Annals of Biomedical Engineering, Vol. 44, No. 8, August 2016 (© 2016) pp. 2464–2479
DOI: 10.1007/s10439-015-1528-8

Novel Conductive Carbon Black and Polydimethlysiloxane ECG Electrode: A Comparison with Commercial Electrodes in Fresh, Chlorinated, and Salt Water

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(*Received 24 July 2015; accepted 8 December 2015; published online 14 January 2016*)

Associate Editor Joel D. Stitzel oversaw the review of this article.

Abstract—In this study, we evaluated the performance of two novel conductive carbon black (CB) and polydimethlysiloxane (PDMS) bio-potential electrodes, with and without an integrated flexible copper mesh, against commercially available electrodes (Polar® textile, Silver-coated textile, and carbon rubber). The electrodes were tested in three types of water (fresh/unfiltered, chlorinated, and salt water). Our testing revealed that our CB/PDMS electrode with integrated copper mesh provided a high-fidelity ECG signal morphologies without any amplitude degradation in all of the types of water tested ($N = 10$). The non-meshed CB/PDMS electrodes were also subjected to a long-term durability test by the US Navy SCUBA divers during which the electrodes maintained ECG signal quality for a 6 h period of continuous use. The results of a material degradation analysis revealed the CB/PDMS composite material does not exhibit significant changes in physical integrity after prolonged exposure to the test conditions. The newly developed meshed CB/PDMS electrodes have the potential to be used in a wide variety of both dry and wet environments including the challenge of obtaining ECG signals in salt water environments.

Keywords—Underwater ECG, Carbon electrodes, Salt water, Textile ECG electrodes, Dry electrodes, Reusable electrodes.

INTRODUCTION

The benefits of electrocardiogram ECG recordings are well known, undisputed in dry conditions, and provide a wealth of physiological information. Such information can be equally important in underwater environments where prolonged hyperbaric exposure, a common occurrence in recreational and technical diving, has been shown to lead to many neurological[1,8,9,11] and cardiovascular[2,4,8,10,11] problems including decompression sickness (DCS)[1,5,8] and performance degradation.[6] For example, Navy divers may need to perform repeated dives even during a time span not recommended by the US Navy Decompression Dive Table, and rescue procedures in a disabled submarine (DISSUB) scenario may not accommodate staged decompression. It is important to detect as early as possible any potential onset of DCS so that recompression can be performed. In our recent work with swine models, we demonstrated that early detection as well as differentiation between cardiopulmonary (non-neurological) and neurological DCS can be made[1,2,13] from an ECG signal. For neurological DCS, we found more than 50% reduction in both the sympathetic and parasympathetic dynamics,[1] whereas significantly elevated parasympathetic and reduced sympathetic tones were seen with cardiopulmonary (CP) DCS.[2] For CP DCS, we also found significant increase in the T-wave amplitude of the ECG waveform and elongation of the QT interval when compared to non-CP DCS condition.[2] Therefore, monitoring of the changes in the dynamics of the autonomic nervous system as well as morphologies of the ECG signal in hyperbaric and full water immersion conditions can play an important role in identifying dangerous conditions and allow for a prompt response while any symptoms are in their early stages.

Electrodes play a key role in measurement and performance of vital signs monitoring devices. The most common ECG electrodes are hydrogel silver/sil-

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ver chloride (Ag/AgCl) electrodes. Although Ag/AgCl electrode usefulness and effectiveness in clinical environments has been unparalleled, their usability is compromised in wet conditions. Furthermore, factors like skin irritation, limited shelf stability, disposability, and signal degradation over time,[12] limit the use of Ag/AgCl hydrogel electrodes in environments outside of the clinical setting. Hydrophobic electrodes should capture all ECG morphological waveforms not only in dry condition, but most importantly in different water compositions where fresh/unfiltered, chlorinated, and salt water are the more relevant types. Each water type has a different conductance due to varying ionic compositions where salt water poses the more challenging environment for ECG recording as the resistance can be as low as 10 Ω for salty water. When exposed to highly ionic environments the impedance between the electrode and skin surface interface is significantly reduced. Therefore, it is critical for an electrode designed for underwater ECG monitoring to be sufficiently isolated from the ionic components of the surrounding environment to function properly.

In our previous study, we presented a novel electrode for underwater ECG monitoring, but the electrodes were not tested in salt water.[12] In that study, we found significant ECG amplitude reduction when the electrodes were tested in a chlorinated water environment. In this study, we reevaluated our electrodes design to overcome this amplitude reduction. Improvements were made by embedding a fine copper wire mesh into the Carbon Black/Polydimethylsiloxane (CB/PDMS) posterior to the electrode contact surface. Additionally, in this study we compared the performance of commercially-available dry electrodes to our newly- and previously-designed CB/PDMS electrodes in three water immersion conditions (fresh/unfiltered, chlorinated and salt water). We studied dry electrodes as it has previously been shown that hydrogel-based electrodes perform poorly underwater.[12] The commercially-available dry ECG electrodes tested included the Polar® textile, a silver-coated textile, and a carbon rubber electrode.

MATERIALS AND METHODS

Meshed CB/PDMS Electrode Fabrication

Hydrophobic CB/PDMS electrodes were fabricated following the procedure described in our previous study.[12] The following steps describe the fabrication procedure of the redesigned CB/PDMS with an embedded copper mesh:

1. The 3D printed Acrylonitrile Butadiene Styrene (ABS) cavity molds (Objet350 Connex, Stratasys, Eden Prairie, MN, USA) were filled with the conductive CB/PDMS composite, and leveled such that no excess material remains.
2. A copper mesh with an attachment point was then affixed on the CB/PDMS mix to allow signal acquisition *via* the monitoring device. Specifically, an insulated and waterproofed wire was soldered to the embedded mesh, and used as a connector to an ECG monitoring device.
3. A PDMS and curing agent mixture was then used to encapsulate the exposed surface with embedded copper mesh.
4. All components were degassed for an additional 15 min in a vacuum chamber.
5. The fasteners were soldered to the exposed end of the wire extending from the electrode.
6. The completed electrode assembly was then placed in a curing oven at 75 °C for 3 h.
7. After the 3 h the molds were removed from the curing oven and subsequently the electrodes were also removed from the cavity molds.

In this study, the contact surface area of the CB/PDMS electrodes was designed to be similar to the commercial electrodes tested. A diagram of the proposed meshed CB/PDMS electrode is shown in Fig. 1.

Electrode–Skin Contact Impedance

Contact impedance of one pair of each electrode type was tested over a frequency sweep from 4 Hz to 1 kHz when applied to a subject's anterior forearm. Each electrode pair was placed parallel to each other, with 2 cm separation, and perpendicular to the midline of the forearm. The skin area was cleaned with 70% isopropyl alcohol. The electrodes were fixed into position with medical adhesive tape (3 M, Transpore, St. Paul, MN, USA). The impedance tests were per-

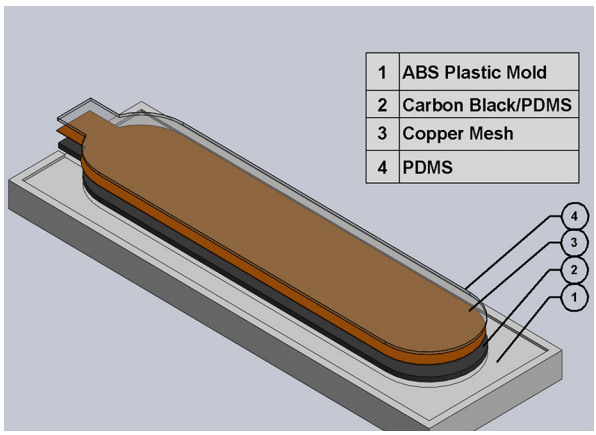

FIGURE 1. Electrode Fabrication cavity mold and fabrication procedure.

formed consecutively within a 1 h time period for dry, water immersion and wet conditions. Data were collected using an impedance analyzer (Hioki IM3570, Hioki E.E. Corp, Nagano, Japan).

Mechanical Properties Evaluation of CB/PDMS Electrodes

Aging in Aqueous Environments

To evaluate the temporal changes in the mechanical properties of the CB/PDMS electrodes, electrodes were rapidly aged by incubating them in a glass beaker for 5 or 14 days at ambient conditions or at 37 °C in one of three simulated liquid environments:

1. Fresh water environment, tap water with pH 7.7
2. Chlorinated water environment
3. Salt water environment simulated with aquarium salt (Instant Ocean, United Pet Group, Blacksburg, VA, USA) in DI water at a specific gravity of 1.022 (measured using a FLUVAL SEA hydrometer).

Dog bone shaped CB/PDMS electrodes were prepared by casting into custom ABS molds with dimensions according to ASTM D412-06a (Standard Test Method for Vulcanized Rubber and Thermoplastic Elastomers—Tension). Following aging, samples were removed from liquid environments and air dried for 24–48 h before tensile testing. Control (non-aged samples) were also prepared and tested within 24 h of fabrication.

Uniaxial Tensile Testing

Uniaxial tensile testing was performed to determine ultimate tensile strength (UTS), strain at failure, and elastic modulus (E) of CB/PDMS samples. The thickness of each sample was measured using digital calipers, and used to calculate the cross-sectional area, assuming rectangular cross-sectional geometry. Dry samples were loaded onto an Instron 5544 uniaxial testing machine (Instron Inc., Norwood, MA, USA) with a 2 kN load cell and screw action grips. Samples with a gage length of 33 mm were strained until failure at a constant rate of 500 mm/min according to ASTM D412-06a. The UTS was defined as the peak stress value on the stress–strain curve for each sample and the corresponding strain value was recorded as the strain at failure. The E was calculated as the slope of a best fit line ($R^2 > 0.95$) for strains from 0 to 40% (or from 0% to failure for samples that fail at strains $<40\%$). Samples that broke in the grip region were excluded from further analysis.

Statistical comparisons of different sample groups at each time point were performed using a one-way Analysis of Variance (ANOVA) with Holm–Sidak *post hoc* analysis. A significant difference between groups was indicated by a $p < 0.05$. Comparisons between the two time points within a particular sample group were made using a Student's t test. A significant difference between groups was indicated by a $p < 0.05$. SigmaPlot (SyStat Software Inc., San Jose, CA, USA) was used to perform ANOVA tests and Microsoft Excel was used to perform Student's t tests.

Cytotoxicity Analyses of CB/PDMS Electrode Media Extracts

Preparation of Material Extracts and Cell Culture

To investigate whether CB/PDMS electrode materials leach cytotoxic residues into the local tissue environment, we analyzed the *in vitro* cytotoxicity of extract liquids obtained from electrode samples. Material extracts were prepared according to ASTM F619-03 (Standard Practice for Extraction of Medical Plastics). Samples each containing five CB/PDMS electrodes (20 mm diameter × 2 mm thickness) were weighed and placed in clean 125 mL glass bottles. An equivalent weight (±0.5 g) of positive control latex disks (19.05 mm × 1.587 mm; McMaster-Carr, Princeton, NJ, USA) and negative control high density polyethylene disks (HDPE; 19.05 mm × 2.381 mm; McMaster-Carr, Princeton, NJ, USA) were also prepared in 125 mL glass bottles. The average total weights of the electrodes, HDPE and latex samples were 3.510 ± 0.093, 3.492 ± 0.281 and 3.377 ± 0.136 g respectively. An appropriate extraction medium (for L929 cells, high glucose DMEM; for NHEKs, KBM-Gold) was added to each bottle at 5 mL medium/g sample weight. An additional extract medium control (no material samples) was prepared by adding 17 mL appropriate extract medium to an autoclaved 125 mL bottle. All material extract samples and extract medium controls were incubated at 37 °C for 5 days on an orbital shaker. After 5 days of extraction, extract medium was removed from material samples and stored in sterile glass bottles for up to 24 h before use in the Extract Cytotoxicity Assay.

For cell culture studies, extract medium cytotoxicity assays were performed on confluent monolayers of L929 mouse connective tissue fibroblasts (CCL-1; ATCC, Manassas, VA, USA) or pooled neonatal normal human epidermal keratinocytes (NHEK; Lonza, Walkersville, MD, USA). L929 cells were cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum

(FBS; Hyclone, Logan, UT) and 100 U-mL/100 mg-mL/2 mM penicillin/streptomycin/L-glutamine (Life Technologies, Carlsbad, CA). L929 cultures were maintained at 37 °C, 5% CO_2 and passaged at 80–90% confluence according to ATCC recommendations. For extract cytotoxicity experiments, L929 cells were seeded at a density of 27,000 cells/cm^2 in a 24-well plate and grown to confluence. NHEK cells were cultured in KBM-Gold medium (Lonza, Walkersville, MD, USA) at 37 °C, 5% CO_2 and were subcultured at 80–90% confluence according to Lonza recommendations. For extract cytotoxicity experiments, NHEK cells were seeded at a density of 20,000 cells/cm^2 in a 24-well plate and grown to confluence.

Extract Cytotoxicity Assay

To assess the cytotoxicity of medium extracted from the electrodes, extract cytotoxicity cell culture evaluation was performed according to ASTM F619-03. Cell culture medium was removed from confluent cell layers and replaced with the extracted medium. Immediately prior to replacement, the L929 extract medium was supplemented with 10% FBS and 1% penicillin/streptomycin/L-glutamine. Samples were incubated with the extracted medium for 24 h, and the morphology of cells was assessed by phase contrast microscopy. Wells with no cells and no added extract medium (fresh medium was added instead) served as background controls.

Cell viability was assessed using an MTT reduction assay to measure cell metabolic activity. Extract medias were removed from wells and cultures were incubated in medium supplemented with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma, St. Louis, MO, USA) at 37 °C, 5% CO_2 for 2 h. Unreacted MTT solution was aspirated. The formazan product was solubilized with 500 μL dimethyl sulfoxide (DMSO; Sigma) per well and the supernatant was diluted 1:4 with DMSO. The optical density of the sample (ODsample) was measured in triplicate in a microplate (100 μL/well) at 540 nm using a SpectraMax 250 plate reader. Extract cytotoxicity experiments were conducted on 9 samples of five electrodes each and 9 samples each of HDPE and Latex controls of equivalent weight for each cell type. Each sample was added to confluent cell layers in triplicate, resulting in $n = 27$ samples for the MTT assay. Statistical differences for cytotoxicity studies were evaluated using SigmaPlot version 12.5 (Systat Software, Inc.). A Kruskal–Wallis one-way ANOVA on ranks with Student–Newman–Keuls *post hoc* analysis was performed. A significant difference between groups was indicated by a p value <0.05.

Underwater ECG Recording

Subjects

Ten ($N = 10$) healthy male volunteers of ages ranging from 21 to 40 years (mean ± standard deviation 28.40 ± 5.91), weight 72.79 ± 11.45 kg, height 175.0 ± 4.66 cm, and body mass index (BMI) 23.82 ± 3.89 were enrolled in this study. The group consisted of students and staff members from Worcester Polytechnic Institute (WPI), MA, USA. The study protocol was approved by the Institutional Review Board of WPI and all volunteers consented to be subjects for the experiment.

Equipment

We designed and fabricated a 1-channel ECG monitoring device for Lead I measurements *via* two electrodes with virtual right-leg driven circuit. This device provides a frequency band at −3 dB from 0.05 to 150 Hz with second- order high-pass and low-pass filters to cover the full ECG range, and a sampling rate of 360 Hz. The ECG signals measured from this device were transmitted to a personal computer *via* Bluetooth wireless communication. A LabVIEW^{TM} software (National Instruments, TX, USA) was developed for wireless transmission of the collected ECG signal, real-time display, and data storage for further off-line data analysis.

Electrodes

Five different types of dry electrodes were tested in this study. For each type, a 2-lead electrode configured as an elastic chest strap was used for all three test conditions. The placement of the 2-lead chest strap put one electrode on the left and one electrode on the right side of the rib cage. The five types of electrodes tested are shown in Fig. 2 and consisted of:

1. Polar® textile electrode, which is fabricated by sewing both silver-coated and normal threads in a lattice pattern.
2. Silver-coated textile electrode which is made by coating silver particles on normal fabric.
3. Carbon rubber electrode which is made by mixing carbon powder and a rubber component.
4. CB/PDMS electrode.
5. Modified copper-meshed CB/PDMS (meshed CB/PDMS) electrode proposed in this study.

The carbon-based electrodes (carbon rubber, CB/PDMS, and meshed CB/PDMS) were placed between skin and chest strap and they were connected directly to an ECG monitoring device *via* insulated copper electrical wires. The commercially-available textile-

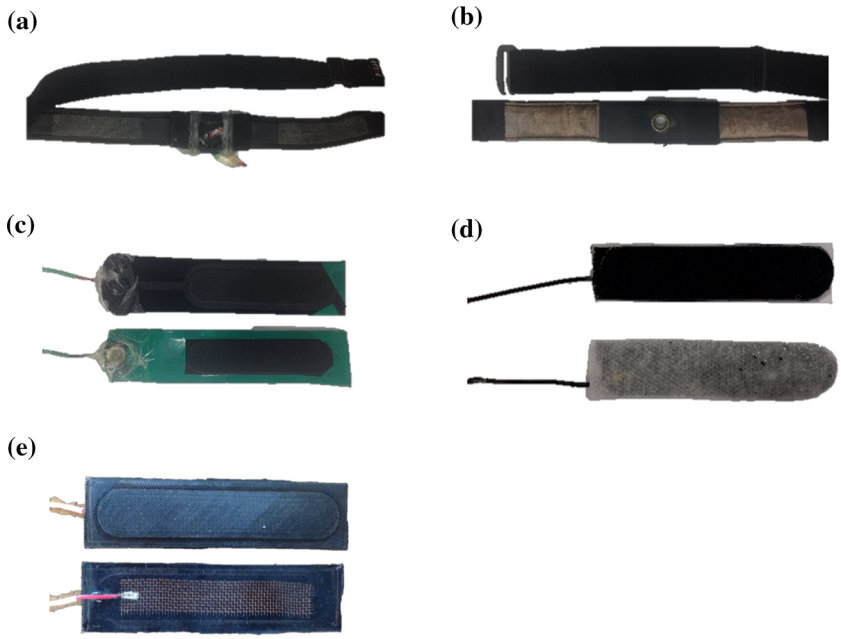

FIGURE 2. Electrode tested in this study (Materials, Size (cm × cm), Manufacturer). (a) Polar® textile (Silver-coated thread + normal thread, 8 × 2, Polar® System), (b) Silver-coated textile (Silver-coated fabric, 7 × 3, Nuga Medical), (c) Carbon rubber (Carbon ++ Rubber, 8 × 2, Wahoo Fitness), (d) CB/PDMS (CB/PDMS, 8 × 2, Self-production), and (e) Meshed CB/PDMS (CB/PDMS with meshed copper, 8 × 2, Self-production).

based electrodes (Polar® textile and silver-coated textile) were already fabricated into a chest strap. Wire connections from electrodes to our ECG monitoring device were waterproofed with a paraffin glue gun. The chest strap was worn such that the electrodes made full contact with the subject. We did not compare Ag/AgCl electrodes in this study since they do not provide discernible signals once water penetrates them.[12]

Experimental Protocols

We compared the performance of the above mentioned electrodes under three different types of water: (1) fresh/unfiltered, (2) chlorinated, and (3) salt water. Each experiment lasted 6 min and was divided into three periods as follows:

I. 2 min in standing position outside water (dry condition). Subjects were instructed to remain relaxed in the standing position outside the water.
II. 2 min in seated position inside water (immersed condition). Subjects were instructed to have their chest fully immersed to fully expose the chest strap to water.
III. 2 min in standing position outside water (wet condition). Subjects were instructed to exit the water after the immersion test and remain relaxed in standing position with the wet electrodes adhered to the chest strap. Data were taken for 2 min immediately after exiting water immersion but after the subject had equilibrated to the new condition which took no more than 10–20 s.

Each subject wore all five types of dry electrodes using the chest straps, one type at the time. All experiments were conducted on the same day for each subject. All recordings were collected at WPI facilities. The fresh and chlorine water tests were conducted at WPI's athletic training and rehabilitation center in a bathtub and swimming pool, respectively. The salt water test was conducted in an inflatable pool. The temperatures of fresh, chlorine, and salt water were maintained at approximately 25 °C. During dry condition, all carbon-based electrodes were kept dry but textile-based electrodes were wetted to ensure good conductance. The first 1 min of data were not used so that we only employ a stable signal and avoiding a possible ringing effect caused by analog 2nd order filters.

Performance Evaluation Using Underwater ECG Recordings

ECG Peak Detection

We found the R and S peaks of the ECG waveforms *via* a threshold detection method.[3] Detected R and S peaks were used to compute information regarding the amplitude reduction of the ECG signals collected with each electrode type at each experimental condition as well as to compute the corresponding ECG templates.

Comparison of ECG Amplitude Reduction

We chose a 30-s ECG segment, containing stable data, for each type of electrodes during each of the three experimental periods and types of water. The amplitude difference between R- and S-peak values from each ECG cycle were denoted as the amplitudes of the ECG signal. Finally, for each subject we obtained the mean ECG amplitude value for all electrode types, all experimental conditions, and all water types.

In order to compare the performance of the different types of electrodes and since their ECG recordings were not performed simultaneously, we normalized the ECG amplitudes with respect to their corresponding one during dry condition, i.e., immersed vs. dry condition and wet vs. dry condition, for each electrode type. The results of this normalization were used to test significant amplitude reduction (normalized values lower than 1) or significant amplitude gain (normalized values higher than 1) during the immersed and wet conditions. When we designed the experiment, we assumed $\alpha = 0.05$ and power of 0.8 and found that these assumptions required a sample size of 9 subjects. Given that normality did not hold for our data, a nonparametric test would require a 15% larger sample size, i.e., 10 subjects; this is the sample size we have used for our study. Normality did not hold for all the data as tested *via* Shapiro–Wilk test, and hence the one-sample Wilcoxon signed rank test with $p < 0.05$ considered as significant was performed. In addition, the recovery in the ECG amplitude from immersed to wet condition was tested using the two-related samples Wilcoxon signed rank test with $p < 0.05$ considered as significant. The null hypothesis was used to test that the mean value of the ECG amplitudes did not differ from the dry environment when electrodes were exposed to water immersion and wet conditions. We also compared the performance of the proposed meshed CB/PDMS electrodes vs. that from each other electrode type for the immersed and wet conditions using the two-related samples Wilcoxon signed rank test with $p < 0.05$ considered as significant. All of this performance analysis was carried out separately for each water condition in IBM SPSS Statistics software (IBM Corporation, Armonk, NY, USA).

Comparison of ECG Signal Quality

To analyze the ECG signal quality of the studied electrodes, we computed the ECG templates for each condition and water type. ECG templates were computed for each ECG segment by creating an ensemble with the corresponding ECG cycles aligned with respect to their R-peak locations and finally averaged at each time instant as described in Ref. 12. The number of beats used to compute the template was based on 2 min segments for all conditions (dry, immersed and wet) which amounted to approximately 140 beats. To quantitatively determine possible morphological changes in the ECG waveforms, we calculated cross-correlation indices of templates between immersed and the dry condition for all electrode types, all experimental conditions, and all water types; the same was done between wet and the dry condition.

Performance in Long Term Underwater ECG Recordings

The ability of the CB/PDMS electrodes to record ECG signals underwater for prolonged periods was tested. ECG signals were collected from Navy SCUBA divers immersed for 6 h at the bottom of a 4.5 m deep pool filled with chlorine water at 30 °C and alternating between 30 min relaxing (sitting upright in a chair) and 30 min biking with a 10 min lunch break on the surface after 3 h of immersion as detailed in Ref. 7. A total of 11 recordings were collected from 4 different divers for this portion of the study, using only the CB/PDMS electrodes. Each diver wore either a tight compression shirt or a wet suit over the chest strap containing the CB/PDMS electrodes. ECG templates were computed as explained in the previous section for each quiescent segment of each recording and their amplitudes were used to test if amplitude reduction occurred at the end of the experiment with respect to the start of data collection.

RESULTS

Electrode–Skin Contact Impedance

The electrode–skin impedance magnitude results of each electrode type are displayed in Fig. 3 as a function of frequency. All electrode types had comparable impedance performance over a wide range sweep of frequency in all conditions. The impedance characteristics of our CB/PDMS (both meshed and non-meshed) electrodes did not change over the sweeping frequency ranges in all conditions, whereas other electrode types had much lower impedances in both immersed and wet conditions when compared to dry condition. For textile-based electrodes, the silver-coated textile electrode had even lower impedance in the immersed condition than Polar® textile electrode. For both textile electrodes, their impedance flattened out immediately as a function of increasing frequency especially when they were exposed to water, but all carbon-based electrodes had linearly-decreasing impedance with increasing frequencies.

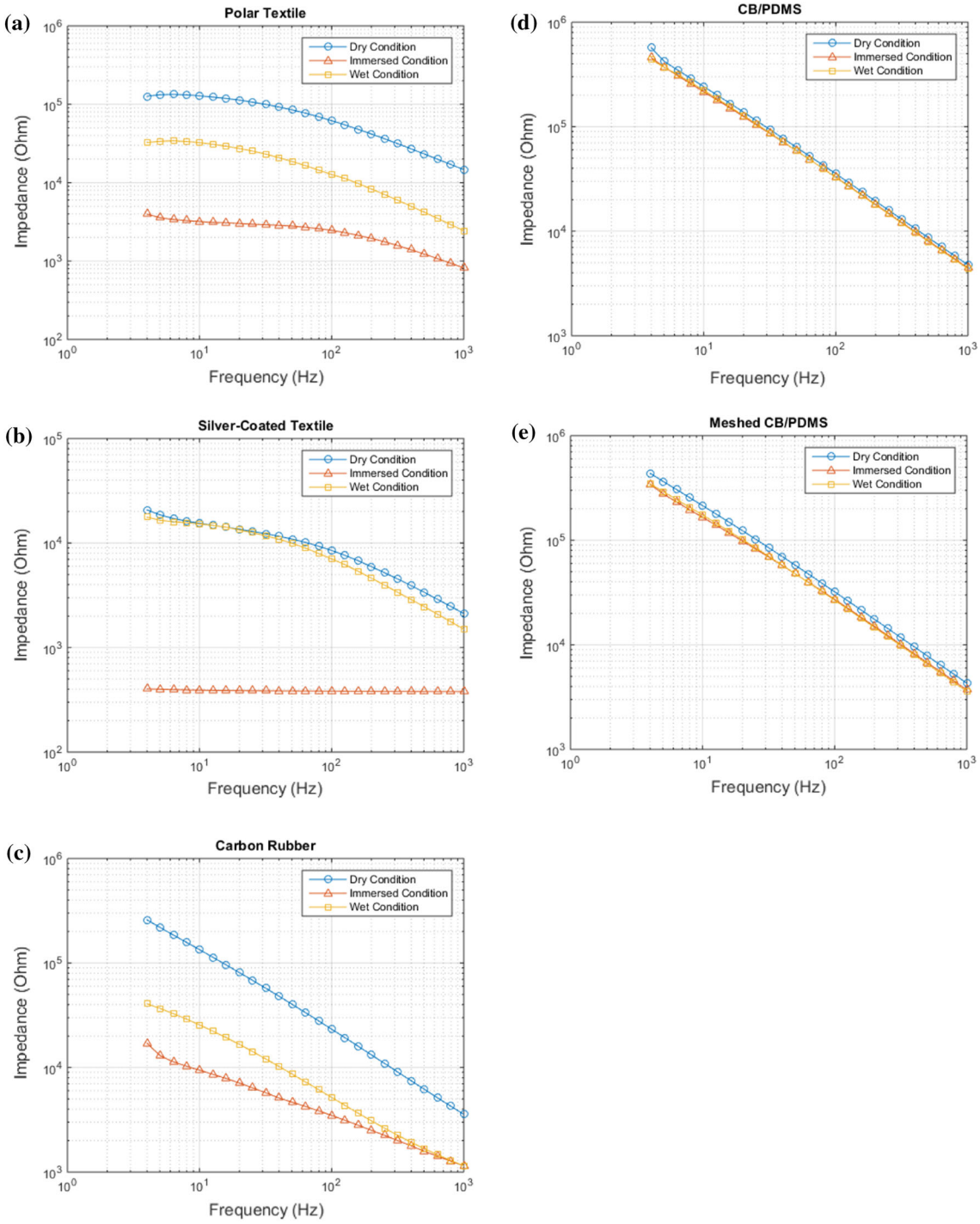

FIGURE 3. Contact Impedance (Ω) against frequency for five electrode types in fresh water. (a) Polar® textile, (b) Silver-coated textile, (c) Carbon rubber, (d) CB/PDMS, and (e) Meshed CB/PDMS.

Mechanical Properties After Aging in Aqueous Environments

Table 1 summarizes the results of uniaxial tensile testing performed on CB/PDMS samples after aging in the liquid environments and in ambient conditions. Representative tensile stress vs. strain curves for each sample (Fig. 4) show a linear region followed by a rapid drop in stress suggesting that CB/PDMS materials deforms elastically until failure. Analyses of UTS, E, and strain at failure (%) data for the CB/PDMS samples indicate that there were no statistical differences between any of the aged and non-aged sample

TABLE 1. Summary of mechanical properties of CB/PDMS aged in different environments.

Sample group	*n*	UTS (MPa)	Strain at failure (%)	*E* (MPa)	Load at failure (*N*)	Cross sectional area (mm^2)
CB/PDMS non-aged	6	1.38 ± 0.37	24.4 ± 5.05	5.22 ± 0.54	31.1 ± 8.75	22.5 ± 0.78
CB/PDMS ambient (5 days)	8	1.48 ± 0.32	28.5 ± 9.02	5.81 ± 0.55	32.1 ± 7.01	21.7 ± 0.94
CB/PDMS-ambient (14 days)	3	1.41 ± 0.42	24.3 ± 3.76	5.29 ± 0.80	30.5 ± 7.32	21.9 ± 1.16
CB/PDMS fresh (5 days)	7	1.35 ± 0.23	24.7 ± 5.32	4.81 ± 0.67	28.4 ± 4.84	21.1 ± 0.37
CB/PDMS fresh (14 days)	4	1.32 ± 0.39	22.8 ± 6.29	5.41 ± 1.05	29.5 ± 8.30	22.4 ± 1.04
CB/PDMS chlorine (5 days)	7	1.63 ± 0.40	31.8 ± 10.7	5.10 ± 0.79	34.8 ± 7.89	21.5 ± 1.25
CB/PDMS chlorine (14 days)	4	1.32 ± 0.31	24.7 ± 6.98	5.03 ± 0.44	29.3 ± 7.51	22.0 ± 1.09
CB/PDMS salt (5 days)	7	1.38 ± 0.22	22.4 ± 4.82	5.57 ± 0.92	28.8 ± 4.72	20.9 ± 0.59
CB/PDMS salt (14 days)	4	1.00 ± 0.54	16.1 ± 9.91	5.77 ± 0.51	20.8 ± 11.6	20.7 ± 0.83

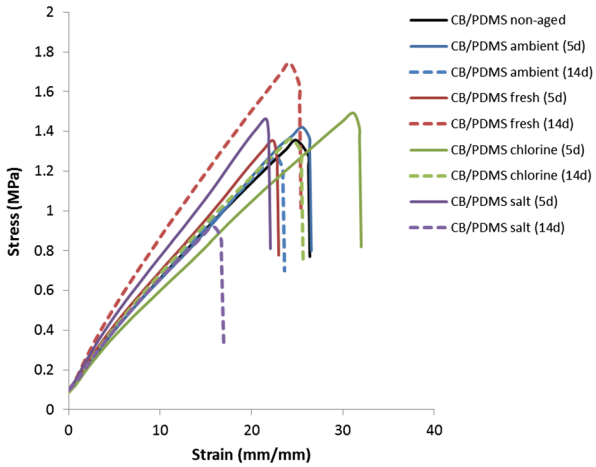

FIGURE 4. Characteristic plots of stress vs. strain for CB/PDMS after aging in various environments.

conditions. These findings suggest that aging CB/PDMS electrodes in aqueous environments does not affect their tensile strengths.

Analysis of Cytotoxicity of Medium Extracted from CB/PDMS Electrode

The cytotoxicity of extracts derived from the CB/PDMS electrodes were evaluated by incubating the extracts with connective tissue cells (L929 cells) and primary human epidermal keratinocytes (NHEK). Microscopic analyses showed minimal cell death in monolayer cell cultures in contact with medium extracted from CB/PDMS electrodes.

Cytotoxic Effect of Electrodes on L929 Cells

L929 cells cultured with medium extracted from the CB/PDMS electrodes remained adhered to the tissue culture plate and were morphologically consistent with cells cultured in the presence of extracts from the negative control material, HDPE, extracted medium only (no material), and the fresh medium control. In contrast, L929 monolayers cultured with medium extracted from the latex positive controls were completely disrupted. The L929 cells that remained had a rounded shape and were not well attached to the tissue culture plate. Statistically, there was no difference between the % cellular activity of L929 cells cultured with medium extracted from the CB/PDMS electrodes and those incubated with the negative control (HDPE) extract. The % cellular activity of L929 cells cultured with medium extracted from the electrodes and cells cultured with medium extracted from the HDPE (negative control) were significantly greater than the % cellular activity of L929 cells cultured with medium extracted from the latex (positive control).

Cytotoxic Effect of Electrodes on NHEK Cells

NHEK cells cultured with medium extracted from CB/PDMS electrodes remained adhered to the tissue culture plate. Additionally, the NHEK monolayers cultured in the presence of the extracts from the electrodes were morphologically consistent with NHEK monolayers cultured in the presence of extracts from the negative control material, HDPE, extracted medium only (no material), and the fresh medium control. By contrast, NHEK monolayers cultured with medium extracted from the latex positive controls were completely disrupted. The NHEK cells that remained had a rounded shape and lacked the cell–cell contact typical of this cell type, indicating cell death. Statistically, there was an increase in % cellular activity of NHEK cells cultured with medium extracted from the CB/PDMS electrodes compared to those incubated with the negative control (HDPE) extract. The % cellular activity of NHEK cells cultured with medium extracted from the electrodes and cells cultured with medium extracted from the HDPE (negative control) were significantly greater than the % cellular activity of NHEK cells cultured with medium extracted from the latex (positive control).

Performance Evaluation Using Underwater ECG Recordings

ECG Peak Detection

Figure 5 shows representative ECG signals collected with each type of electrode during the three conditions of the salt water immersion experiment. Note the significant R-peak amplitude reduction especially with both Polar® and silver-coated textile electrodes during salt water immersion. The meshed CB/PDMS electrodes perform well in all of water immersion conditions as there is no apparent R-peak amplitude reduction; in fact, for this particular subject, there was amplitude amplification in all types of water. In general, ECG amplitudes were similar between the dry and wet conditions for all types of electrodes but for this particular subject, the ECG amplitude from silver-coated textile electrodes did not recover fully to the dry condition.

Comparison of ECG Amplitude Reduction

Table 2 shows the signal amplitude reduction values for each electrode type between dry and immersed conditions, while Table 3 shows the corresponding values between dry and wet conditions. Figure 6 depicts the mean ECG amplitude values normalized to dry condition for each electrode type in fresh, chlorine, and salt water, respectively.

For the immersed vs. dry condition, the amplitude reductions of textile-based electrodes (Polar® and silver-coated) are significantly greater than those of carbon-based electrodes (rubber, CB/PDMS and meshed CB/PDMS). Between textile-based electrodes, the amplitude reduction of silver-coated electrodes was the more severe. Among all electrode types, the meshed CB/PDMS electrode was the most successful in preserving the ECG signal amplitude in all types of water. Overall, for all types of electrodes, the signal amplitude reduction values in fresh water were lowest followed by those from chlorinated water; salt water yielded the greatest reduction values. There were significant amplitude reductions for textile-based electrodes ($p < 0.003$) and significant amplitude gain from the meshed CB/PDMS electrode ($p < 0.015$) under fresh water, as shown in Fig. 6a; there were significant

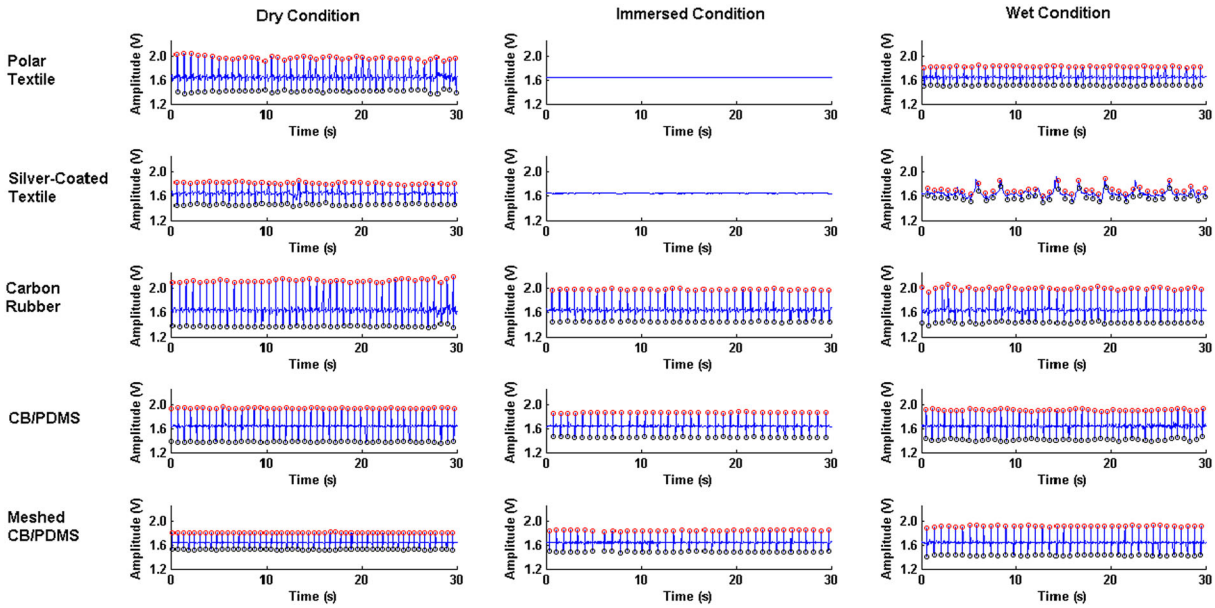

FIGURE 5. Example of ECG signals collected from one of the subjects in salt water for each type of electrode and tested condition. Red and black circles indicate the R-peak and S-peak locations, respectively, as determined by the automatic peak-detection algorithm.

TABLE 2. Mean of amplitude reduction (%) between dry and immersed conditions (N = 10 subjects).

Electrodes	Fresh water	Chlorine water	Salt water
Polar® textile	−66.69	−93.81	−99.38
Silver-coated textile	−79.94	−98.33	−100.00
Carbon rubber	−10.20	−35.07	−46.45
CB/PDMS	+1.85	−5.36	−32.22
Meshed CB/PDMS	+24.84	+15.19	+1.97

TABLE 3. Mean of amplitude reduction (%) between dry and wet conditions ($N = 10$ subjects).

Electrodes	Fresh water	Chlorine water	Salt water
Polar® textile	+27.20	−16.13	−37.91
Silver-coated textile	+13.41	−16.04	−51.98
Carbon rubber	+8.10	−2.67	−22.99
CB/PDMS	+12.87	−2.46	−14.17
Meshed CB/PDMS	+31.77	+0.48	+3.60

amplitude reductions in all types of electrodes ($p < 0.003$ for textile-based, and $p < 0.015$ for carbon rubber) except for CB/PDMS based-electrodes under chlorine water, as shown in Fig. 6c, and there were significant amplitude reductions in all types of electrodes except meshed CB/PDMS electrodes ($p < 0.001$ for silver-coated, $p < 0.002$ for Polar®, $p < 0.007$ for carbon rubber, and $p < 0.015$ for CB/PDMS) under salt water, as shown in Fig. 6e.

For the wet vs. dry condition, among carbon-based electrodes, the meshed CB/PDMS electrode had better performance than the others as there was no amplitude reduction after immersion in all types of water. For textile-based electrodes, we observed they exhibited significant amplitude reductions in the wet condition after immersion in either chlorinated or salt water. Overall, for all types of electrodes, signal amplitude reductions after fresh water were lowest and those after salt water were highest. There were significant amplitude gains in carbon rubber ($p < 0.03$), CB/PDMS ($p < 0.003$) and meshed CB/PDMS ($p < 0.003$) electrodes in the wet condition after fresh water immersion as shown in Fig. 6a, and there were significant amplitude reductions in textile-based electrodes ($p < 0.05$ for silver-coated, and $p < 0.015$ for Polar®) in the wet condition after chlorine water, as shown in Fig. 6c, and there were significant amplitude reductions for all types of electrodes except meshed CB/PDMS electrodes in the wet condition after salt water immersion ($p < 0.003$ for textile-based, $p < 0.004$ for carbon–rubber, and $p < 0.03$ for CB/PDMS), as shown in Fig. 6e.

Regarding the statistical comparison among each type of electrode in the immersed condition, we found that the normalized ECG amplitude of the meshed CB/PDMS electrode was significantly higher than that of the others ($p < 0.004$) in all types of water, as shown in Figs. 6b, d, and f. In the case of statistical comparison among the different types of electrodes in the wet condition, we found that normalized ECG amplitudes of meshed CB/PDMS electrodes were significant higher than those of carbon rubber electrode ($p < 0.025$) after fresh water, as shown in Fig. 6b, and were significant higher than those of textile-based electrodes ($p < 0.025$ and $p < 0.004$ for silver-coated; $p < 0.03$ and $p < 0.009$ for Polar®) after chlorine water and salt water, respectively, as shown in Figs. 6d and f.

We also examined the amplitude recovery from immersed condition to wet condition. In fresh water, normalized ECG amplitudes were significantly lower in the immersed condition than in the wet condition for textile-based electrodes ($p < 0.003$) and the carbon rubber electrode ($p < 0.02$), as shown in Fig. 6a. Similar results applied to these electrodes in chlorine and salt water types ($p < 0.003$ for textile-based electrodes, and $p < 0.025$ for carbon rubber electrode). Because there was not significant amplitude reduction while immersed in fresh water, no statistical significant recovery was found for both CB/PDMS based-electrodes. In chlorine water, normalized ECG amplitudes of all of the electrodes except CB/PDMS-based electrodes were significantly lower in the immersed condition than in the wet condition ($p < 0.003$ for textile-based electrodes, $p < 0.025$ for carbon rubber electrodes), whereas those of meshed CB/PDMS electrodes were significantly higher ($p < 0.05$), as shown in Fig. 6c. In salt water, the normalized ECG amplitudes of all types of electrodes except meshed CB/PDMS electrodes were significantly lower immersed than in the wet condition ($p < 0.003$ for textile-based electrodes, $p < 0.025$ for carbon rubber electrodes, and $p < 0.009$ for CB/PDMS electrodes), as shown in Fig. 6e.

Comparison of ECG Signal Quality

Figure 7 shows the ECG templates computed for all experimental conditions with the meshed CB/PDMS electrode in fresh, chlorinated, and salt water. All ECG morphologies are well delineated including the QRS complex, *P* and *T* waves in all experimental conditions and water types. Cross-correlation test results are shown in Table 4 to quantitatively assess if there were any indications of ECG waveform morphological change when the electrodes were exposed to water. Only the CB/PDMS-based electrodes (both meshed and non-meshed) showed high correlations for both immersed and wet conditions for all water types, whereas textile-based electrodes showed no indication of any ECG waveform resemblance to the dry condition in all water conditions. The carbon rubber electrodes showed high correlation in chlorinated water, but a marked decrease when exposed to salt water.

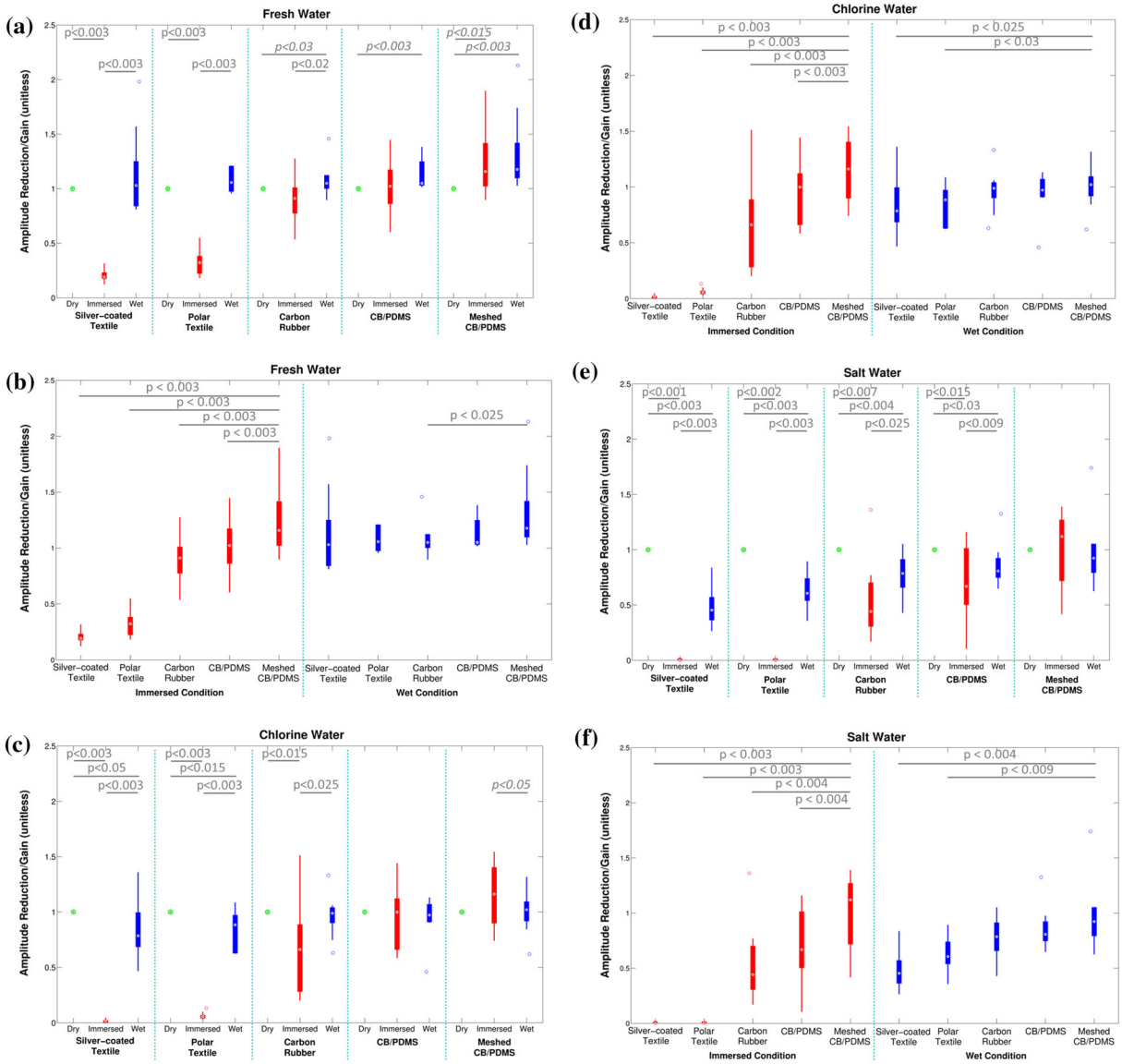

FIGURE 6. Comparison of normalized ECG amplitude for each type of water. (a) In fresh water: Statistical comparison among different conditions for each electrode. Asymptotic significant values displayed. First two rows indicate statistical significant amplitude reduction or gain (italic) compared to initial dry condition. Third row indicates statistical significant amplitude recovery from immersed to wet condition. (b) In fresh water: Statistical comparison of meshed CB/PDMS electrodes vs. other electrode types in both immersed and wet conditions. Asymptotic significant values displayed. (c) In chlorinated water: Statistical comparison among different conditions for each electrode. Asymptotic significant values displayed. First two rows indicate statistical significant amplitude reduction or gain compared to initial dry condition. Third row indicates statistical significant amplitude recovery or decrement from immersed to wet condition. (d) In chlorinated water: Statistical comparison of meshed CB/PDMS electrodes vs. other electrode types in both immersed and wet conditions. Asymptotic significant values displayed. (e) In salt water: Statistical comparison among different conditions for each electrode. Asymptotic significant values displayed. First two rows indicate statistical significant amplitude reduction compared to initial dry condition. Third row indicates statistical significant amplitude recovery from immersed to wet condition. (f) In salt water: Statistical comparison of meshed CB/PDMS electrodes vs. other electrode types in both immersed and wet conditions. Asymptotic significant values displayed.

Performance in Long Term Underwater ECG Recordings

The results presented so far are based on short-term (minutes) ECG recordings underwater. From top to bottom panels, Fig. 8a shows an example of a long-term (hours) ECG recording, the heart rate time series, and the corresponding ECG templates during each 30-min resting and exercising intervals. Figure 8b shows the boxplot of the amplitude of ECG templates taken at the first and last resting periods of each of the 11 dives in this study. No significant ECG template amplitude reduction was found ($p = 0.2345$) between

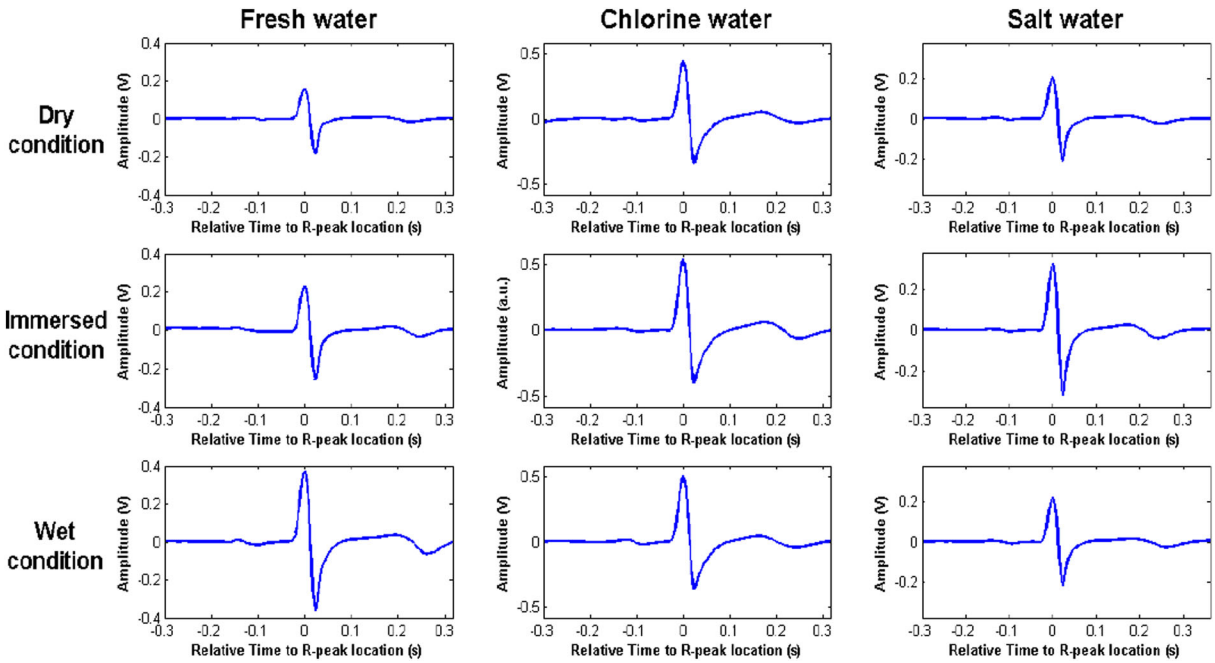

FIGURE 7. Example of ECG templates from a subject recorded with copper-meshed CB/PDMS electrodes during different conditions and types of water.

TABLE 4. Mean and standard deviation of cross correlation indexes of templates between the dry and water exposed conditions (N = 10 subjects).

	Fresh water		Chlorine water		Salt water	
Electrode	Immersed	Wet	Immersed	Wet	Immersed	Wet
Polar® textile	0.943 ± 0.017	0.963 ± 0.043	NA ± NA	0.989 ± 0.019	NA ± NA	0.928 ± 0.172
Silver-coated textile	0.942 ± 0.042	0.969 ± 0.021	NA ± NA	0.982 ± 0.017	NA ± NA	0.842 ± 0.338
Carbon rubber	0.978 ± 0.017	0.989 ± 0.010	0.971 ± 0.023	0.989 ± 0.005	0.861 ± 0.344	0.907 ± 0.263
CB/PDMS	0.978 ± 0.013	0.978 ± 0.018	0.957 ± 0.031	0.957 ± 0.058	0.915 ± 0.170	0.960 ± 0.047
Meshed CB/PDMS	0.986 ± 0.011	0.985 ± 0.011	0.982 ± 0.010	0.992 ± 0.005	0.976 ± 0.028	0.984 ± 0.019

the beginning and end portion of the experiment as measured with a paired t test. This suggests that despite more than 5 h of exposure to chlorinated water, CB/PDMS electrodes maintained their signal quality.

DISCUSSION

Our previously-developed CB/PDMS electrode[12] was proposed for ECG monitoring underwater, but it had the shortcoming that amplitudes of ECG signals were attenuated nearly 50%. This was to a large extent due to our oversight in not water-proofing the snap connectors between the CB/PDMS electrodes and the ECG monitoring device. Hence, while the CB/PDMS electrodes themselves were designed to be nearly waterproof, the snap connectors were not insulated from water, which resulted in ECG amplitude reduction. In addition, the small snap connector placed in the middle of the CB/PDMS electrode was not efficient in gathering all bio-potential from the skin surface. Hence, despite lower impedance with a large electrode size, we were getting bio-potentials only from a small surface area surrounding the vicinity of the snap connector. In this study, to mitigate these issues, we made two modifications to our previously-developed electrodes: (1) embedded a fine and flexible copper mesh all along the surface of the CB/PDMS electrode; lowering the impedance in comparison to our previous CB/PDMS electrode as shown in Fig. 3, and (2) soldered a waterproof copper wire directly to the copper-mesh for connection with an ECG device instead of an external snap connector; insulation against water penetration of the mesh and solder joint was done using PDMS as

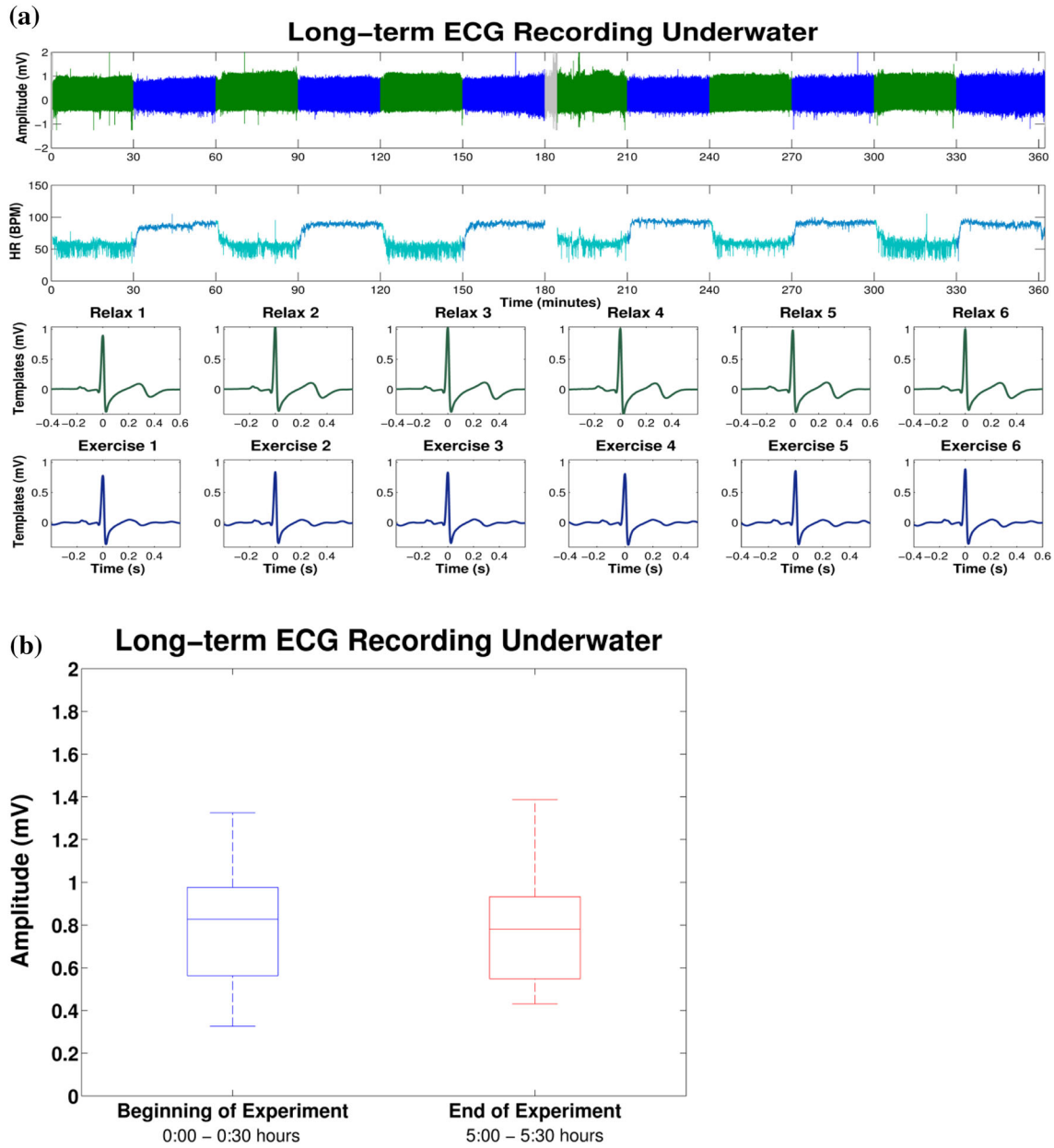

FIGURE 8. (a) Example of long-term ECG recording underwater. Top: 6-h ECG recording. Green portions correspond to relaxation segments and blue segments to exercise segments. The gray segment in the middle indicates the period when the subject was eating refreshments on the surface. Middle: Heart rate time series. Bottom: ECG templates for the corresponding relaxation and exercise segments. (b) Boxplot of ECG templates' amplitudes for the first and last relaxation segments of long-term ECG recordings collected during 11 water immersion experiments.

shown in in Fig. 2. Our new CB/PDMS electrode design ensures that the bio-potential from all areas of the electrode's contact to the skin surface are collected. Moreover, a direct wire connection to the mesh, which is embedded inside the waterproof CB/PDMS electrode, has made the lead waterproof. In summary, our new electrode has two major advantages. First, our electrode preserves its low impedance characteristics even underwater because the CB/PDMS electrode is nearly hydrophobic. Second, collection of electrical bio-potentials from the entire electrode's surface is now possible.

To evaluate the performance of the newly-designed electrodes, we compared them to some of the existing so-called dry (without hydrogel) commercial electrodes: Polar® textile, silver-coated textile, and carbon

rubber, as well as CB/PDMS without copper mesh. We performed the comparison during dry, water immersion and wet (post-immersion) scenarios; the immersion experiments were performed in fresh, chlorinated and salt water. We found that ECG amplitudes were significantly reduced during immersion for all electrode types except for the copper-meshed CB/PDMS, with salt water causing the largest reduction. While the CB/PDMS electrode without the copper mesh performed better than other non-CB/PDMS electrodes, the signal degradation was significant for both chlorinated and salt water as the ECG amplitudes decreased by 5.36 ± 30.85 and $32.22 \pm 34.50\%$, respectively, with respect to the dry condition. For the modified copper-meshed CB/PDMS electrode, not only did the amplitudes of the copper-meshed CB/PDMS electrodes not decrease for all types of water, in fact, the mean ECG signal amplitude values increased by 24.84 ± 33.91, 15.19 ± 27.28, and $1.97 \pm 32.13\%$ (mean $\pm$ standard deviation) in fresh, chlorine, and salt water, respectively. This amplification of R-peak amplitude for the meshed CB/PDMS electrodes can be explained by two means. First, the skin impedance in water immersion is lower than in dry conditions; this applies for all types of electrodes. Second, non-hydrophobic (especially textile-based) electrodes experience significant bio-potential leakage to the surrounding water. Because the CB/PDMS electrodes are nearly hydrophobic, the biopotentials measured at the electrode surface do not dissipate very much to the surrounding area. Hence, given the lower impedance of the skin with water immersion, this can lead to higher ECG amplitudes provided that the electrodes are sufficiently waterproof. Certainly, given that the meshed CB/PDMS electrodes provided amplification of the ECG signal in some subjects and no deterioration of the signal in others, it can be concluded that the hydrophobicity of these electrodes has led to their better performance than other electrodes compared in this study.

To obtain ECG morphological waveforms from the human body, the impedance of an electrode needs to vary as a function of frequency. The magnitude of ECG amplitude depends on the impedance or biopotential difference between at least two electrodes at the electrode–skin interface. As shown in Fig. 3, only the CB/PDMS-based electrodes maintain a biopotential difference and vary as a function of frequency, in immersion and wet conditions. Note that only the CB/PDMS-based electrodes exhibit the same impedance characteristics of dry electrodes when exposed to water. Due to the nearly complete hydrophobicity of the CB/PDMS electrodes, they are able to maintain their impedance characteristics even when exposed to water thereby resulting in non-negligible reduction in the ECG signal amplitude (due to difference in impedance or biopotential between two electrodes) and no discernible changes in the waveform morphologies. The hydrophobicity characteristics are not maintained especially for the textile electrodes with constant impedance for the silver-textile electrodes which yields the worst results in terms of amplitude reduction and destruction of ECG waveform morphologies. The Polar textile electrodes fared slightly better than silver-textile electrodes in terms of the ECG signal morphologies but they also had similar significant reduction in the ECG amplitude. Because the water penetrates the two electrodes and conducts across their span, the impedance or biopotential difference at the skin–electrode interface between the two electrodes is very small, which results in nearly zero ECG amplitudes.

It is well known that salt and chlorinated water have higher conductance than fresh water because they include many more ions such as sodium and chlorine compared to fresh water. Hence, it is not surprising that the observed decrease in ECG amplitudes with all electrodes except for the meshed CB/PDMS electrodes became more apparent with salt and chlorinated water. Even for meshed CB/PDMS electrodes, there was less amplification of ECG amplitudes when immersed in salt and chlorinated water. Because of the higher conductance of salt and chlorinated water when compared to fresh water, there was slightly more bio-potential leakage with the meshed CB/PDMS electrodes to the surrounding water than they experienced in fresh water. However, again due to the hydrophobicity of the meshed CB/PDMS, the bio-potential leakage was not significant compared to the other electrodes, which is why the other electrodes had significant amplitude reduction with salt and chlorinated water.

When electrodes were wet, meaning, immediately out of full water immersion, there was significant amplification of the ECG amplitude with meshed and non-meshed CB/PDMS only for fresh water. This amplification effect was not seen for both salt and chlorinated water for meshed CB/PDMS electrodes, but their amplitudes remained at the level of the dry condition. For both textile electrodes for all water types, because the fabrics were soaked, there was enough bio-potential leakage that the amplitudes were significantly lower than the amplitude values found in the dry condition. For both non-meshed CB/PDMS and carbon electrodes, their amplitude values recovered to the dry condition level only for fresh water immersion. Hence, the newly designed meshed CB/PDMS electrodes again showed superior performance over all other electrodes tested due to their hydrophobicity.

In our previous study based on the non-meshed CB/PDMS electrodes, we found no cytotoxicity of these

electrodes.[12] In this study, we were interested in examining skin irritation as well as degradation of the electrodes based on repeated and long-term usage. Our *in vitro* analyses of cellular responses to leachable constituents from the CB/PDMS materials showed that medium extracts from the samples were not cytotoxic to connective tissue (L929) or keratinocyte (NHEK) cells. Further, no subject complained of skin irritation based on continuous wearing of CB/PDMS electrodes for 6 h and there was no degradation of the ECG signal after nearly 6 h of continuous recordings under water even with repeated use. These electrodes were re-used within a 1-month time span but we did not find any notable deterioration. We used only the non-meshed CB/PDMS electrodes for long-term ECG monitoring as we performed these experiments before meshed electrodes were developed. However, since the main difference between the non-meshed and meshed CB/PDMS electrodes is the embedding of meshed copper wires, we expect at least a similar performance with meshed electrodes. These findings are consistent with the results of our electrode aging studies that showed that the mechanical strengths of the CB/PDMS electrode materials did not decrease when they were subjected to various aqueous conditions for as long as 14 days. While our electrodes are flexible, continuous usage and severe bending of these electrodes will eventually lead to deterioration that can compromise signal quality. Certainly, a full claim of reusability of the proposed electrodes would require repeated use and wiping of the electrodes with alcohol, and provide a definite time limit for various water exposure conditions. This will be a topic of future study.

CONCLUSION

We developed a novel copper-meshed CB/PDMS electrode which has been found to have superior performance in all water immersion conditions than any other electrodes compared in this study. Our CB/PDMS electrodes either maintained or in some cases amplified the ECG signal during water immersion when compared to the level of the dry condition. No other electrodes tested have had this superior performance. We are not aware of any ECG electrodes that work in full water immersion without the use of waterproof tape over the electrodes and the skin. Other advantages of our electrodes are that they are reusable and should not cause skin irritation since adhesives are not used. Given that these CB/PDMS electrodes work in both dry and water immersion conditions, they are versatile and can potentially be used in many sports applications such as monitoring of athletes' vital signs like heart rates or other performance measures. They will work when sweaty but also do not require being wetted with water for good conduction. Moreover, for dry and even immersed or exceedingly moist use, these electrodes can be very useful for long-term recording of ECG for detecting arrhythmia such as paroxysmal atrial fibrillation which is known to be one of the most common heart problems today in America.

ACKNOWLEDGMENT

This work was supported by the Office of Naval Research work unit N00014-12-1-0171 and N000 1414WX20184.

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