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Smart Fatigue Phone: Real-time estimation of driver fatigue using smartphone-based cortisol detection

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ABSTRACT

Numerous studies reported that psychological fatigue is one of the main reasons leading fatal road crashes. In order to quantify fatigue level of each subject, we measured a concentration of salivary cortisol from 4 subjects (20–40 years of age) using the *Smart Fatigue Phone*, which consists of a lateral flow immunosensor and a smartphone-linked fluorescence signal reader, during 50-min driving session. Since the salivary cortisol needs to be measured below 1 ng/mL to distinguish the subjects from awaken-drivers, we have employed the fluorescence detection module (Limit of detection: 0.1 ng/mL). To validate correlation between fatigue status and salivary cortisol concentration measured by the *Smart Fatigue Phone*, the electroencephalogram (EEG) signal was simultaneously obtained from the participants. As a result, alpha wave and concentration of cortisol over time was highly correlated, reflecting that quantification of salivary cortisol can be used for real-time monitoring of driver fatigue ($p < 0.05$). The *Smart Fatigue Phone* is expected to be a useful tool for drivers to recognize their fatigue status and subsequently to make a decision for driving a car. Thus, we assume that this fatigue detection system will consequently minimize road crashes by quantifying salivary cortisol in real time in the near future.

1. Introduction

Saliva, secreted from the salivary glands, comprises a myriad of biomolecules related to various disorders, from severe to benign symptoms, thus applying salivary biomarkers to diagnostic purposes (Shin et al., 2018). The interest of saliva-based *in-vitro* diagnostics (IVD) related studies has tremendously increased in recent year with respect to several advantages, fulfilling cost-effectiveness and non-invasive sampling. Furthermore, the innovative smartphone technologies are recently applied to salivary diagnostics as alternatives to blood for the point-of-care-testing, which plays a key role in the clinical fields (Zhu et al., 2013; Lee et al., 2014; Choi et al., 2014, 2017; Zangheri et al., 2015; Shin et al., 2017; Yang et al., 2017; Choi et al., 2019). The authors have previously developed a noble method to quantify a degree of psychological stress with a concentration of salivary cortisol, which is also known as a key biomarker for determination of fatigue status, by using a smartphone-based colorimetric analysis system (Choi et al., 2014, 2017; Yang et al., 2017). Since fatigue has been considered as one of the major causes that induces fatal crashes on roadway, several research teams focused on analyzing data from either electroencephalogram (EEG) or driving simulation to study fatigue patterns of drivers (Kar et al., 2010; Simon et al., 2011; Li et al., 2012). Craig et al. reported that EEG activation increases in theta and alpha frequency band and decreases in beta frequency band with respect to time during fatigue condition (Craig et al., 2012). In addition, a few studies showed the correlation between fatigue condition and concentration of salivary fatigue biomarkers, such as cortisol and alpha-amylase (Roberts et al., 2004; Yamaguchi et al., 2006). Shin et al. reported that human salivary cortisol can be used for an indicator of emotional and fatigue status (Shin et al., 2018). The main purpose of this study is to design the smartphone-based fluorescence detection system, fulfilling the high-sensitivity of a lateral flow immunosensor because less than 1 ng/ml of salivary cortisol is required to distinguish the subjects in fatigue condition from awaken-drivers. In addition, previous studies reported that low cortisol concentrations were observed in patients, who suffered from chronic fatigue syndrome (Cleare, 2004; Roberts et al., 2004). Then, the fatigue level assessment of the subjects was conducted with electroencephalogram (EEG) measurement to validate our fatigue detection system. As shown in Fig. 1, we have measured salivary cortisol in real time by using the *Smart Fatigue Phone,* which comprises a fluorescence reader, a lateral flow immunosensor, and an android-based fluorescence signal application, as well as EEG analysis. In this

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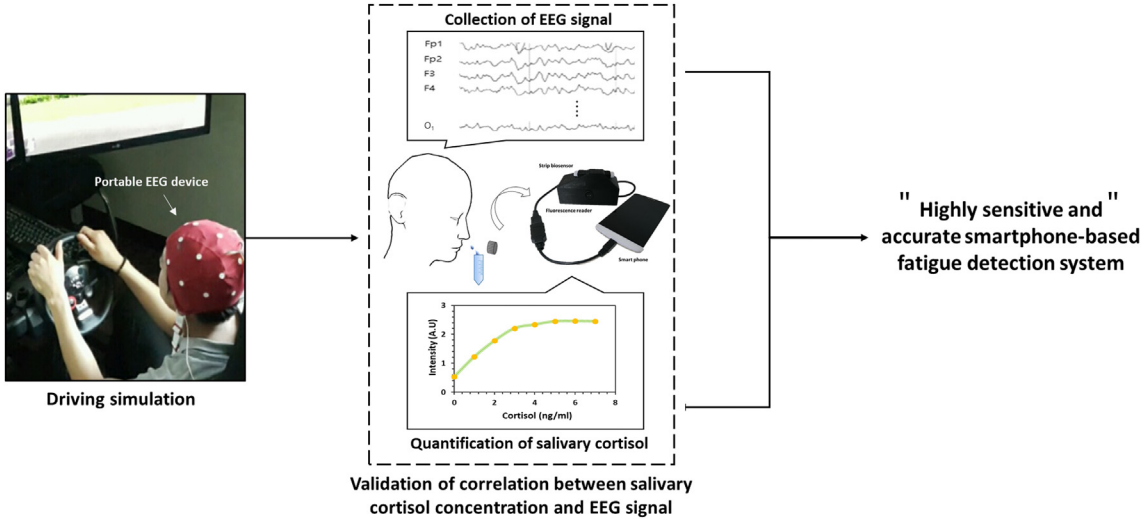

Fig. 1. The experimental procedure for driver fatigue assessment using the smartphone-based fatigue detection system. During 50-mins of the experiment, saliva and EEG signal from the subjects were simultaneously collected and measured every 15 min, respectively. After collection of both signals, the correlation between concentration of salivary cortisol and EEG signal was analyzed.

study, we have enhanced the sensitivity of a lateral flow immunosensor for cortisol detection by using fluorescence-based assay (Limit of detection (LOD): 0.1 ng/mL) instead of application of colorimetric assay (LOD: 1.0 ng/mL). In addition, the *Smart Fatigue Phone* was designed to keep drivers away from driving a car in fatigue condition. This is the first attempt to determine a degree of fatigue from drivers in quantitative manner using smartphone-based fatigue detection system by the combination of two different modalities (salivary cortisol and EEG signal). The fatigue detection system separately consists of a smartphone-based cortisol detection system (*Smart Fatigue Phone*) and a portable EEG measurement device. Based on these results, we demonstrated the meaningful study of the smartphone-based cortisol detection system for fatigue quantification.

2. Materials and methods

2.1. Methods with subsection as design of experiment

The subjects, who were graduate school students (3 males and 1 female), participated in this study with the following criteria: age between 20 and 40 years old (average 29.5 and SD = 1.64), possession of a valid driving license, and more than one-year driving experience. On the other hand, participants with substance abuse, psychiatric and sleep disturbances, or those taking more than 400 mg of caffeine per day were excluded. All subjects were tested for 50 min after signing a written consent before the experiment. Our study was carried out under the human research guidelines of human subjects established by the Institutional Review Board (IRB) of Yonsei University, South Korea. Experiments were conducted at 1:00 pm when participants arrived at the site by providing a driving simulator and a driving environment interface, which are 3D-realtime VR software programs, UC-Win/road, at Yonsei University. The virtual driving scenario was carried out on three large LED monitors (LG Display 27 inches), providing a 130-degree field of view and two side mirrors. The accelerating pedal, steering wheel, brake, driver's seat and the body frame of the car were purchased from Logitech Inc. The entire session of the experiment consisted of 50 min, including a 5-min practice run and three 15-min driving assignments. Thereafter, the saliva samples of each subject were then collected at the end of each test (total 4 times). A stand for holding a conical tube as a saliva collector was placed in front of each participant so that we were able to minimize the influence on the attention from subjects. Finally, we measured the concentration of salivary cortisol using a smartphone-linked fluorescence detecting system. Additionally, cortisol concentration of each subject was detected using a commercial ELISA kit (Cambridge, MA, USA) to validate our measurement system. A standard competitive ELISA method was applied to measure optical density values at 530 nm through multiple label plate readers (VictorX5, PerkinElmer). The absorbance of plate was correlated with concentration of cortisol captured in human saliva.

2.2. Design of smartphone-based fluorescence detection system for quantification of fatigue

2.2.1. Fabrication of fluorescent lateral flow immunosensor

The antibody vial was diluted to a concentration of 0.25 mg/mL of monoclonal cortisol antibody (abCAM, ab1949), which has negligible cross-reactivity with cortisone (~ 0.6%), by the addition of 0.1 M sodium bicarbonate (pH 9.3). The diluted solution (1 mL) was then added to a Cy3-containing vial (Amersham GE healthcare Inc.) and was incubated at 23 °C for 2 h with a rotator speed of 120 rpm. The Cy3 conjugated antibody should be between pH 6.5 and 8.5 by adjusting the pH of the compound with HCl (0.1 M). The spin column of the microcentrifuge tube was centrifuged at 1000 g for 30 s to add 250 μL of the dye-removing resin. Finally, 250 μL of Cy3 conjugated antibody was added to the spin column and centrifuged under the same conditions. The intensity of the diluted antibody (9-fold) was measured with a fluorescence spectrophotometer (Nanodrop 3300) to determine the optimal conditions for the conjugated antibody. The synthesized compound between high concentration of Cy3 dye and cortisol antibodies results in being clogged on the membrane, thus rarely reaching to the test line, where cortisol-BSA is immobilized (Fitzerald, USA). Therefore, the concentration of each conjugated antibody at 0.001, 0.005, 0.01, 0.02, 0.04, 0.05, 0.08, 0.1, 0.25, 0.5, and 1.0 mg/mL was applied to the binding pad, respectively, to evaluate the optimal concentration of the synthesized antibody. Finally, the disposable fluorescence based lateral flow immunosensor was fabricated with a conjugation pad (Ahlstrom, Spain), an absorbance pad (Ahlstrom, Spain), and a nitrocellulose membrane (Millipore, USA) which contains a cortisol-BSA and IgG antibodies on a test line and control line, respectively, as shown in Fig. S1(a). In order to detect fluorescence signal from the test line, the collected saliva samples after 2:1 dilution with Phosphate Buffered Saline (PBS) were dropped to the strip biosensor for 10 min at 23 °C.

2.2.2. Smartphone-based fluorescence reader

The 3D-printed smartphone-based fluorescence reader was designed by using CATIA (Dassault Systèmes Inc, Catia V5R14) and a 3D printer (Measurement Korea Corp., Wiiboox). Dimensions and weight of the reader were measured to be 115 mm × 65 mm × 58 mm and 137 g, respectively. Two green LEDs at 530 nm wavelength (Cree inc., XPEBGR-L1-0000-00E03) were employed for the excitation light source. The LED light was filtered with optical filters (Chroma Technology Corp., AT540/25x) to minimize the leakage of LED light through the emission filter, and subsequently directed to a strip biosensor with an incident angle of 45-degree. The fluorescence emission from the strip biosensor was then collected by an achromatic lens (Thorlabs Inc., AC080-010-A-ML) with a focal length of 10 mm, passed through the emission filter (Chroma Technology Corp., AT605/55m), and subsequently measured by a two dimensional (2D) charge-coupled device (CCD) sensor (HANJIN DATA Corp., 1321191). The field of view was determined by 5.7 mm × 3.2 mm (1920 × 1080 pixels) by adjusting the magnification of the fluorescence imaging system at 1x. A 9V battery was employed to supply a stable DC voltage (3.3 V) to the LEDs by a voltage regulator (Texas Instruments Inc., LM1117IMPX-3.3). The 2-dimensional fluorescent image was transferred to a smartphone (LG Electronics Inc., LG-F400L) using a micro 5-pin connector.

In addition, a strip biosensor (Fig. S1(a)) was loaded into a slot of the smartphone-linked fluorescence reader. The customized application was designed to capture a fluorescence image and to quantify the salivary cortisol concentration. In order to generate the signal output from the strip biosensor, the captured image was first converted to a grayscale image by computing the average of pixel values over 3 color channels (i.e. red, green and blue channels) as shown in Fig. S1(b). The sensor output was then calculated by evaluating the mean of pixel values in the regions of interest (ROI) (I_{ROI}) and reference (I_{ref}), then subtracting both parameters (I_{ROI} - I_{ref}). We evaluated the difference between I_{ROI} and I_{ref} to compensate for the intensity fluctuation in the LEDs and noises arising from the image sensor. The ROI sizes of the measurement and reference spot were 3 mm × 1 mm and 0.3 mm × 0.3 mm, respectively. Then, we have decided the locations for both regions on the strip biosensor, as depicted in Fig. S1(c). In fluorescence measurement experiments, the exposure time was set to 1/16 s, which is the smallest coefficient of variation (CV) value (0.75%). The detailed description on the noise performance of the reader is provided in Fig. 2. The smartphone application, named as the *Smart Fatigue Phone*, based on the android OS was designed for quantification of the salivary cortisol. By pressing the capture button, the embedded application automatically converted the output signal (I_{ROI}-I_{ref}) into salivary cortisol concentration using a calibration curve of the *Smart Fatigue Phone*. In addition, a total measurement time was within 7 s, which includes capturing images, computing cortisol concentration, and displaying a driver status on the smartphone.

2.3. EEG measurement

Neurons, neurite cells and blood brain barrier mainly determine the electrical activity of the brain. Power spectrum analysis is usually used to classify frequencies according to Electroencephalograms (EEG) measurement. This analytical method assumes that EEG signal is a linear combination of simple oscillations of a particular frequency and decomposes each frequency component of the signal to represent the amplitude. The EEG is generally divided into θ (theta wave, 3–7 Hz), α (alpha wave, 8–12 Hz), β (beta wave, 13–29 Hz), and γ (gamma wave, 30–100 Hz) wave in relation to the range of frequency (Fitzgibbon et al., 2004). Theta and alpha are dominant in deep sleep, emotionally stable and relaxed states, whereas Beta and gamma waves are mainly observed in mental instability and complex problem solving, respectively. The parietal lobe near the forehead has a somatosensory cortex responsible for movement and sensory information, and the occipital lobe plays an important role in primary visual processing. The EEG was recorded via a 20-channel cognitive wireless EEG system (Quick-20 system, Cognionics, USA), with the following settings: 1 kHz sampling and band pass filtering at 0.05–100 Hz. The silver electrodes were attached to Fp1, Fp2, F3, F4, F7, F8, C3, C4, T3, T4, P3, P4, T5, T6, O1, and O2 according to the international 10–20 method (Okamoto et al., 2004). In addition, we set up low pass filtering at 50 Hz, thereby sampling EEG data. The following band frequency was analyzed and quantified in absolute power for 3 consecutive 15 min of the driving test.

3. Result and discussion

3.1. Noise performance of fluorescence reader

We measured noise characteristics of LEDs and image sensor to figure out the optimal exposure time for highly accurate detection system for salivary cortisol concentration. In order to characterize dark and readout noise from the *Smart Fatigue Phone*, numerous images were captured as varying detector exposure times. Then, average of each pixel value over the entire detection area, where antibodies and antigens were conjugated, was calculated. As shown in Fig. 2(a), we captured 20 images at each condition with measurement of noise outputs and standard deviations. The dark noise and readout noise of the fluorescence detection system were the smallest at the exposure time of 1/16 s. In addition, we examined the intensity fluctuation of the LED light source. The light emitted from the LED was directly reached to a photodetector (Thorlabs Inc., PDA36A-EC). Subsequently, the fluorescence intensities were measured for 2.6 s at a sampling rate of 500 Hz. The power spectrum of the measured intensity fluctuation is shown in Fig. 2(b), along with the intensity fluctuation in the inset. Interestingly, the smallest value for intensity fluctuation was detected at 16 Hz frequency. Our experimental results for both readout noise and LED

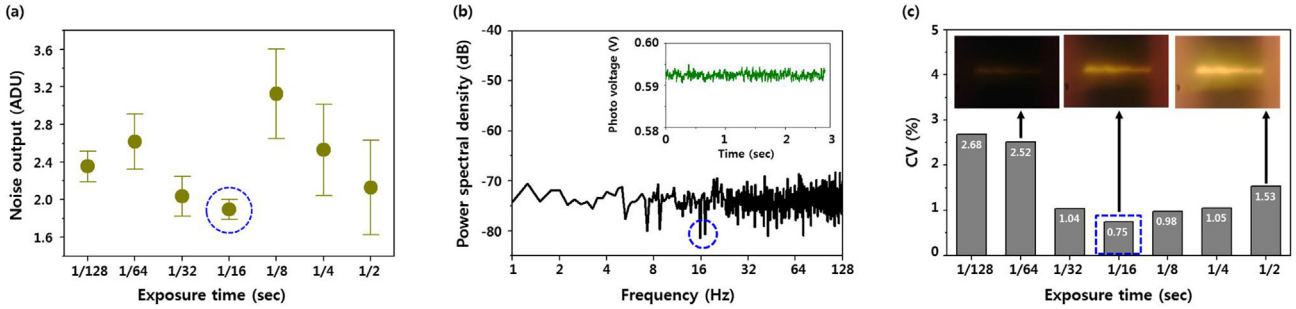

Fig. 2. (a) Measured dark and readout noise outputs from the image sensor at different exposure times. The error bars indicate the standard deviation obtained with 20 measurements. (b) The power spectral density of the measured LED intensity fluctuation. The inset shows the measured intensity of the LED light acquired for ~2.6 s at a sampling rate of 500 Hz. (c) Measured CV values of the sensor outputs at various exposure times. All of the results denote that measurement at the exposure time of 1/16 s would provide fluorescence signal with the highest precision.

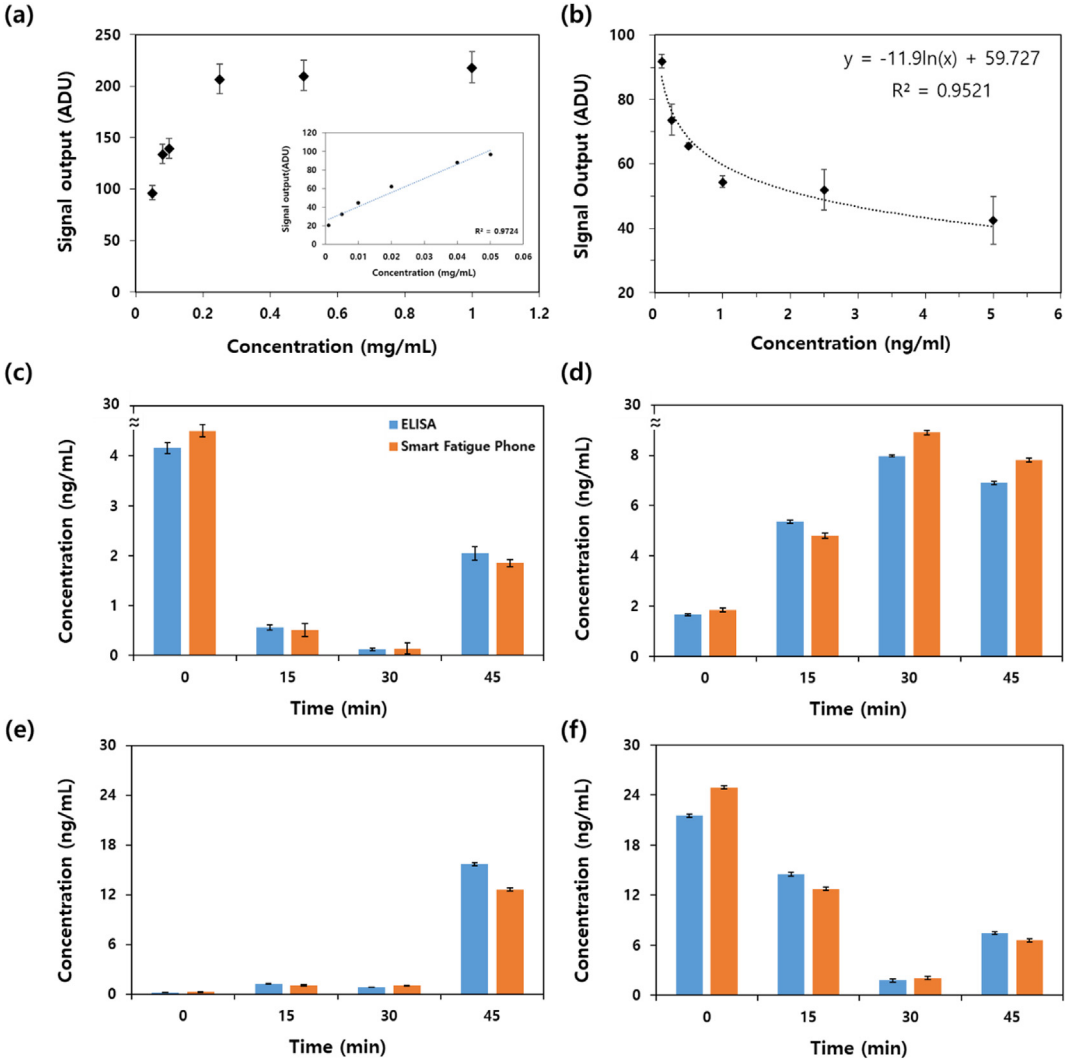

Fig. 3. Determination of the optimum concentration for Cy3 dye conjugated cortisol antibodies. (a) The conjugation of multiple different concentrations from low to high (0.001, 0.005, 0.01, 0.02, 0.04, 0.05, 0.08, 0.1, 0.25, 0.5, and 1.0 mg/mL) and Cy3 dye, respectively, were reacted with cortisol-BSA that is placed on a test line of the lateral flow immunosensor. (b) The calibration curve of the strip biosensors at various concentration (0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 ng/mL) are presented with high coefficient of determination (r^2 = 0.9521) by using the *Smart Fatigue Phone*. (c–f) The comparison of cortisol concentration for each subject over time, obtained from ELISA and the *Smart Fatigue Phone*, is described.

intensity fluctuation denoted that the measurement with the highest precision could be achieved at the exposure time of 1/16 Hz. In order to validate these results, we loaded strip biosensor into the fluorescence reader, measured variation of intensities from a strip biosensor under various exposure times, and evaluate Coefficient Variation (CV) as shown in Fig. 2(c). The smallest CV was detected at the exposure time of 1/16 s with 0.75%. Based on these results, our study was performed at the exposure time of 1/16 s.

3.2. Validation of smartphone-based fluorescence detection system compared to ELISA kit

In this study, we were able to identify the fluorescence signals from each lateral flow immunosensor where various concentrations of cortisol antibody (0.001, 0.005, 0.01, 0.02, 0.04, 0.05, 0.08, 0.1, 0.25, 0.5, and 1.0 mg/mL) were conjugated with Cy3 dye as shown in Fig. 3(a). The fluorescence intensity in response to each antibody concentration showed that the intensity no longer increased by more than 0.25 mg/mL (Fig. 3(a)). In addition, a smear of Cy3-conjugated antibodies on the membrane was appeared around a test line at the highly concentrated condition above 0.25 mg/mL in Fig. S2. Due to interatomic aggregation among highly concentrated cortisol antibodies, the enlarged compounds, conjugated with Cy3 dye, are stuck into the membrane. In the light of the above, we determined the optimum concentration of Cy3-conjugated cortisol antibodies as 0.25 mg/mL. The calibration curve of each concentration (0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 ng/mL) of salivary cortisol was described with high coefficient of determination (r^2 = 0.9521) in Fig. 3(b). The equation (1), where x denotes the concentration of cortisol and y refers to signal output (fluorescence intensity), was then applied to the *Smart Fatigue Phone* to evaluate the cortisol concentration from the captured images.

$$x = e^{(y-59.727)} \div 11.9 \quad (1)$$

Furthermore, the comparison of concentration for salivary cortisol from each subject measured by commercial ELISA kit and the *Smart Fatigue Phone* was presented on Fig. 3(c–f). The error rates of the results between strip biosensors and ELISA kit were measured within 10% (± 4%) as 7.69, 9.45, 12.14, and 10.58% in figure (c), 10.0, 11.67, 10.51, and 11.65% in figure (d), 13.88, 16.31, 18.98, and 24.28% in figure (e), and 13.58, 13.66, 12.74, 13.86% in figure (f), respectively. The significantly high error rates were monitored in a few strips since the different amount of cortisol-BSA was immobilized on a test line of

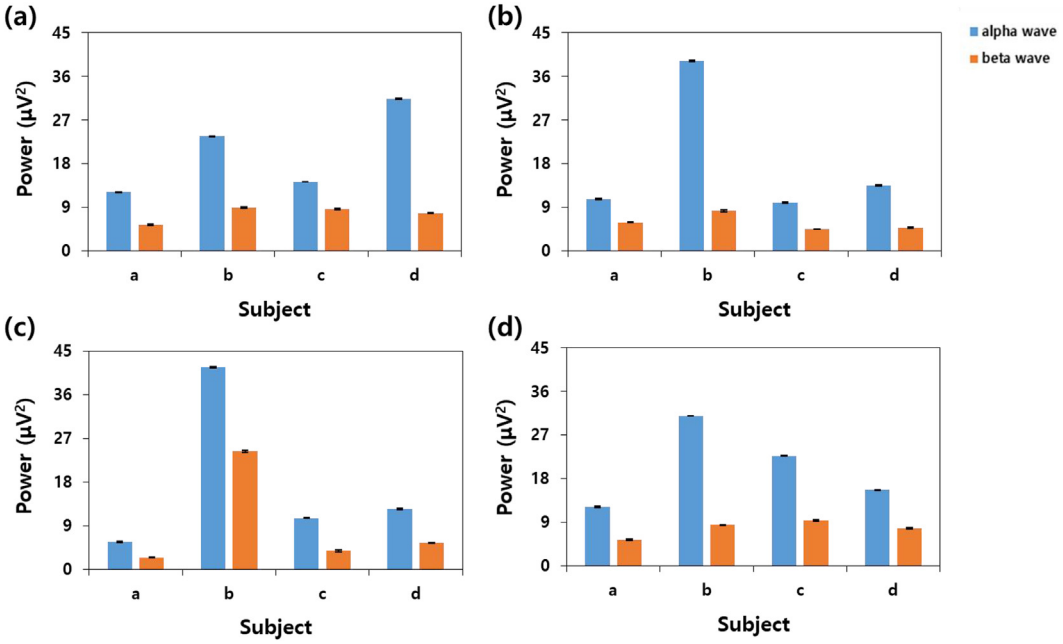

Fig. 4. (a–d) The comparison of average power spectrum (alpha and beta wave) of four participants in response to time (0, 15, 30, and 45 min, respectively).

each strip biosensor by using a strip dispenser, thereby resulting in low conjugation between Cy3-conjugated antibodies and cortisol-BSA. On the other hand, the similar trend of a concentration shift of cortisol for every 15 min was observed in both measurement methods. In addition, standard deviation (SD), coefficient of variation (CV), and error rate of cortisol concentration, measured by ELISA kit and the *Smart Fatigue phone* from 4 different participants, were calculated as shown in Table S1.

3.3. Correlation between salivary cortisol concentration and alpha wave signal

EEG signals measured during 60 s (± 30 s at the measurement point) were averaged to minimize the error rate for signal noise caused by subject movement during saliva collection. As shown in Fig. 4(a–d), the significant differences were found in alpha and beta wave for the subjects. This observation suggested that brain activity (alpha wave) of the frontal lobe increased in the fatigue status. This finding is consistent with roles of the frontal lobe rule in attention. All of the subjects had high alpha and low beta frequency band during the driving session (every 15 min). In other words, the low amplitude in beta frequency band appeared in the sleep group. The SD and CV of alpha and beta frequency power were calculated (Table S2). In addition, each concentration of cortisol of the subjects was measured by two different methods: ELISA kit and *Smart Fatigue Phone*. The proportional correlation between alpha wave and cortisol concentration with respect to driving time was observed in Fig. 5(a–d). The collected data were analyzed statistically using ANOVA (analysis of variance) to investigate the correlation between alpha wave and cortisol in fatigue state. The ANOVA in the alpha frequency bands showed high interaction between EEG and salivary cortisol ($p < 0.05$). The fatigue effect appeared immediately in that the increase in frontal Fp1, Fp2, F3, F7, O1 and O2 amplitudes was significant in the entire driving session analysis ($p < 0.05$). This finding likely reflected the alpha wave and concentration of cortisol depending on a degree of fatigue in response to driving time. In addition, we were able to determine the fatigue status of each participant based on reference values where less than 1 ng/mL of cortisol and range from $8\mu V^2$–$12\mu V^2$ of EEG signal are considered as fatigue state (Cleare, 2004; Roberts et al., 2004; Fitzgibbon et al., 2004).

4. Conclusion

In this study, we have successfully developed the smartphone-based fluorescence reader for salivary cortisol detection and evaluated significant correlation between cortisol concentration and the EEG signals in response to the fatigue condition during performing a driving simulation. In order to enhance the sensitivity of the sensor, the fluorescence detection system (a smartphone-based reader and a lateral flow immunosensor) was applied because the low level of cortisol (less than 1.0 ng/mL) is generally detected in the fatigue condition. Furthermore, we successfully enhanced the sensitivity of lateral flow immunosensor until 0.1 ng/mL, which enabled to distinguish the subjects in the fatigue condition from the awaken people. Our system possesses significant pros compared with ELISA, electrochemical sensor, Raman spectroscopy, and surface plasmon resonance in terms of ease-to-use, rapid, and cost-effective methods. On the other hand, we were only focused on salivary cortisol level regarding fatigue status in this study; additional fatigue-related biomarkers, such as alpha-amylase and lactate, are needed to be studied for correlation with cortisol concentration to enhance accuracy of the fatigue detection system. In addition, the system has a cumbersome disadvantage of diluting saliva collected from subjects into buffers (PBS) and dropping the solution into a strip biosensor. In order to improve the drawback of the *Smart Fatigue Phone*, we are currently developing an all-in-one strip biosensor that allows collecting saliva sample and detecting cortisol concentration simultaneously. Thus, we fully expect our system to be a practical tool for determination of fatigue status of drivers to avoid fatal crashes on roadway before driving a car.

Declaration of interests

All authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

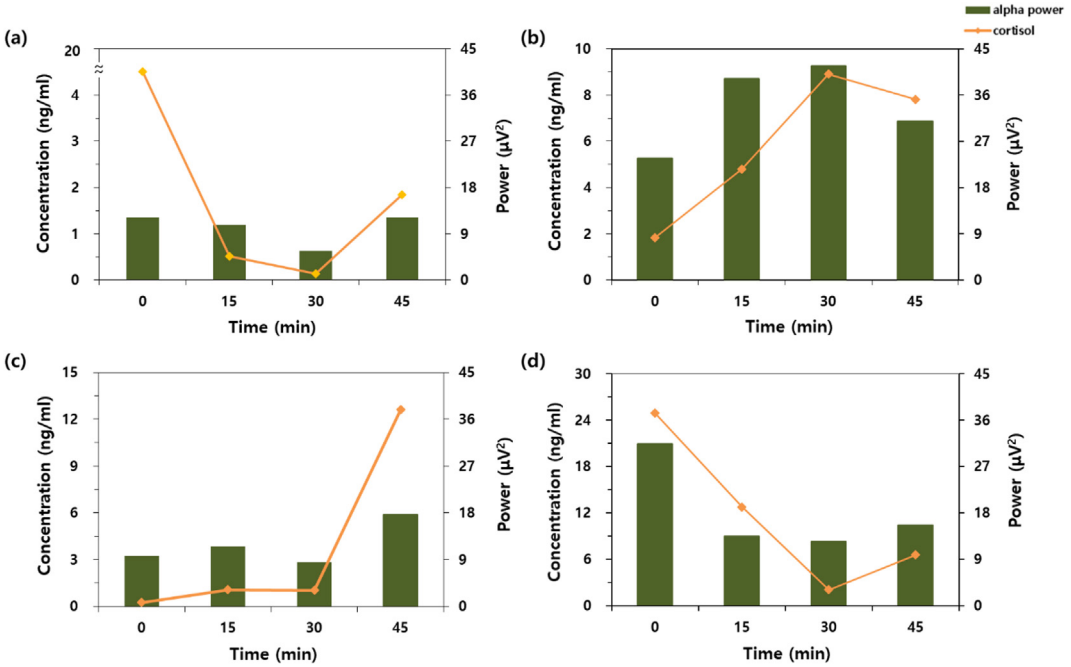

Fig. 5. (a–d) Correlation between alpha power spectrum and concentration of cortisol in response to time from four different subjects.

CRediT authorship contribution statement

Joonchul Shin: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Soocheol Kim:** Conceptualization, Software, Validation, Formal analysis, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Taehee Yoon:** Validation, Formal analysis, Resources, Data curation, Writing - review & editing. **Chulmin Joo:** Investigation, Writing - review & editing, Visualization, Supervision, Funding acquisition. **Hyo-Il Jung:** Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.04.046.

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