research article

A prototype of a flexible grid electrode to treat widespread superficial tumors by means of Electrochemotherapy

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Background. In recent years, superficial chest wall recurrence from breast cancer can be effectively treated by means of electrochemotherapy, with the majority of patients achieving response to treatment. Nevertheless, tumor spread along superficial lymphatic vessels makes this peculiar type of tumor recurrence prone to involve large skin areas and difficult to treat. In these cases, electroporation with standard, small size needle electrodes can be time-consuming and produce an inhomogeneous coverage of the target area, ultimately resulting in patient under treatment.

Materials and methods. Authors designed and developed a prototype of a flexible grid electrode aimed at the treatment of large skin surfaces and manufactured a connection box to link the pulse applicator to a voltage pulse generator. Laboratory tests on potato tissue were performed in order to evaluate the electroporation effect, which was evaluated by observing color change of treated tissue.

Results. A device has been designed in order to treat chest wall recurrences from breast cancer. According to preliminary tests, the new flexible support of the electrode allows the adaptability to the surface to be treated. Moreover, the designed devices can be useful to treat a larger surface in 2–5 minutes.

Conclusions. Authors developed the prototype of a new pulse applicator aimed at the treatment of widespread superficial tumors. This flexible grid needle electrode was successfully tested on potato tissue and produced an electroporation effect. From a clinical point of view, the development of this device may shorten electrochemotherapy procedure thus allowing clinicians to administer electric pulses at the time of maximum tumor exposure to drugs. Moreover, since the treatment time is 2–5 min long, it could also reduce the time of anesthesia, thus improving patient recovery.

Key words: electrochemotherapy; electrode; flexible support; breast cancer recurrence

Introduction

Electrochemotherapy (ECT) is an effective local therapy in use for unresectable skin cancers as well as cutaneous metastases from different tumor histotypes such as melanoma, head and neck cancer, soft tissue sarcomas and breast cancer.[1-3] During the procedure, high voltage pulses are applied to a needle pair implanted into tumor tissue in order to generate an electric field aimed at increasing cell membrane permeability and the uptake of chemotherapeutic drugs (bleomycin or cisplatin). In fact, electric fields over a suitable threshold allow the opening of transient aqueous pores on the cell membrane, thus inducing a temporary permeabilization (reversible electroporation).[1,4-8]

In recent years, ECT has shown efficacy in several tumors types and has been adopted by several

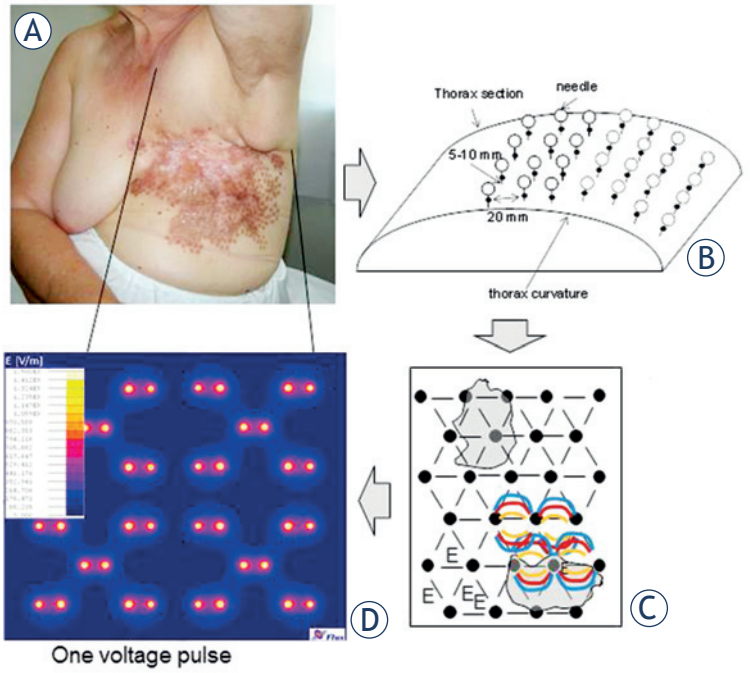


FIGURE 1. Chest wall recurrence from breast cancer and development of the grid electrode. **(A)** An example of a breast cancer patients who underwent repetitive ECT cycles to treat cutaneous metastases. The extension of electrode-induced skin marks highlights the extension of the treatment field and the need for more effective pulse delivery. **(B)** Sketch of the device, **(C)** resulting electric field lines of a grid electrode and (**D**) electric field color map.

centers for the treatment of skin tumors and, most of all, superficial metastases with the aim to improve local tumor control without discontinuation of concomitant systemic treatments.[4,8-13] Among ECT indications, breast cancer (BC) represents a promising, but challenging field. Chest wall recurrence (*i.e.* the occurrence of skin / soft tissue metastases on the chest wall after previous mastectomy) is an uncommon, but not negligible, pattern of recurrence observed in BC patients. It may occur also after optimal multidisciplinary management (*e.g.* mastectomy, radiation and systemic therapy). Its occurrence is more frequent (up to 45%) if the primary tumor was advanced in stage, while drops to 2–15% if adjuvant radiotherapy was applied after mastectomy. About 40–50% of chest wall recurrences occur around the mastectomy scar.[14-18]

ECT represents a promising treatment option in these patients and its efficacy in achieving an effective tumor control is particularly high when disease is limited in size.[10,19,20] Nevertheless, when chest wall recurrence is multifocal and widespread, it poses a therapeutic challenge to the treating oncologist.

Generally, during ECT procedure a 7-needle electrode, arranged in hexagonal geometry, is used to apply the electric fields to tumor tissue.[8,11] This type of electrode covers a surface close to 3 cm^2 at each single application.

Since it is well known that bleomycin maintains a sufficiently high concentration in tissue for a limited time interval (*i.e.*, 20 minutes according to Standard Operative Procedures[8,21]), the voltage pulses have to be applied within this interval. Consequently, large tumor-involved areas can be managed only by applying the 7-needle electrode several times during the 20-minute interval which follows the infusion of bleomycin. In theory, the standard hexagonal array needle electrode can be applied indicatively 100–120 times during a single ECT procedure, thus allowing for the coverage of approximately an area of 200–360 cm^2. In the clinical practice, this surface area may prove to be insufficient for effective treatment of patients with widespread skin tumor infiltration of the chest wall (Figure 1A).

As a consequence, a larger area can be managed only planning an additional ECT cycle, which inevitably will require a new anesthesia and an additional administration of drugs. As a consequence, many patients need to undergo repetitive ECT cycles in order to completely treat their chest wall recurrences or to treat newly occurred metastases outside treatment field (Figure 1A).

To avoid this problem, some electrodes designed to treat surfaces up to tens of cm^2 have been proposed.[22-24] Among these solutions, a composition of triangular configurations or arrays of parallel needles mounted on rigid support has been indicated[25-28]. An alternative strategy is represented by the use of planar antenna technology, thus avoiding needles insertion and developed in Nenzi *et al*.[29]

In this paper we present the prototype of a grid electrode suitable for not-plane surfaces such as chest wall and the treatment of recurrences from BC. The concept is based on a grid device including several electrodes arranged in a regular mesh (Figure 1). The new device is mounted on a flexible support that can be adapted to the skin surface and is equipped with removable needles that can be positioned one by one on the treating area.[44] As a result, the proposed device is a grid composed by a several needles that can be positioned before electric field application. For instance, the flexible version of the prototype tested in Ongaro *et al*. [30] has 13 electrodes and can cover an area of 50 cm^2. The treatment time in this case can be equivalent to one application of the 96 voltage pulses sequence prescribed by ECT Standard Operative Procedures[8,21] for hexagonal electrodes. Then, by using such de-

vice, the electric field could be applied in a shorter time interval compared with the currently used procedure which requires multiple, juxtaposed electrode placements (*e.g.* at least 16 applications for an area of 50 cm^2 considering the area covered by the standard electrode of 3 cm^2). In this paper, a prototype with 67 needles mounted in a flexible support covering an area of 225 cm^2 is proposed. The aim of this device is to apply the electric field more homogeneously in the treatment field and to respect the suggested time interval of 20 minutes after bleomycin infusion. Also the electric connection to the voltage pulse generator is here described.

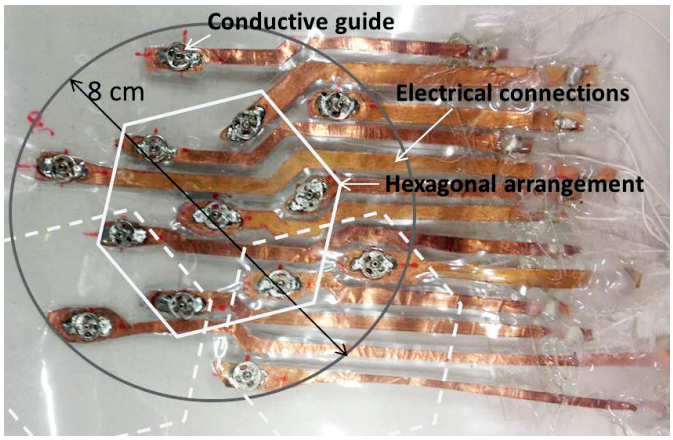
The prototype of the new flexible device consists of a grid of needles distant 2 cm apart, *i.e.* the needle configuration use hexagonal geometry of needle as in standard ECT needles, but their distance has been increased in order to reduce the number of needles per cm^2. The amplitude of the electric field generated by this needle configuration has been verified by simulation and compared with the one obtained with lower distance needles.[30] Moreover, the effect of the field in term of electroporated cells has been also verified using potato tissue tests and in vitro tests.[28,30,31] Finally, in order to verify the electroporation, the results of simple preliminary tests carried on vegetable tissue are presented.

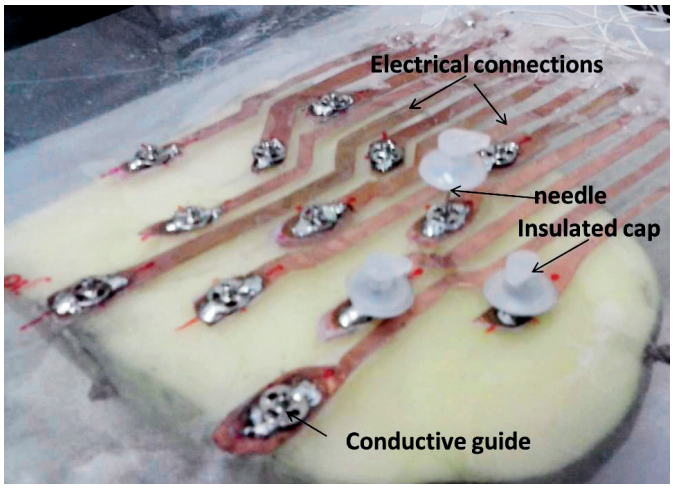
Materials and methods

The prototype of the grid electrode (Figure 2) is composed by a flexible support and 67 (5 or 10 mm-long) stainless steel needles that can be inserted one by one and linked to the electrical connection of the support.

The flexible support is equipped with some electrical conductive strips with some holes where the needles can be inserted. Each hole is provided with an insertion guide. The guides allow maintaining the perpendicularity of the needle with the surface where they are inserted. The guides center is positioned in points belonging to the vertex of adjacent equilateral triangles like in[28,30] with side 2 cm long.

The connection guides are arranged in hexagons as shown in Figure 2A in order to reproduce the standard hexagonal electrode geometry.[8,21,28,30] The guides allow both the insertion and electrical connection of the needle electrodes. The flexible plastic support is also provided with electric connections to the voltage pulses generator. The flexible electrode in Figure 2A has the electrical connections formed by copper strip, whereas in the electrode





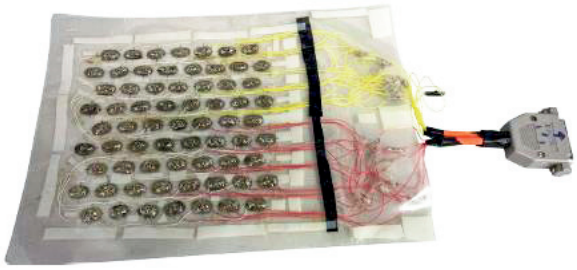


FIGURE 2. Prototypes of the flexible electrode: **(A)** device with diameter 8 cm with hexagonal electrode disposition, **(B)** removable needles inserted in electrical connection and **(C)** square device 15 x 15 cm with hexagonal arrangement of conductive guides highlighted.

of Figure 2C the electrical connections are made, for sake of simplicity, with copper wires. Figure 2B shows the insertion of the needles in the guides. The connection guides are realized in order to easily place and remove the needles. The needles are provided with an insulant cap (Figure 2B) and the flexible support by needle guides in order to allow the normal penetration of the tip. Moreover, for ensuring a user friendly, safe and effective appli-

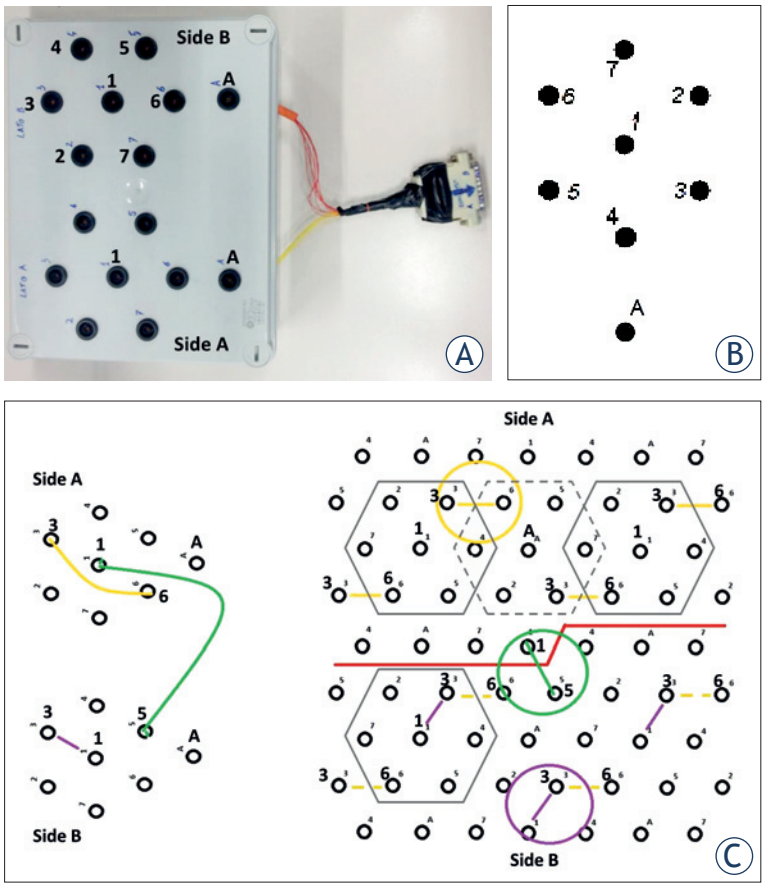


FIGURE 3. (A) Connection box to interface the flexible electrode to the voltage pulse generator. **(B)** Schema of the arrangement of clamps and **(C)** examples of connections.

TABLE 1. Example of supply needle pair sequence

#STEP	Needle pair	#STEP	Needle pair
#1	1–2	#14	A-3
#2	1–3	#15	A-4
#3	1–4	#16	A-5

cation on chest wall BC recurrences, needle length was limited to 5–10 mm. In practice, it is expected that, when the flexible plastic support is fixed on a curved surface (e.g. the thoracic wall), the short length of the needles and the 2 cm distance will limit the approach of their tips. Finally, the device is connected to the pulse generator by means of fast connectors.

The prototype of the grid electrode has been realized in two different sizes. The first has a diameter of 8 cm with 13 needles and the second one is a square of side 15 cm with 67 needles that covers an area of 225 cm^2.

Electrode supply

The new grid electrodes are supplied by a voltage pulse generator manufactured by Igea S.p.A. (Carpi (MO), Italy).[32,33] In each test performed the voltage generator delivers sequences of 10 rectangular pulses between 0 and 2000 V with pulse duration of 100 μs at 100 Hz (total time of 10 msec). In this work the voltage has been fixed to 2000 V for all test performed.

Figure 3A shows the connection device. It is a box equipped with 16 plug clamps that can be connected two by two to the voltage pulse generator. The 16 clamps are arranged in groups of 8 each one identified by a number except the eighth that is identified by the letter 'A' like in Figure 3B.

The needles in the flexible electrode are arranged in hexagon and numbered consequently following the scheme in Figure 3B. This schema is formed by a hexagonal structure of numbered points and a point named 'A' (corresponding to the clamping numbers). Continuous gray hexagons in Figure 3C highlight the hexagonal scheme in Figure 3B in which the central electrode is named '1'. Considering the dotted hexagons, the central point is named 'A', whereas the other points of the hexagon correspond to the points identified by numbers. Figure 3C shows also the connection scheme of the flexible grid electrode with 67 needles to the voltage pulse generator. Some examples of supplied electrode pairs are presented in the same Figure. For instance, Table 1 reports the supply sequence where the needle pairs selected involve needle '1' or needle 'A'.

The clamps are subdivided in two groups that supply a half of the needle pairs. In fact, since the average resistance at electrode ends, R_e, is 130–200 W (value measured at needle extremities during pulses application) considering a current of 40 A (maximum value deliverable by the voltage pulse generator, I_{max}) and an applied voltage, V_A, of 2000 V the maximum number of electrode pairs that can be parallel connected, N_m, is between 3 and 5.[28]

$$N_m = R_e \frac{I_{max}}{V_A} \quad [1]$$

For instance, like shown in Figure 3C for the pair 3–6, the number of electrode pairs between needles with the same number is up to 8. Consequently, the larger grid electrode is divided into two areas where there are up to 4 electrode pairs between needles with the same number.

Each group of eight clamps in the box in Figure 3A supplies half of the grid electrode, and

the electrode pairs on the boundary are supplied by connecting two clamps belonging to different group as it has been highlighted for pair '1–5' in Figure 3C. All the electrodes, both the activated and not-supplied ones, are inserted in the treating area before pulse delivery. They remain inserted during the entire treatment. In fact the influence of the not-supplied electrodes is irrelevant, as reported in a previous work, since they act as an 'open circuit'[30] and any current can flow.

Potato test procedure

The two models of the grid electrode have been tested on a phantom made of potato tissue. In fact, it is well known that after few hours of application of voltage pulses, the potato tissue appears dark if cell membranes have been electroporated.[28,30,34,35] For instance in Castiello *et al*.[28] and Ongaro *et al*.[30] have observed potato color after 24 h even if the darkening started after few hour after pulses application.

Voltage pulses have been applied to the potato tissue using the pulse generator and applying 2000V at each electrode pair following the pair-sequence described in Standard Operative Procedure.[8,21] A sequence of 10 pulses has been applied to each possible electrode pair. Potatoes tissue was preserved at room temperature for 24 hours after application of voltage pulses and then observed looking for the electroporation effect; pictures of the investigated tissue were also taken (Figure 4A,B). The electroporated potatoes appear dark only in the areas where the voltage pulses have been applied (Figure 4A), whereas any color change did not occur for the potato cut and maintained at room temperature (Figure 4B) as described in Ongaro *et al*.[30]. The intense dark coloration is due to the electroporation and not to the cut. Nevertheless, this technique cannot discriminate between reversible or irreversible electroporation and is a simple qualitative test to visualize the area where the electroporation was occurred.

The two prototypes of the device have been tested on two type of phantoms: a single potato tuber for the 13 needles electrode, whereas the larger device with 67 needles has been tested using more potatoes piece immerged in Meat Liver Agar gel (46379 Fluka Analytica) dissolved in hot water at 5% and cooled at room temperature. Agar gel allows electrical conduction between adjacent potato pieces. Potatoes are arranged as shown in Figure 5A.

The flexible electrode is positioned over the phantom, a single half of potato tuber for the 13 needle device or the arrangement in Figure 5A for the large electrode. The needles are positioned manually one by one in each contact as in Figure 5B. Figure 5C shows the flexible electrode with 67 needles ready to apply the voltage pulses to all possible needles pairs. Finally, Figure 5D shows the connection of larger flexible electrode to the connection box.

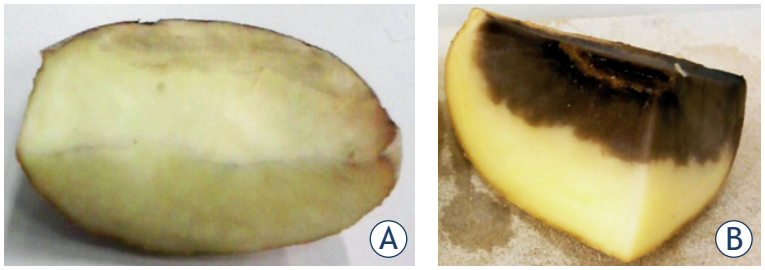


FIGURE 4. Potato tuber after 24 h preserved at room temperature: **(A)** no voltage pulses (control) and **(B)** treated with voltage pulses.

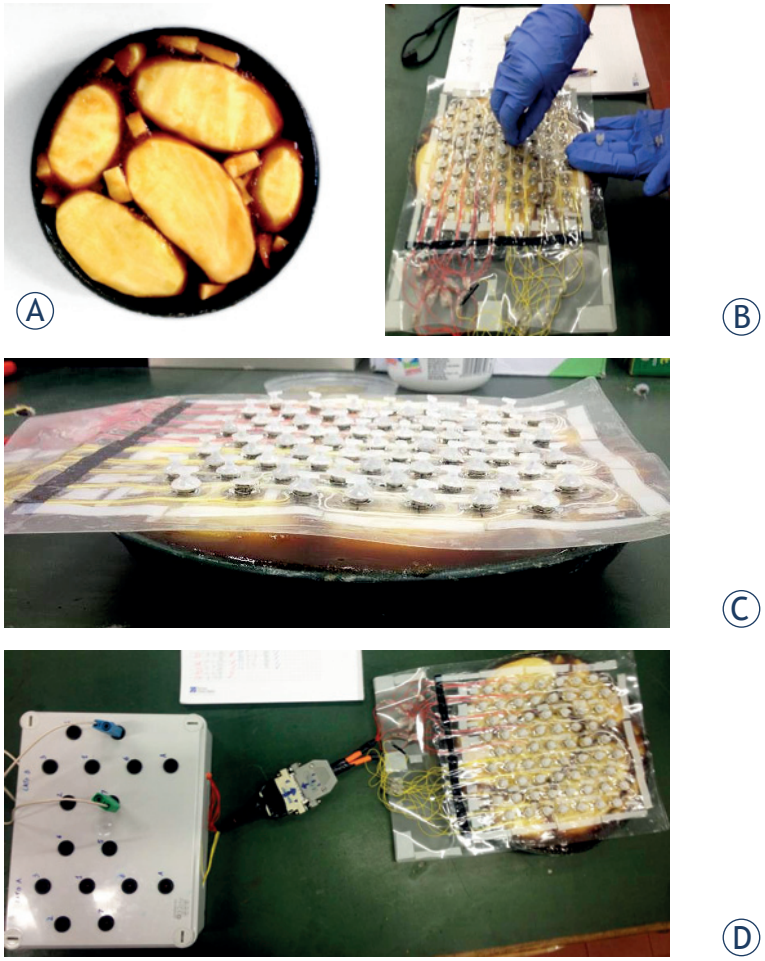


FIGURE 5. (A) Phantom used to test the square flexible electrode with side 15 cm. **(B)** Manual insertion of needles, **(C)** device ready for pulse application and **(D)** connection of the electrode with the connection box.

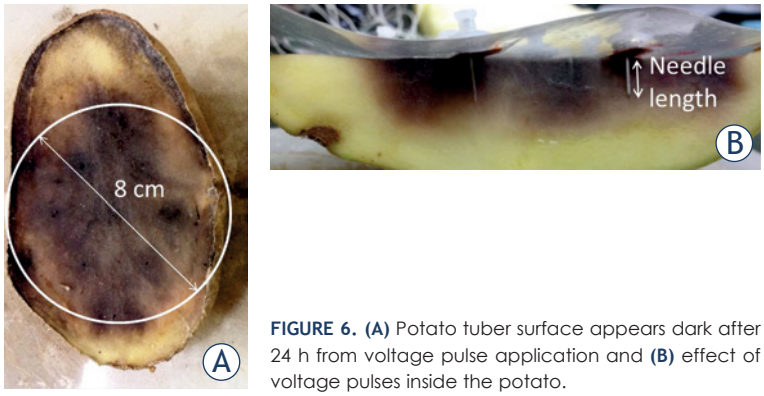


FIGURE 6. (A) Potato tuber surface appears dark after 24 h from voltage pulse application and **(B)** effect of voltage pulses inside the potato.

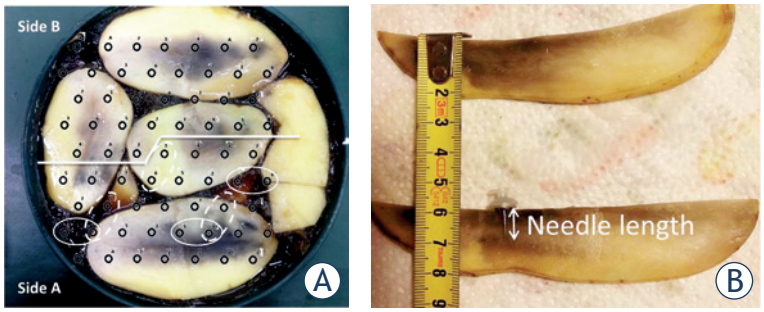


FIGURE 7. (A) Potato phantom surface appears dark after 24 h from voltage pulse application and **(B)** effect of voltage pulses inside the potato.

Results

Figure 6 shows the electroporation effect observed 24 hours after the application of voltage pulses by means of the 13-needles flexible electrode presented in Figure 2A. The 15 cm^2 area of potato surface appears dark (Figure 6). After 24 h the potato has been cut vertically in order to evaluate the effect of electroporation inside the phantom. The tissue appears dark for a depth larger than the electrode length as shown in Figure 6B. In this case, the needle is 10 cm long and the dark area has a depth double than the needle length (close to 20 mm). This effect has been described also in Castiello *et al*.[28] and in Ongaro *et al*.[30] In fact, the electrode distance affects the electric field distribution in the treated volume: using electrode with a larger distance the electric field can penetrate more deeply in the tissue as it has been demonstrated in Ongaro *et al*.[30] by means of numerical modeling by means of finite element method.[30,36-39]

Figure 7A shows the surface of phantom in Figure 5A used to test large electrode, captured 24 hours after voltage pulse application. It is evident the electroporation effect produced by the device from the dark color of the tissue surface. Nevertheless, in some areas the electroporation did not occur. Considering the pairs underlined with continuous or dotted line in Figure 7A, it appears that in these cases almost one needle pair is entirely immersed in Agar gel that is more conductive than potato tissue. In these cases, the pulse generator has not delivered the current to the load because the internal control of the device has measured a current greater than the maximum allowed (I_{max} = 40 A).

Finally, pieces of potato in the phantom have been vertically cut 24 h after the voltage pulse application showing the appearance of dark areas inside the tissue.

The electrode has 67 needles and can treat an area of 225 cm^2 (a square with side of 15 cm). Also in this case the electroporation depth is larger than the electrode length as shown in Figure 7B, where the needle length is 10 mm and the electroporation depth is 20 mm.

Tests on potato tissue are very easy and cheap. Nevertheless, they are not sufficient to explore if the electroporation is reversible or irreversible even though some authors have tried to couple simulation results and dark color gradation[40-42] and searched for the dark intensity for which the electric field overcame the irreversible electroporation threshold. The evidence whether electropoation effect is reversible or irreversible can be obtained by means of in-vitro tests using cell culture as reported in previous experiences.[30,31]

Discussion

Chest wall recurrence from BC represents a peculiar type of tumor recurrence, and, when superficial and widespread (due to lymphangitic diffusion), it is crucial, during ECT, to apply electric fields on a wide and thin surface instead of a target volume.

The preliminary experimental results show that the flexible device is able to electroporate larger tissue surfaces with respect to the standard hexagonal electrode. In fact, the designed device covers an area from 50 cm^2 (by using 13 needles) to 225 cm^2 (by using 67 needles). From a practical point of view, the plastic flexible support allows the adaptability to non-planar surfaces as the chest wall and can be applied to the skin as a plaster. Its main drawback is represented by the loss of parallelism between adjacent needles because of the curved surface. Nevertheless, this kind of device has been primarily conceived to treat superficial tumors of the chest wall, which has a limited radius of curvature; moreover, this an anatomical area, especially

in mastectomy patients, is characterized by the presence of a rigid, underlying plane represented by the rib cage, which limits the penetration of needle electrodes. Moreover, and importantly, tumor growth in these patients follows a superficial pattern of spread and tumor thickness is limited to 4–5 mm from the superficial skin layer; consequently, needle electrode should be inserted only few millimeters, thus limiting the convergence of their tips. With the proposed device, needle electrodes can penetrate only few millimeters (maximum 10 mm), and the distance between two points of insertion was fixed at 20 mm, so that the effect of parallelism loss is limited.

On the other hand, single needle insertion may be more user friendly for the clinician in presence of fibrous tissue. In fact, in this case, it is simpler to insert one needle instead of 7 needles at the same time. Moreover, each needle is inserted using a rigid guide provided by the insertion mask in order to allow parallel penetration of tips. Moreover, the device has been designed to use needles with a length between 5 and 10 mm, so that the effect on electric field intensity due to tip convergence should be limited. During the procedure, when the needle tips are too close (*e.g.* by visual inspection or by resistance evaluation before pulse application), applied voltage can be accordingly reduced.

The device and its positioning have been designed in order to contain the time of ECT procedure and to permeabilise tumor cell membrane when drug concentration is higher. To make electrode application time-sparing, the needles of the new device can be pre-positioned on the flexible support, before pulse delivery. In this way, the repetitive, operator-dependent, placement and displacement of standard electrodes could be avoided and voltage pulse delivery may be performed within the optimal time interval from the infusion of bleomycin.

Authors are aware that the present study has several drawbacks. In fact, the phantom used to test the electrode shows areas with large difference in conductivity, likely due to Agar gel between potato pieces. These areas have caused the failure of the electroporation procedure since the pulse generator in some case has not delivered voltage pulses for its proper current limitation (internal control detected low impedance and blocked pulse delivering). Nevertheless, in previous works a lower scale device with 52 needles 1 cm apart has been already tested on a single potato tissue and experimental results are in Ongaro *et al*.[28]. These tests on single potato piece did not show any drawback due to high conductivity area.

Since, the designed electrode can be considered an extension of existing devices for clinical ECT treatments (*e.g.* hexagonal array electrodes), then current and electric field thresholds are borrowed from standard protocol for ECT.[4,8,20,21,43]

In future experiments, the new grid electrode will be tested in animal models in order to verify its efficacy. Moreover, it will be tested also a prototype with more distant needles (2 cm apart) with respect to standard electrode as well as the increment of electric field depth, up to two times the electrode length shown in Figure 7B.

The flexible device has the potential to reduce the time required for the application of electric pulses. For instance, the 13 needles device which, can treat an area of 50 cm^2, is supplied by means of two 96-pulse sequences for hexagonal electrode described in Standard Operative Procedures.[8,21] Therefore, the 50 cm^2 area can be treated in less than 2 min (one 96-pulse sequence has a time duration approximately of 20 ms) considering the time to arm the pulse generator. Moreover, the 67 needles electrode, which covers an area of 225 cm^2, can be supplied by means of 5 sequences of 96 pulses as described in the Electrode supply paragraph. Considering the time to change connections and arm the pulse generator, the treatment can be performed in approximately 5 min. This short time may assure a higher drug availability in tumor tissue during tumor electroporation. The new device is advantageous since the clinician can repeat the 96-pulse sequence up to 150 times moving the standard electrode several times to cover all the chest wall surface. Considering, for instance, an application time of 20 ms per sequence (*i.e.* 96 pulses) and the time required to move the electrode and arm the generator of approximately 15s it gives a treatment time of approximately 40 min.

Conclusions

A flexible device has been designed in order to manage chest wall recurrence from BC, which usually involves large skin areas in mastectomy patients. A prototype of a grid electrode aimed to treat widespread superficial tumors has been designed and tested in preclinical preliminary experiments. This report presents the results obtained with a flexible device that can be used to treat by means ECT skin areas between 50 cm^2 and 225 cm^2. The tip approach effect is limited by needle distance, which

was fixed to 2 cm, and by needle lenght, which was set between 5 and 10 mm. Moreover, the possibility to pre-insert all the needles before pulse delivery has several advantages: it may allow to reduce the duration of the procedure and anesthesia, to expose tumors to higher drug concentration and, hopefully, to increase the antitumor effectiveness of ECT in a challenging subgroup of BC patients.

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