



Technical note

Development and testing of acoustically-matched hydrogel-based electrodes for simultaneous EMG-ultrasound detection

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ABSTRACT

In this manuscript we describe the development and testing of a bipolar electrode for the simultaneous acquisition of ultrasound (US) images and surface electromyograms (EMGs) from the same muscle region. The developed electrode (bEMG-US) consists of two circular sensing regions (20 mm diameter) with fixed inter-electrode distance (3.5 cm, center-to-center). Both the sensing regions and the external structure of the electrode are made of hydrogel layers separated by insulating materials. The electrical properties (i.e., impedance and noise of the electrode-skin interface) and the quality of EMGs detected with the developed electrodes during electrically elicited contractions were assessed and compared with those provided by commercially available EMG electrodes. The effect of the bEMG-US electrode on US images was evaluated by comparing images detected from the same muscle region with and without the electrode interposed between the US probe and the skin. Tests on five subjects showed that the electrode-skin impedance of bEMG-US electrodes was higher than that of conventional EMG electrodes (mean (range): 15.6 (8.5–21.1) k Ω vs. 8.2 (4.9–16.5) k Ω). Despite higher impedance values, both electrode systems provided comparable, electrode-skin noise levels (1.4 (1.1–1.7) μ V vs. 1.3 (1.0–1.5) μ V) and *M* waves (normalized mean square error: 2.6 (0.6–6.8)%). The quality of US images detected with and without the bEMG-US electrode between the US probe and the skin was comparable, as demonstrated by the low errors in the estimation of anatomical variables in the two experimental conditions (range: (0.37–2.35) deg for pennation angle and (–0.31–0.1) cm for muscle thickness). Results demonstrate that bEMG-US can be used to acquire concurrently EMGs and US images from the same muscle region with a negligible effect on the quality of the two detected signals, thus allowing for a simultaneous, multimodal evaluation of muscle activation.

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1. Introduction

Surface Electromyograms (EMGs) provide a measure of muscle activity that has found application in the field of muscle fatigue, myoelectric control of prosthesis, movement analysis and others [1–5]. Ultrasound imaging allows for the in-vivo assessment of muscle architecture, often considered in clinical applications and basic research [6–9]. By combining EMG and US imaging it would be possible to fully characterize the electromechanical properties of the muscle, from the neural excitation to the resulting muscle tissue displacement, and their possible adaptations with aging, rehabilitation, neurological disease, and injury. This integration may be obtained by positioning the two detection systems alongside

each other. However, for some body regions with high muscle density, e.g., the forearm, this arrangement is not viable, as EMGs are localized in correspondence of the surface projection of the muscle [10] and the US probe located beside the electrodes would sample from different muscles. For large muscles, this issue seems less relevant; however, there is a growing number of evidence describing regional patterns of muscle activation from EMGs [11–13] as well as regional variations in fascicle architecture and properties [14–16]. Hence, even within the same muscle, EMGs and US images detected from different portions may reflect distinct physiological and/or geometrical events. The optimal approach for a joint EMG-US investigation of muscle activity seems therefore to demand the recording of both signals from the same muscle region, which requires the US probe to lie over the electrodes.

Recording surface EMGs and US images concurrently from the same muscle region requires specific technological solutions to avoid artefacts in both EMGs and US images. The acoustic

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impedance of the electrode, of its supporting material and of the skin must match; otherwise, reflections of the ultrasound beam would hinder the visualization of muscle structures in US images. Henceforth, we use the term *acoustically-matched* to indicate the acoustic impedance matching between different materials. Moreover, the detection system must adhere to the skin to avoid the formation of air bubbles at the skin interface. While these features ensure the detection of clear US images, high quality EMGs demand the insulation of electrodes and connections, avoiding therefore short circuits and low impedance paths generated by the US coupling gel.

In 2013 we developed a grid of 32 electrodes for simultaneous detection of EMGs and anatomical muscle features [17]. Each electrode consists of a stainless steel wire (100 μ diameter) exposed within a small, circular cavity (4 mm diameter) housed in a substrate of polymeric material. The electrical contact between wires and skin is ensured by filling cavities with a water-based conductive gel or paste. The mechanical properties of the polymer (i.e., density and adhesion to the skin) and the relatively small size of the cavities ensure a negligible reflection of the US beam, thus making the detection system suitable for simultaneous EMG-ultrasound detections. This grid finds application in studies investigating the spatio-temporal properties of motor unit activation (through multichannel EMG) and the associated mechanical muscle tissue strain (through US imaging). For instance, it has been used to investigate the spatial relationship between excitation and muscle motion onsets in biceps brachii [18] and it is being used for the EMG-US based identification and classification of fasciculation potentials in triceps surae [19]. For some applications or contexts, however, the use of a multichannel detection system may not be necessary nor feasible. Providing a couple of electrodes samples EMGs from a representative muscle region [20], for example, the overall degree of muscle activation may be adequately estimated from bipolar electrodes. Moreover, the production of acoustically-matched grids of electrodes is expensive and demands considerable time. Simpler and more affordable detection systems (e.g., bipolar electrodes) are therefore useful for the concurrent acquisition of surface EMGs and US images. If the development of bipolar electrodes for simultaneous EMG-US recordings is to be pursued, the cavity-based technology used for the grids of electrodes is unlikely appropriate as a somewhat large cavity volume would have to be filled with conductive gel. However, with respect to the grids, the greater size of the electrodes and larger inter-electrode allow for different technological solutions not suitable for the small, grid electrodes. Here, we present and test an innovative, potential solution for a more affordable production of acoustically-matched bipolar electrodes.

In this note we describe the technical procedures necessary for the realization of bipolar, hydrogel-based electrodes, suitable for the simultaneous acquisition of surface EMGs and US images. The test of the device in terms of electrode-skin impedance, contact noise, and quality of EMGs and US images collected is presented.

2. Methods

An EMG electrode consists of a sensing region (i.e., the transducer), usually a metal plate that interfaces the skin through a layer of electrolyte solution, and a supporting material that includes sensing region and connections and provides the mechanical stability to the device. In some cases, a pair of sensing regions share the same supporting material, resulting in a single bipolar electrode with fixed inter-electrode distance. The device developed in this study, hereafter referred to as bEMG-US electrode, includes two sensing regions integrated in the same support, and the electrical connections to an external acquisition system.

2.1. System requirements

The main functional requirement of an electrode for simultaneous acquisition of surface EMG and US from the same muscle region is the ability of transmitting ultrasound with a minimal attenuation. It is well known that reflection, scattering and adsorption attenuate the ultrasound beam, depending on the material properties (e.g., size, density, presence of inhomogeneity). While the reflection due to biological tissues is at the basis of ultrasound imaging, the interaction with other structures along the propagating path of the ultrasound leads to a loss of energy and to the generation of artefacts in the ultrasound image. The characteristics of the material constituting this kind of electrodes should thus comply with two requirements: (i) be homogeneous, to reduce the number of transitions (and reflections) through different materials, and (ii) having an acoustic impedance similar to that of biological, soft tissues, which is very close to that of the water [21]. In this respect, the presence of porous structures containing air (e.g., foam) or highly dense materials (e.g., metal) is critical, as they would generate artefacts in the ultrasound image. Using a layer of US coupling gel to improve the acoustic interface between the electrodes and the skin is overtly inappropriate, as it would reduce the impedance between electrodes. For this reason, the entire device (electrodes and supporting material) must be highly adhesive on the skin side, ensuring a good acoustic, electrode-skin interface without the need of US coupling gel.

As regard to the EMG detection, geometrical properties of the electrodes should be comparable to those of commercially available electrodes (1–2 cm diameter of the conductive area and 2–3 cm inter-electrode distance).

2.2. Electrode description

Hydrogel was selected for designing the sensing regions and the external structure of the bEMG-US electrode, as it fulfills the main project requirements: (i) it is a water-based material and thus suitable to transmit the ultrasound beam with low attenuation, (ii) it is adhesive, ensuring a good mechanical and acoustic contact with the skin, and (iii) its conductivity makes it a suitable interface between the skin and the metal part of the electrode [22,23]. However, being hydrogel a conductive material, its use as supporting material for the external electrode structure requires the electrical insulation between the two sensing regions that would otherwise be short-circuited. A possible solution to have insulating elements without the use of additional materials is to reduce the concentration of salts in the hydrogel; however, preliminary experiments revealed reduced salt concentration did not reduce hydrogel conductivity sufficiently (at least 1/100 of the conductive material) to create insulating layers. It was therefore necessary to use additional material to insulate the sensing regions. Fig. 1A shows a schematic representation of the bEMG-US structure. The first hydrogel layer is 0.8 mm thick. Within this layer, two insulating rings (silicon rubber, 20 mm diameter) define the shape of the two sensing regions and avoid short circuits through the first, hydrogel layer. The center-to-center distance between the two sensing regions (i.e., inter-electrode distance) is 35 mm. A thin (0.1-mm diameter) Ag/AgCl wire, whose insulation is removed in correspondence of the hydrogel disc, connects each sensing region with an external connector. A thin sheet of bi-adhesive material (0.08 mm thick) ensures the top, hydrogel layer (0.8 mm thick) does not short circuit electrodes. An additional, outward sheet of low-density polyethylene (0.06 mm thick) serves as external protective layer. By removing this last sheet, the external surface of the device becomes adhesive. In this condition, the US probe may

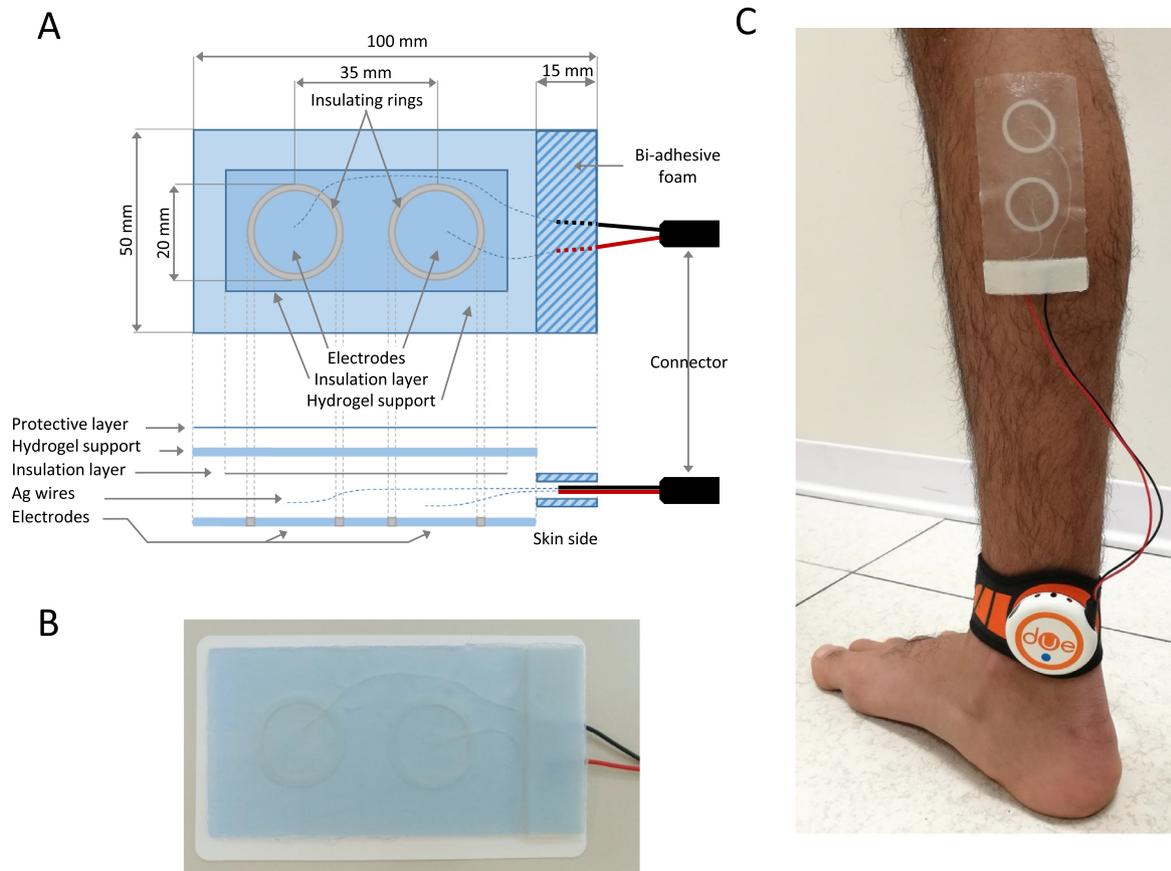


Fig. 1. bEMG-US Electrode. (A) Schematic representation of the bEMG-US electrode (planar view and longitudinal section). (B) Picture of the device represented in the same dimensions of panel A. (C) bEMG-US electrode positioned over the medial gastrocnemius.

be attached over the electrode and the hydrogel of the electrode replaces the ultrasound gel as coupling material with the skin. Fig. 1B shows a picture of the bEMG-US electrode.

2.3. Electrode testing

An experimental protocol was designed and applied to five male volunteers (range values: age 24–37 year, height 170–187 cm, body mass 61–85 kg), after they provided written informed consent. Data collection was carried out at Politecnico di Torino, the study conformed to the Declaration of Helsinki and was approved by the Ethics Committee of ASL TO1, Torino, Italy. The aims of this protocol were to: (i) characterize the electrodes in terms of electrode-skin impedance and noise, (ii) evaluate the effect of the proposed electrodes on the quality of US images and surface EMGs recorded simultaneously from the same muscle region. All measurements were performed on medial gastrocnemius (MG) with the subject lying comfortably in a prone position over a padded bed.

2.3.1. Impedance and noise measures

The electrode-skin impedance of bEMG-US electrodes was measured in two experimental conditions: with and without ultrasound probe and gel over the electrodes. With these measurements, we tested whether the presence of US gel could generate a low impedance path between the sensing regions, thus reducing the inter-electrode impedance. Impedance and noise measures obtained from bEMG-US electrodes were compared with those obtained from conventional, bipolar electrodes classically used in EMG recordings (CDE02401500BX, Spes Medica, Battipaglia, Italy).

To ensure a like-with-like comparison, the selected conventional electrodes had the same shape (circular), size ($d = 20$ mm) and center-to-center distance (35 mm) of bEMG-US electrodes. Moreover, the material interfacing the skin of conventional electrodes was the same, with the same thickness (0.8 mm) of the bEMG-US electrodes.

After cleaning the skin with abrasive and conductive paste (Everi cream, Spes Medica, Battipaglia, Italy), both bEMG-US and conventional electrodes were positioned, one at a time, over the same MG region. Electrodes were positioned over the superficial aponeurosis of MG, on average 5 cm distally from the popliteal crease, along the proximal-distal axis of the muscle (Fig. 1C). The noise of the electrode-skin interface was measured with a custom-made noise amplifier (LISiN, Politecnico di Torino, Italy) with the following characteristics: 12,000 amplification; 100-dB CMRR; 100 G Ω /2 pF input impedance; 0.8 μ V RTI noise; 0.1–1000 Hz bandwidth. The amplified noise was acquired (USB-6210, sampling frequency: 10 kHz, 16 bits A/D converter) and then band-pass filtered (10–1 kHz, 2nd order Butterworth filter). The root mean square (RMS) of the noise was computed over a 10 s epoch. Afterwards, the impedance between the two electrodes (i.e., the sum of two electrode-skin impedances and the tissue impedance) was measured with a custom-made impedance meter (LISiN, Politecnico di Torino, Italy). This device injects a sinusoidal current (200 nA peak-to-peak amplitude) and measures the voltage drop between the electrodes tested. The magnitude and the phase of the impedance are then quantified as the amplitude ratio and the phase shift between the measured voltage and injected current, for a frequency sweep in the range 10 Hz–1 kHz. The impedance magnitude at 50 Hz was considered to compare the three experimental conditions.

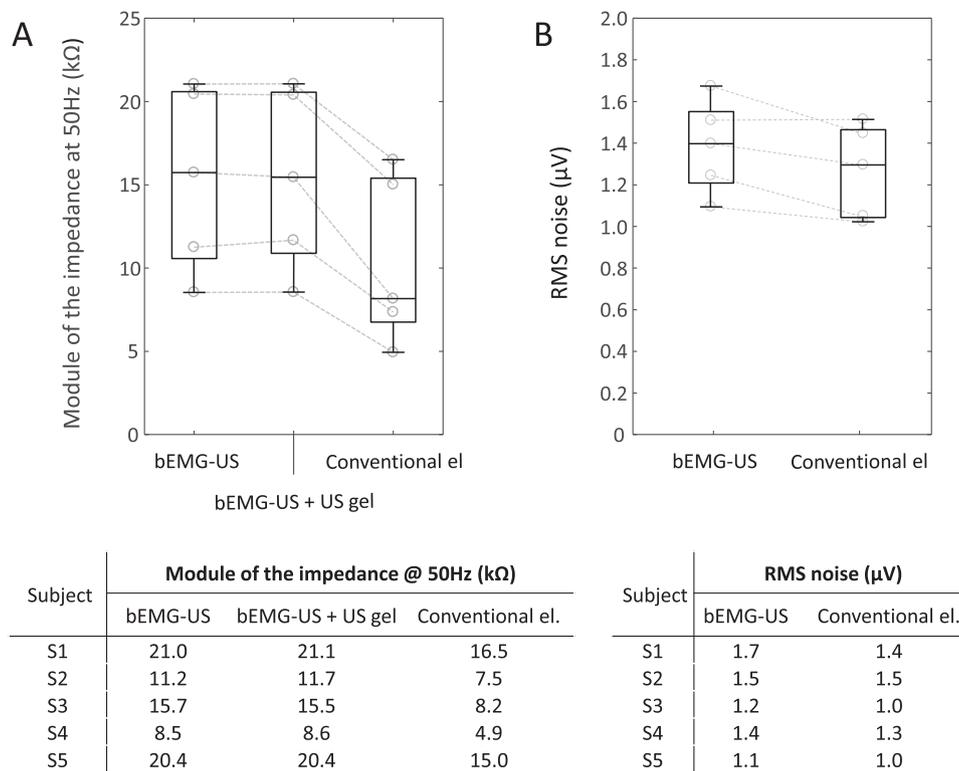


Fig. 2. Impedance and noise measures. (A) Comparison between the module of the electrode-skin impedance measured in three experimental conditions: (i) between the two sensing regions of bEMG-US electrode, (ii) in the same condition as (i), but with a layer of US gel covering the top of the device (i.e., bEMG-US + US gel), and (iii) between two conventional electrodes for EMG recordings with the same diameter and inter-electrode distance of bEMG-US electrodes. Data are reported as magnitude of the impedance at 50 Hz. (B) Root mean square (RMS) noise of the electrode-skin interface for bEMG-US and conventional electrodes. Noise was estimated in the 10Hz-1 kHz frequency band. For both electrode-skin impedance and RMS noise, data from individual subjects are reported below the figures.

2.3.2. EMG quality assessment

We quantified how much the detection system (bEMG-US only, bEMG-US with US gel, and conventional bipolar electrodes) affects the quality of EMGs. Quality assessments were made during electrically elicited contractions, ensuring differences in the detected EMGs were mostly due to differences in the detection system [17]. Immediately after the noise and impedance measurements, a circular, pre-gelled cathode electrode (10 mm diameter) was positioned over the branch of the tibial nerve supplying the MG muscle and a large anode electrode (80 × 50 mm, damp cloth) was fixed to the leg anterior aspect, just above the patella, using elasticized bandage [24]. Afterwards, a train of 10 supramaximal current stimuli (rectangular pulses, 200-μs duration) was delivered at 1 pps. *M* waves were detected and wirelessly transmitted to a personal computer with a bipolar EMG amplifier (Due, OT-Bioelettronica, Torino, Italy), separately for each of the three detection systems. After band-pass filtering (2nd order, zero-lag Butterworth filter, 20–400 Hz), the 10 *M* waves were averaged and differences in the averaged *M* waves between detections systems were assessed with the Normalized Mean Square Error (NMSE) [17].

US image quality assessment: The aim of this measurement was to evaluate the influence of the bEMG-US electrodes on the US images. US images were detected in two experimental conditions: without and with the bEMG-US electrodes positioned between the US probe and the skin. The first measure was performed at the beginning of the experiment (i.e., before electrodes' application), whereas the second measure was done after the *M*-wave detection using bEMG-US electrodes. The position of the probe was marked on the skin to ensure a consistent probe repositioning in the two experimental conditions. The overall quality of the image and the possibility of observing muscle features such as muscle thickness, subcutaneous layer thickness and pennation angle were regarded

as qualitative comparison criteria. In order to quantify the effect of the detection system on the quality of US images, we measured and compared muscle thickness and pennation angle estimated from US images [25] collected with and without the bEMG-US electrode interposed between the US probe and the skin.

3. Results and discussion

3.1. Impedance and noise measures

Impedance though not noise of the electrode-skin interface was affected by the detection system. Fig. 2 shows the comparison of electrode-skin impedance and noise measured in the experimental conditions described in the methods. In this experiment, the impedance was measured between two electrodes. Therefore, it reflects the sum of the impedances of two electrode-skin junctions and the impedance of the tissue between the electrodes. Similar values of electrode-skin impedance were measured with (15.6 (8.6–21.1) kΩ) and without (15.5 (8.5–21.1) kΩ) ultrasound gel over the bEMG-US electrodes. This result suggests that the presence of gel over the bEMG-US electrodes does not generate low impedance paths between the two sensing regions. In the comparison between bEMG-US and conventional electrodes, a higher module of the impedance at 50 Hz was observed for bEMG-US electrodes: 15.6 (8.5–21.1) kΩ vs. 8.2 (4.9–16.5) kΩ. The physical dimension of the electrodes' components may explain this difference. The type and the dimension of the hydrogel disk interfacing the skin are the same for both kind of electrodes (0.8 mm thickness, 20 mm diameter). Therefore, if the contact area between the metal (Ag/AgCl for both electrodes) and the hydrogel was the same for both the bEMG-US and conventional electrode, their electrode-skin impedance would be likely comparable. However,

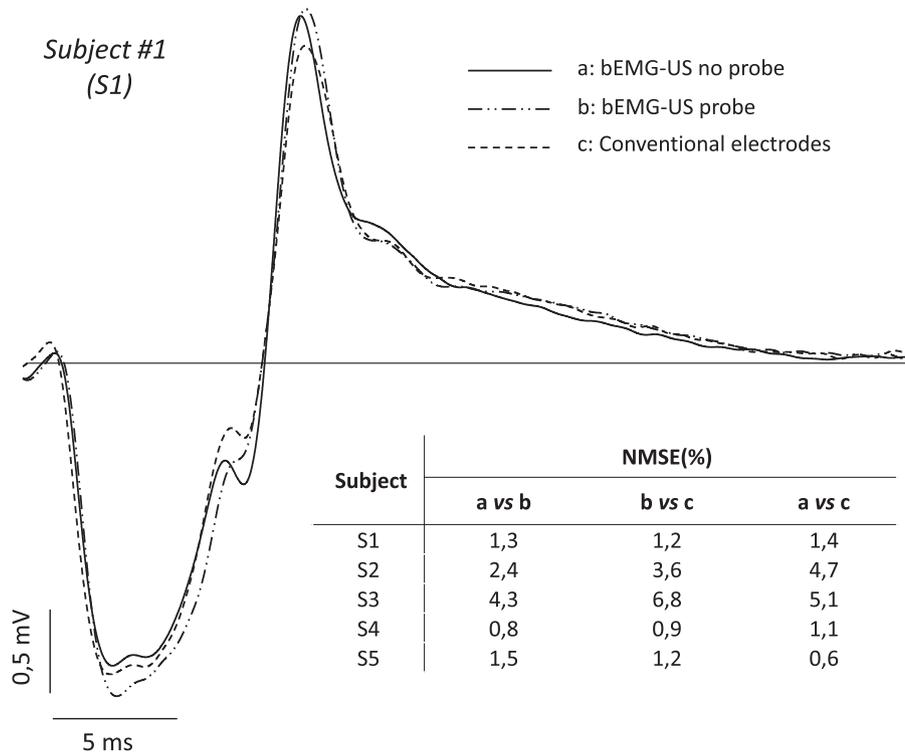


Fig. 3. Quality of the detected EMGs. *M* waves detected from conventional electrodes and from bEMG-US electrodes in two conditions: with and without the presence of the US probe and US gel over the top of the device. Representative *M* waves are shown for an individual subject (S1). The Normalized Mean Square Error (NMSE) between pairs of *M* waves detected in the different conditions is reported for all subjects in the table.

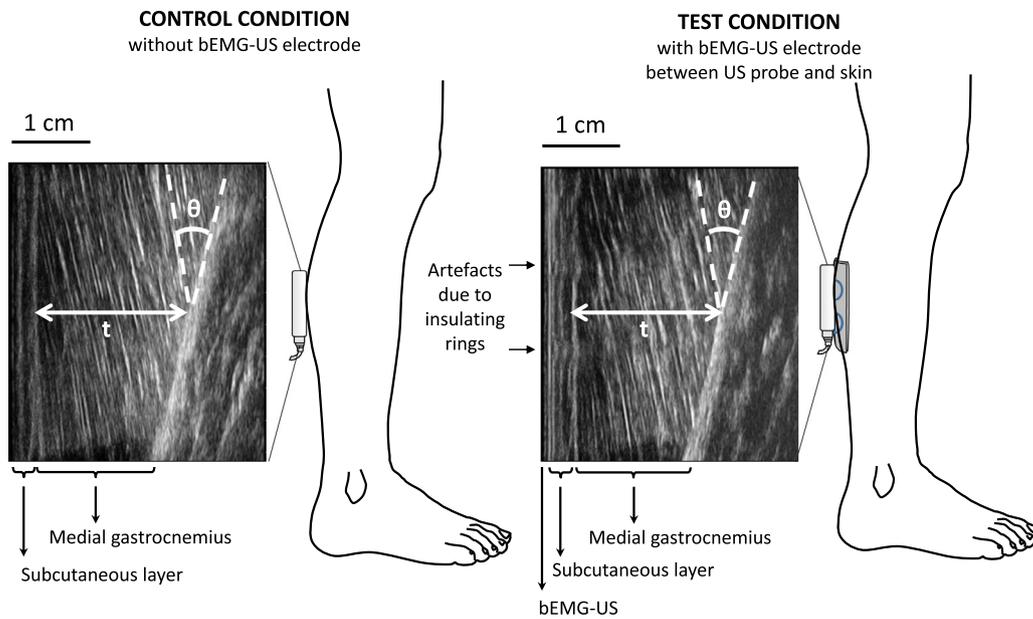


Fig. 4. Effect of bEMG-US electrodes on the US images. US images detected from the central part of medial gastrocnemius in two experimental conditions: without and with the presence of bEMG-US electrodes between the US probe and the skin. Horizontal arrows in the right panel indicate two regions of slight image distortion due to the presence of bEMG-US insulating rings. t = muscle thickness; θ = pennation angle.

the metal-hydrogel contact area is markedly smaller for bEMG-US electrodes. Although our experiment was not designed to quantify the contribution of different electrode materials on the electrode-skin impedance, our results suggest that the size of the metal-hydrogel interface may account for the differences observed in the electrode-skin impedance. Notwithstanding the higher electrode-skin impedance of bEMG-US electrodes, the RMS noise (1.4 (1.1–1.7) μ V) was comparable to that of conventional electrodes (1.3

(1.0–1.5) μ V), as shown in Fig. 2B. The observation of comparable noise values for bEMG-US and conventional electrodes is consistent with the notion that the electrode-skin noise mainly originates at the electrolyte-skin interface and that it is highly dependent on the electrolyte used to interface the skin [26]. Indeed, in this study the type, the shape and the size of the electrolyte layer were the same for the two tested electrodes, possibly generating a comparable noise level.

3.2. EMG quality assessment

The electrode type affected marginally the shape of maximal M waves, as shown in Fig. 3. The NMSE between M waves detected with conventional electrodes and bEMG-US electrodes was consistently smaller than 7%: 2.6 (0.6–6.8)% (see b vs. c and a vs. c in Fig. 3). Moreover, the presence of the US probe and gel over the bEMG-US electrodes modified to a low extent the detected, M-wave shape, as confirmed by the low NMSE values 2.1 (0.8–4.3)% (see a vs. b in Fig. 3). These NMSE values are comparable with those reported in our previous study for a grid of small, EMG-US electrodes [17]. Current results indicate that, despite the larger electrode-skin impedance observed for bEMG-US electrodes, the quality of EMG signals detected with the proposed electrodes is comparable with that of conventional electrodes.

3.3. US image quality assessment

Visual inspection of US images revealed that the presence of bEMG-US electrode did not affect the image quality. Fig. 4 shows an example of US images detected with and without the bEMG-US electrode positioned between the probe and the skin. From the representative example depicted in Fig. 4, it can be observed that muscle thickness, fascicle orientation and location of aponeuroses can be similarly identified in images acquired in both experimental conditions. The main difference between the two images is the presence of an additional layer introduced by the bEMG-US electrode and two regions affected by a slight image distortion associated with the insulating material of the bEMG-US electrodes (see Fig. 4 right panel). The difference between medial gastrocnemius thickness and pinnation angle values estimated from US images detected without and with bEMG-US electrodes ranged from -0.31 to 0.10 cm and from 0.37 to 2.35% , respectively. These figures are within the 95% limits of agreement for repeated measured of muscle architecture with US [25], suggesting that the error due to the presence of the bEMG-US electrodes is lower than that due to the repositioning of the probe directly over the skin.

4. Conclusions

A bipolar electrode for concurrent acquisition of electrophysiological signals and ultrasound images from the same muscle region (bEMG-US electrode) was developed and tested. The electrode is made of hydrogel layers separated by thin, insulating structures. Experimental results revealed that bEMG-US electrodes may be used to acquire concurrently surface EMGs and US images from the same muscle region with a negligible effect on the quality of the two detected signals. This electrode may be used to investigate electromechanical events occurring during a muscle contraction and to characterize their possible changes associated with neuromuscular diseases, ageing, training or rehabilitation.

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Conflict of interest

There are no competing interests.

References

- Merletti R, Botter A, Cescon C, Minetto MA, Vieira TMM. Advances in surface EMG: recent progress in clinical research applications. *Crit Rev Biomed Eng* 2010;38:347–79. doi:10.1615/CritRevBiomedEng.v38.i4.20.
- Gazzoni M, Botter A, Vieira TMM. Surface EMG and muscle fatigue: multi-channel approaches to the study of myoelectric manifestations of muscle fatigue. *Physiol Meas* 2017;38:27–60. doi:10.1088/1361-6579/aa60b9.
- Samuel OW, Zhou H, Li X, Wang H, Zhang H, Sangaiah AK, et al. Pattern recognition of electromyography signals based on novel time domain features for amputees' limb motion classification. *Comput Electr Eng* 2018;67:646–55. doi:10.1016/j.compeleceng.2017.04.003.
- Oliveira LF, Matta TT, Alves DS, Garcia MAC, Vieira TMM. Effect of the shoulder position on the biceps brachii emg in different dumbbell curls. *J Sports Sci Med* 2009;8:24–9.
- Minetto MA, Holobar A, Botter A, Farina D. Discharge Properties of motor units of the abductor hallucis muscle during cramp contractions. *J Neurophysiol* 2009;102:1890–901. doi:10.1152/jn.00309.2009.
- Kawakami Y, Ichinose Y, Fukunaga T. Architectural and functional features of human triceps surae muscles during contraction. *J Appl Physiol* 1998;85:398–404. doi:10.1152/jappl.1998.85.2.398.
- Onambele GL, Narici MV, Maganaris CN. Calf muscle-tendon properties and postural balance in old age. *J Appl Physiol* 2006;100:2048–56. doi:10.1152/jappphysiol.01442.2005.
- Harding PJ, Loram ID, Combes N, Hodson-Tole EF. Ultrasound-based detection of fasciculations in healthy and diseased muscles. *IEEE Trans Biomed Eng* 2016;63:512–18. doi:10.1109/TBME.2015.2465168.
- Minetto MA, Caresio C, Menapace T, Hajdarevic A, Marchini A, Molinari F, et al. Ultrasound-based detection of low muscle mass for diagnosis of sarcopenia in older adults. *PM&R* 2016;8:453–62. doi:10.1016/j.pmrj.2015.09.014.
- Gallina A, Botter A. Spatial localization of electromyographic amplitude distributions associated to the activation of dorsal forearm muscles. *Front Physiol* 2013;4:1–8. doi:10.3389/fphys.2013.00367.
- Wolf SL, Segal RL, English AW. Task-oriented EMG activity recorded from partitions in human lateral gastrocnemius muscle. *J Electromyogr Kinesiol* 1993;3:87–94.
- Staudenmann D, Kingma I, Daffertshofer A, Stegeman DF, van Dieën JH. Heterogeneity of muscle activation in relation to force direction: a multi-channel surface electromyography study on the triceps surae muscle. *J Electromyogr Kinesiol* 2009;19:882–95. doi:10.1016/j.jelekin.2008.04.013.
- Vieira T, Botter A, Minetto MA, Hodson-Tole EF. Spatial variation of compound muscle action potentials across human gastrocnemius medialis. *J Neurophysiol* 2015;114:1617–27. doi:10.1152/jn.00221.2015.
- Ando R, Saito A, Umemura Y, Akima H. Local architecture of the vastus intermedius is a better predictor of knee extension force than that of the other quadriceps femoris muscle heads. *Clin Physiol Funct Imaging* 2015;35:376–82. doi:10.1111/cpf.12173.
- Lopata RGP, van Dijk JP, Pillen S, Nillesen MM, Maas H, Thijssen JM, et al. Dynamic imaging of skeletal muscle contraction in three orthogonal directions. *J Appl Physiol* 2010;109:906–15. doi:10.1152/jappphysiol.00092.2010.
- Blazevich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J Anat* 2006;209:289–310. doi:10.1111/j.1469-7580.2006.00619.x.
- Botter A, Vieira TMM, Loram ID, Merletti R, Hodson-Tole EF. A novel system of electrodes transparent to ultrasound for simultaneous detection of myoelectric activity and B-mode ultrasound images of skeletal muscles. *J Appl Physiol* 2013;115:1203–14. doi:10.1152/jappphysiol.00090.2013.
- Dieterich A V, Botter A, Vieira TM, Peolsson A, Petzke F, Davey P, et al. Spatial variation and inconsistency between estimates of onset of muscle activation from EMG and ultrasound. *Sci Rep* 2017;7:1–11. doi:10.1038/srep42011.
- Botter A, Vieira TM, Hodson-Tole EF. On the identification of plantar flexor's fasciculation potentials from US imaging and multichannel sEMG. In: *Proceedings of the 11th international motoneuron meeting*. Boulder, CO, USA; 2018.
- Vieira TM, Botter A, Muceli S, Farina D. Specificity of surface EMG recordings for gastrocnemius during upright standing. *Sci Rep* 2017;7:1–11. doi:10.1038/s41598-017-13369-1.
- Goss SA, Johnston RL, Dunn F. Comprehensive compilation of empirical ultrasonic properties of mammalian tissues. *J Acoust Soc Am* 1978;64:423–57. doi:10.1121/1.382016.
- Alexe-Ionescu AL, Barbero G, Freire FCM, Merletti R. Effect of composition on the dielectric properties of hydrogels for biomedical applications. *Physiol Meas* 2010;31. doi:10.1088/0967-3334/31/10/S02.
- Freire FCM, Becchi M, Ponti S, Miraldi E, Strigazzi A. Impedance spectroscopy of conductive commercial hydrogels for electromyography and electroencephalography. *Physiol Meas* 2010;31. doi:10.1088/0967-3334/31/10/S01.
- Vieira TMM, Minetto MA, Hodson-Tole EF, Botter A. How much does the human medial gastrocnemius muscle contribute to ankle torques outside the sagittal plane. *Hum Mov Sci* 2013;32:753–67. doi:10.1016/j.humov.2013.03.003.
- De Oliveira VB, Carneiro SP, De Oliveira LF. Reliability of biceps femoris and semitendinosus muscle architecture measurements obtained with ultrasonography. *Rev Bras Eng Biomed* 2016;32:365–71. doi:10.1590/2446-4740.04115.
- Huigen E, Peper A, Grimbergen CA. Investigation into the origin of the noise of surface electrodes. *Med Biol Eng Comput* 2002;40:332–8. doi:10.1007/BF02344216.