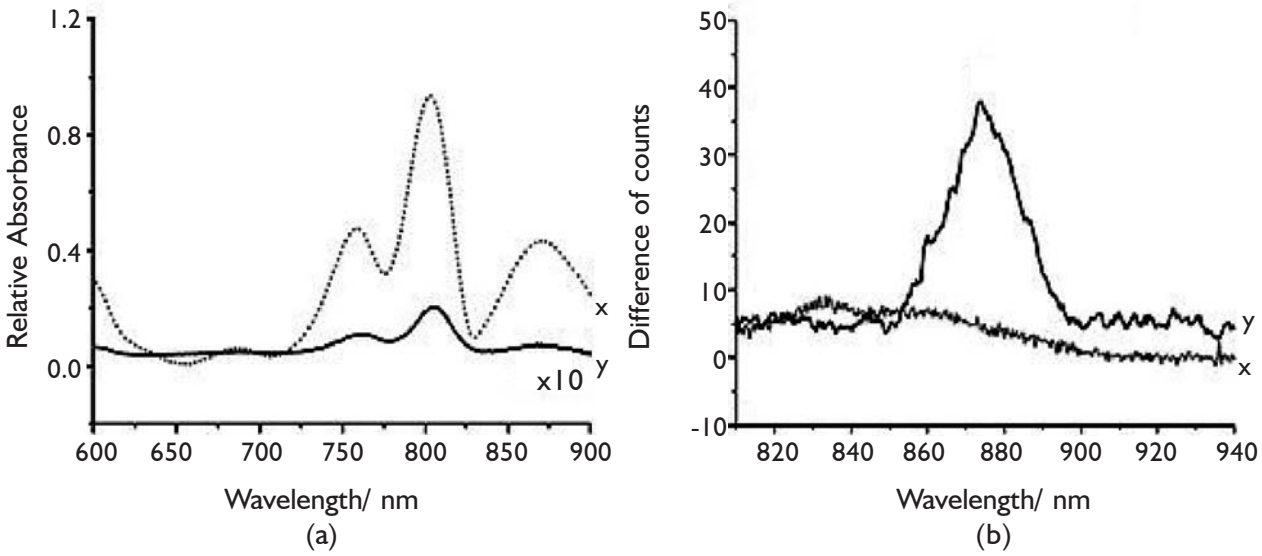

Fig. 4. Steady absorption spectra and fluorescence emission spectra of the RC/TiO_2 composite film. (a) NIR-visible absorption spectra (x, dotted line) and the RC/ TiO_2 film (y, solid line). Absorption of the bare TiO_2 film was subtracted as background. (b) Fluorescence emission spectra of the RC-free TiO_2 film (x, dotted line) and the RC/TiO_2 film (y, solid line) at 293 K, excited at 800 nm and detected at 870 nm

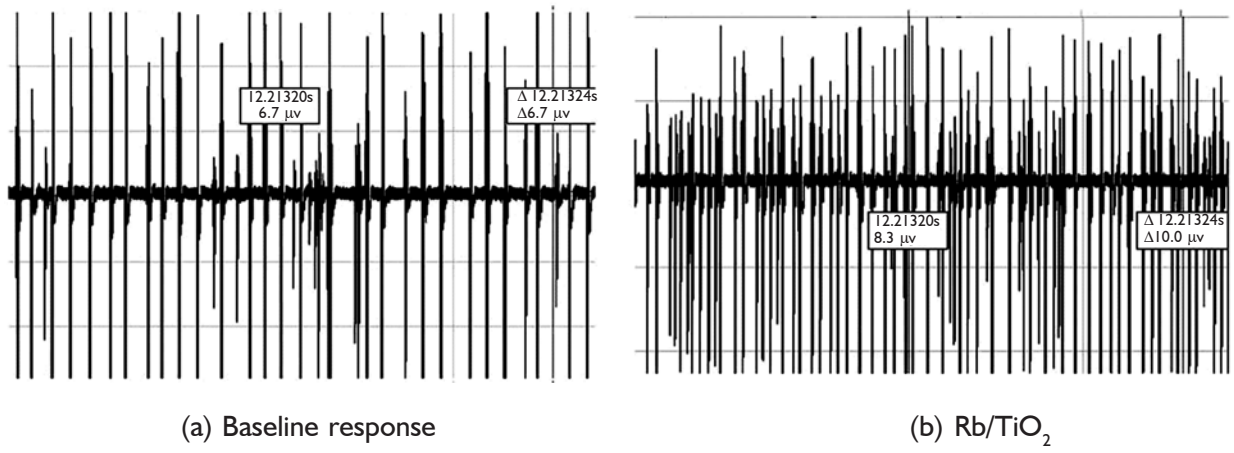

Fig. 5. Cultured cardiac cell responses measured through microelectrode arrays (a) A baseline responses before using Rb/TiO_2(b) cells showing a chronotropic response to Rb/TiO_2

equilibrium condition (resting membrane potential) is reached.

In Rb, the redox potential of light-initiated primary electron donor P is about 0.5 mV. The band gap between the ground state and the excited state of P was reported about 1.1～1.3 eV. When the RC/TiO_2 composite patch is illuminated by photons with energy exceeding the band gap of RC, the electrons from the light-induced P* quickly inject the conduction band of TiO_2. The photo-generated negative charge carriers can effectively transfer due to the matching energy level of TiO_2 and RC. On the other hand, the simultaneously generated positive holes (P^+) congregate at the interface between the composite patches. High efficient light harvesting capability of the NIR light energy by RC compensates the negligible light absorption of TiO_2

at long wavelength region, which enables the derived composite patches to capture the light energy effectively.

Proton uptake upon the photo reduction of the secondary quinine (QB) in RC is a key step in establishment of proton-electrochemical potential, the driving force for ATP formation. As mentioned previously, gating of ion channels can be triggered by attainment of a specific transmembrane potential (threshold) or by binding of a ligand to the channel. This ligand binding is often dependent on G-proteins (nucleotide regulatory proteins) which can couple cell surface receptors to catalytic units depending on the surface charge. Thus, at this point the net surface charge of RC changes from the positive to the negative as the channel permeability of the medium become higher than the isoelectric point of RC (about 7 ~ 8). Since TiO_2 matrix also has a negative surface charge in this pH region, it is considered that the stability of the RC/TiO_2 composite patch would be destroyed. In view of the two factors, the optimal pH range for the I_{sc}generation of RC at λ>600 nm apparently exists in the neutral pH region, as shown in Fig. 4 (a). Figure 4 (b) depicts the relationship between the Isc measured at λ>600 nm with the applied bias in pH 8.0. These catalytic units after photoelectric excitation from RC/TiO_2 create intracellular second messengers that travel through the cytosol and bind to the channels causing them to open. G-proteins can also couple receptors directly to ion channels to control gating. For the purposes of this work, a more detailed understanding of these ligand binding mechanisms is not necessary. Awareness of their existence and recognition that they can affect channel permeability in the same manner as transmembrane voltages is sufficient. For RC, the applied bias deeply influences the sequence of photo-induced electron transfer between the electron donors and acceptors inside the protein.

Since, cardiac cells grow in close contact to one another and form what are called *gap junctions*. Integral proteins (called *connexons*) form channels through the membrane of the cell. When the connexon in the membrane of one cell lines up with connexon in another cell, a channel is formed between the cells through which substances can pass without entering the extracellular solution. This acts as an electrical connection through which the depolarization in one cell can initiate depolarization in a neighboring cell. If this depolarization is sufficient to trigger opening of the Na^+ channels, then the neighboring cell will free its own action potential. In this way the action potential is propagated through cardiac tissue. Contraction of the tissue is initiated by the action potential. The action potential triggers the release of internal stores of Ca^{2+} which binds to *troponin* C (troponin C inhibits the interaction between actin and myosin). With the troponin C bound, the actin-myosin interaction is able to occur. Thus, allowing contraction of actin filaments. This forms the basis of contraction in cardiac tissue by artificial stimulation of RC/TiO_2bio-nanocomposite photoelectric patches.

4. Conclusion

The successful entrapment bacterial photosynthetic proteins (RC) from *Rb. Sphaeroidesstrain* RS601 on the surface of nanocrystalline TiO_2 matrix give a very useful functional and conformational probe of the photosynthetic proteins. The fluorescence emission of RC modified nanocrystallineTiO_2 film also suggests the well-remained native activity of the photosynthetic proteins. The NIR-visible absorption and fluorescence emission spectra displayed that the structure and activity of RC remained unaltered on the surface of TiO_2 film. Thus, the need of electrical discharge for defibrillation using NIR wavelets is fulfilled by bio-hybrid RC/TiO_2 material to correct the heart rhythm of the patient. Such strategy of fabricating photosynthetic proteins onto specific matrix provides an alternative way to probe the photo induced electron transfer of photosensitive chromophores contained composite films. However, we are still working on developing an ideal delivery system for the optimized protein. We hope further research in this direction may one day help pave the way to a better way to treat patients.

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