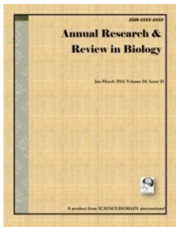

Annual Research & Review in Biology
4(20): 3054-3070, 2014

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From therapeutic Electrotherapy to Electroceuticals: Formats, Applications and Prospects of Electrostimulation

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Authors' contributions

MEK, ZZ, GL and KP contributed in the literature searches, drafting the manuscript, proofreading and editing it. The outline of the review was set by MEK and KP. All authors read and approved the final manuscript.

Review Article

Received 31st March 2014
Accepted 18th May 2014
Published 31st May 2014

ABSTRACT

Introduction: Electrical modalities ~~a~~were used therapeutically since the 18th century till 1900s to reemerge after World War II and to come to prominence after the 80s. Applications include wound healing of injured/molested/burnt tissues, pain management, exercise enhancement, cardiac arrest management, and hearing enhancement.
Modus Operanti: Electrostimulation acts by generating currents and voltages similar to innate ones in cell/tissue/organs. Electrosensitive moieties of the cell membrane are excited and signals transduced to regulate gene expression and metabolism, while provoking vectored movement by orienting the cytoskeleton.
Therapeutic Applications: The highly diversified applications of electrostimulation created the notion of "electroceuticals". Conductive applications use electrodes to transmit~~ting~~ current; inductive applications use fields to induce currents in the tissues. Different forms and kinds of field and current combine with temporal parameters to create a multitude of modalities, as do the material of the electrodes. Last come the treated ailments. At present the focus is ~~to~~onwound healing, in burns, sports or other traumatic injuries and ulcers due to underlying disease.
Microbiological parameters: Electrostimulation affects microflora both in biotechnological and clinical contexts. Different electrodes and forms of electrostimulation seem to affect

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differently Gram (-) and Gram (+) bacteria; the effect on biofilms and yeasts is encouraging for treatinghospital infections as is the combination with biochemical compounds.
Wireless Microcurrent Stimulation: A new modality, free of conductive approaches' drawbacks (electrochemical instability, risk of infection and electric burns, pain/irritation). It uses charged air molecules to create very low intensity current, of the level of single-digit microAmperes, thus being well-tolerated.
Conclusion: Electroceuticals become very sophisticated and diversified and should be viewed in terms of electrodynamics and electrokinetics, so as to be properly integrated in modern therapeutic schemes either as a supplement or as an alternative to biochemical compounds.

Keywords: Electrotherapy; electrostimulation; electroceuticals; wound healing; bone fractures; microbial growth.

1. INTRODUCTION

Modern medicine used to focus for intervention on three main (although not exclusive) approaches: surgery, irradiation and, most frequent of all, (bio)chemistry [1]. This trinity does not capitalize on one modality long known for its key role in many functions in cell and, most important, in whole organism level: electric currents. There is indication enough that therapeutic use of electric currents or fields (which result in currents) may exert its therapeutic potential at an upstream level compared to chemicals and thus such protocols may be used uniformly for multiple ailments with a common basis; such grouping may be much larger and more generalized than similar ones currently attainable by biochemical pharmaceuticals. Moreover, as there are many formats and a multitude of tried protocols based on electricity [2], with many more possible ones to try, there is an obvious issue of different applicability; these will probably be wider than applicabilities of families of biochemical compounds, but a universal electricity-based protocol is not deemed attainable. Thus, the concept of "electroceuticals" [3] emerges, which is used to describe different modalities, differing in principle, form, quantity, time and other parameters, but focused on administering an external or induced electrical current for amending impaired biological functions. On the other hand, the use of electric modalities for treating neurological disorders, which was initiated early on [4-6], never fell completely out of favor. Its specifics, as today it is gaining momentum, are too differentiated and complicated to be included in this study, as multiple issues of both science and morality rise [7, 8]. Nevertheless, its use tends to become more targeted and stochastic compared to rather "dumb and brute" applications of trial-and-error [9] and this holds true, more or less, for the whole range of ES treated ailments [10].

1.1 Electrotherapy in The Mist Of Times: From Asklepeia and the 19th Century to the Decline of 1900

Empiricism on the therapeutic use of electricity is detectable much earlier than its discovery: the cult of Asclepius in ancient Greece was building their *Asklepeia*, whenever possible, near waterfalls, where the ionizing and spraying effect of the waterfall was considered to improve both the mood and the health of the patients. At 420 BC, Hippocrates prescribed electroshocks from black torpedo fish for analgesia, while Egyptians used the similar Nile catfish [11,12]. Moreover, during the 17th century, physicians were using charged golden leaves to resolve smallpox lesions [1,4,13,14]; the popular belief was that the gold, the noble metal, exercised the therapeutic function, but it could have been more a matter of charge than of the metal. The reinvigorating result of the electric energy is stereotyped by the lore of Frankenstein, where dead body parts of cadavers were assembled into a full body, returned to life by the force of lightning bolts. Currents were successfully used during the 19t^{h} century by physicians to treat broken limbs and psychic ailments [4-6], introducing what is now termed Electrostimulation (ES), that is the use of electricity to cause physiological results. A full four generations of ES were developed at both sides of the Atlantic and used within the 160 years [1740-1900] of the golden era of ES. Starting with the Franklinism of B. Franklin which used static electricity developed by friction to deliver shocks in 1750s, the Galvanism of the 1800s used direct currents (DC), to be superseded by Faradism of 1832 where pulsed (PC) or alternate current (AC) was used, to culminate to d'Arsonvalis~~il~~ationof 18~~9~~88 where high frequency currents were used [11,12]. But after the turn of the 20th century, due to a number of cases that today might have been termed simply "malpractice", the Carnage Foundation formed a commission to review relevant data and results, which in 1910 simply imposed the discontinuation of such practice (Fleiner Results), leaving chemical compounds as the only non-surgical option of the day [1,11,14]. Despite such developments in the United States (US), in Europe the ES had not been very widely used, but was not extinct neither, as is obvious by the development of ES devices during the 30s; a French-developed and produced apparatus is shown in Fig. 1.

Fig. 1. A French ES device of the 30s manufactured by Holo Electron

2. THE COMEBACK OF THE '90s

Things were slow to change. Despite the very early realization of~~n~~ the electrochemical nature of physiology down to cellular level [1,15], and important discoveries during the 80s, the electrotherapy made a slow, progressive but definite comeback within the 90s. Its applications, other than coping with cardiac arrest, included: pain management [16-18], bone regeneration [19] and various athletiatric issues [20], such as relief of the soreness after training [21,22], training enhancement (such as the belts for building abs) / training substitution [23] and injury rehabilitation [24]; the last two under the "galvanic exercise" concept developed by the US during World War II (WWII) [25].

Such applications did not earn any decent name for the electrotherapy, as the scope was limited and the use marginal if not peculiar; but the drug design crisis, escalating at the turn of the century, permitted small-scale experimentation of novel approaches. The new area electrotherapy was meant to explore had been still within the regeneration scope, but this time the focus was on the epithelium, not the bone, nor muscle. Epithelia are by definition regenerating tissue of prime importance; failure to do so –or do so effectively enough- means life-threatening conditions-mainly through infections. As ulcers were becoming an increasing problem, being in a way a civilization ailment, the regenerative effect of electricity was tested in extended open wounds (burns and ulcers), preferably whenever other approaches had already met with failure due to underlying conditions. A brief chronological summary of the abovementioned is presented in Table 1.

Table 1. Brief evolutionary history of Electrostimulation

Chronology	Accomplishments-events
1st millennium BC	Amber and magnetite (static electrism) used by Egyptians for headache and arthritis. [11,12]
420 BC	Hippocrates prescribes shocks from torpedo fish. [11, 12]
5th c BC	Asklepeia near ionized water environment (falls).
17th c AD	Golden artifacts, charged, to treat smallpox lesions. [1]
1752	Franklinism by Ben Franklin; static electricity to relieve pain. [11. 12]
1800s	Galvanism; DC to relieve pain. [11,12]
1825	Sarlandiere and Berlioz combine Galvanism ES and acupuncture. [11, 2]
1832	Faradism; use of AC for ES; Duchenne employs it for muscle stimulation. 11,12]
1850	Publication of the use of ES for bone fractures in US. [6]
1888	D’Arsonvalisation: use of high frequency currents. [11,12]
1900	Carnage foundation establishes Fleiner committee. [1,14]
1910	Fleiner results discontinue ES in the US. [1,14]
1930s	ES modalities actively marketed in Europe. [1, 11]
1944	Galvanic Exercise for wounded personnel of US Armed Forces. [25]
1957	Electric properties of the bone first published by Fukada& Yasuda. [1]
1960s	ES effect on cell wall-principle of electroporation. [70]
1967	Wall &Sweat: 100Hz ES in skin proved analgesic. [11]
1970s	Bruce Lee perfects galvanic exercise for accelerated training.
1980s	Regular ES use in sports injuries and muscle atrophy; discovery of skin battery potentials; antimicrobial effect of ES *in vivo*, usual treatment of bone fractures. [4,14,20]
1982	Cheng *et al* publish the impact of ES to ATP generation. [1]
1990s	Wound healing by ES becomes prominent. [1,4,14]
2000s	Development of NCCT combining different ES schemes’ advantages. [63]
2010s	Massive development of different ES schemes and approaches.[1,11]
2013	Concept of electroceuticals. [3,9]

3. THE OPERATING ENVIRONMENT

Human physiology is electrochemical in nature. A multitude of electro-dependent phenomena are affected by external electric challenges, such as current influx or exposure to electric fields and electromagnetic radiation and waves. The pulse of both sensor and motor neurons and muscle cells is but one. The tuning of the heart is a second. Less well-known and far lower of intensity is the “skin battery”, a difference of potential between the surface of the epidermis and deeper tissues of living dermal cells such as dermal and subcutaneous ones [1,14,26]. This potential is created due to the charged residues of epidermis and the ions secreted primarily by perspiration and thanks to the isolative effect of keratin, which in turn permits low amperage currents streaming along the skin (skin current). Thus, whenever an external injury occurs, the streams of skin current are discontinued at the wound site, and a Current of Injury (CoI) [4,14] occurs in its stead; this current is not only due to the electrical leaks through the wound proper, but also due to alteration in the distribution and function of ion channels in affected cells, both possibly due to depolarizations caused by the initial electric leak, which is thus amplified.

The CoI is caused by the different potentials of dermis and epidermis, and whenever conductivity is attained, as by rupture of the insulating tissue, current flows outwards, effectively short-circuiting the skin battery; there is a significant difference between the edges of the wound and points of the skin 3mm away, thus creating a lateral voltage gradient [4]. This very current of injury is thought to attract regenerative and immune cells, to activate them by regulating the signal transduction through membrane pumps and channels, to affect changes in gene expression, to upregulate the metabolism and to induce extracellular matrix production and emplacement; once healing has reached the re-epithelization level, isolating dermal and epidermal areas, the current of injury stops [4, 27]. In the event the CoI is discontinued, for example due to the isolative effect of necrotic tissue in the wound bed, without the healing having been accomplished, the latter is impaired and this fact lies in the heart of chronic wounds [28].

The reason immune and regenerative cells are activated and attracted by the CoI is that the cell proper is prone to electrical events. Different cell types are attracted by different polarities [1], allowing a more detailed management of an ailment. Differences of potential have been identified along the plasma membrane of the same cell, especially after injury [29], thus creating a cellular polarity which serves as a compass and induces cytoskeletal restructure. This way, reshaping of the cell and/or movement towards or away from the electric stimulus is attainable [28]. But, most importantly, differences of potential have been discovered across the cell membrane (“action potential”), since the distribution of electrolytes (especially K^+) differs at the two sides of the cell membrane. These differences produce a Voltage of 100 mV [30], with the intracellular environment being more electronegative due to phosphates and carboxylates in glucans and membrane surface proteins or protein moieties.These two kinds of potential differences practically duplicate the abovementioned respective ones of the skin. Moreover, the additive effect of the potentials of different cells creates voltages of cell aggregates (tissues, organs) at the millivolt (mV) range which may produce, whenever a conductive entity is found, currents at the range of microAmpere (μA) [1]. The action potential is the principle of the transportation function of the cell membrane: it is known to trigger cells into transmitting signals (electrical signals in the form of a cascading depolarization) along the plasma membrane for excitable cells [2] and into secretion of cytokines, hormones and other chemical signals in non-excitable cells.

Electro stimulation works because of its ability to stimulate cellular physiology and growth by applying low energy electric stimuli (fields-currents), similar in intensity to endogenous ones [1]. According to [31], it could increase Adenosine Tri-Phosphate (ATP) generation by almost 500% and enhance amino acid transportation and protein synthesis. This leads to extracellular matrix production, cell proliferation and neogenesis of different tissue types. The upregulation of different cell types and oftheir metabolism results in increases in oxygenation levels and membrane permeability, thus enhancing damage control and restoration in tissues [1].

Electric stimuli in the level of μA and mV cannot depolarize the membranes [32], but may well cause transmembrane proteins, forming pumps and channels, to activate (voltage-sensing proteins, [2]) allowing ion exchange; this ionic movement through the membrane (gating current – [33]) triggers specific cellular enzymes which initiate the signal transduction cascade, resulting in cellular responses of various kinds: cell proliferation, DNA synthesis, gene expression, ATP production, membrane excitation, cytokine secretion, metabolism up- or down-regulation [4, 11, 28, 34, 35]. Moreover, altered electric density in the extracellular environment may change the affinity of ligands to the receptors and alter the distribution of receptors on the membrane both in physical and conformational terms, affecting their availability [28]. Such events affect the signaling routines of the cell, causing quantitative and qualitative differentiation in gene expression; as far as the latter is concerned, the first genes found to respond to ES are Phosphatase and Tensin homolog (PTEN) and phosphatidylinositol-3-OH-kinase-γ [36]. Though, the dynamics of such an approach require attention: after a threshold the stimulation does not simply reach a plateau; it actually becomes counterproductive [31]. Increasing current actually decreased the results. For bone regeneration the advantageous window is between 5-100 μA [49]; under 5 μA there is no stimulatory result, over 100 μA the result is necrosis instead of stimulation, and the extend of the necrosis, if not proportional to the increase of the intensity, at the very least is positively correlated to it; previous studies[37, 38] had put the upper threshold with DC between 20 and 30 μA.

4. THE APPLICATIONS

4.1 Forms and Methods: Currents, Fields and Intensities

There are two main ways to employ electrotherapy: the first option is to rush current by electrodes to the body, the setup usually being the injured body part being in between the electrodes or one of the electrodes being placed within the wound bed (in external wounds) and the other at its periphery. This approach is called “conductive coupling” [39], as the hardware conducts electricity to the treated body part. Different settings are possible, depending on: i) the duration of treatment, ii) the administered current intensity -usually at the range of μA and not milliamperes (mA), so as to be similar to endogenous currents and avoid thermal/ohmic injury of the tissues, as the ones mentioned by Duchenne in 1855 [25] and iii) the form of the current: continuous-pulsed, monophasic-biphasic, anodal/cathodal[1]. Such parameters, infringing to the rate and polarity of the electric charges applied, constitute the electrokinetic aspect of the regimen, as opposed to accumulative and net charge (for DC) and amplitude (for other forms, such as PC and AC), which constitute the electrodynamic aspect of a regimen.

The alternative is the “inductive coupling”, where the wounded member is exposed to suitable fields (electric [28, 40] or electromagnetic- such as Pulsing Electro Magnetic Fields-PEMF [14, 28]), which cause a current to flow strictly within the tissues. It is obvious that both approaches entail pros and cons and offer plenty of leeway for differentiation. The conductive coupling allows precise control of electrodynamic (such as charge and, where applicable, amplitude) and electrokinetic (intensity, pulse frequency and duration where applicable) parameters. It does, however cause pain or irritation and has inherent infection risks due to the contact with wounded tissue, and in some formats there is high possibility for burns, complicated by the different tolerance of specific individuals. The inductive approach, on the other hand, entails no pain, infection or burn risk. Nor is there any possibility of unforeseen electrochemical reactions, as is the case with contact electrodes

[28]. Though, it is questionable whether the same field settings, which can be regulated with the outmost precision, cause the same values of electric responses to the individual every time-not to mention differences and deviations among different individuals or between different limbs of the same individual. There is also the everlasting debate between the use of electric *versus* magnetic fields.

Although fields do not cause burns, nordo they present infection risks, the resulting currents are dissimilar to endogenous ones. DC currents present the highest degree of similarity to bioelectricity, but alternate and pulsed currents fully exploit the different galvanotactic properties of different cell types [4,41] which fulfill different functions, such as: (i) solubilization of necrotic tissue by positive [42] or negative [43] current inducing autolysis, (ii)phagocytosis of infectious elements by activated leukocytes attracted by negative current, (iii)production of extracellular matrix by negative current, and (iv)cell migration and propagation by positive [42] or negative [4]current. Such differentiation of polarity allows combinations which accelerate the healing process and, in addition, instigate antimicrobial effects [14,42]; though, it is obvious that DC of negative polarity may also perform most of the tasks on itsown[4], thus explaining the successful use of DC and negatively charged air gases (see paragraph 5, Wireless Micro Current Stimulation-WMCS).

4.2 Ailments, Indications and Contraindications

4.2.1 Indications

Chronic ulcers (i.e. ulcers that are unresponsive to initial therapy or that persist despite appropriate care) are prominent amongst the ailments which respond well to ES. Lower extremity ulcers, attributed to diabetes, venous disease, or arterial disease comprise a substantial proportion of chronic ulcers and may lead, if not successfully treated, to amputation or death. Treatment modalities and wound care therapies are often selected based on the ulcer characteristics as well as patient factors, past treatment, and provider preference. A large and growing array of advanced wound care therapies of different composition and indications have been developed. If ulcers do not adequately heal with standard treatment, additional modalities may be required – these are often termed "advanced wound care therapies". Treating ulcers with ES has been the first widely adopted use of ES since its reemergence into therapeutic prominence (by 1989 there were review articles already, focused on lower limb [44]); most of the treated cases presented increased ratio and rate of healing- especially when ES was adjunctive to standard wound treatment [1]. Pulsed currents were long used successfully [42,45], although DC currents were used even earlier and with as much success [1,14]. Currently, ulcers are the indication *par excellence* for ES therapy since it is covered by the main US healthcare providers [4,46].

Bone fractures, especially non-union cases of long bones, had been a privileged field for electric modalities, being among the first modern therapeutic uses of electricity [5,6]. Moreover, the first post-war study which paved the way for experimentation in the field has been [47], which showed that the bone as such was electricallyactive and sensitive to electric stimuli. The bone continued to be a very common indication for ES: by 1995 massive evidence was available and the most used schemes were either implanted stainless steel electrodes with direct current or PEMF (Pulsed Electro-Magnetic Fields) which were non-invasive and used a coil or transducer [14]. The dynamics and kinetics of ES therapy where also advanced by bone-related research: [48] observed that osteoblasts and osteoclasts move to the opposite sides of an electric field, while [49] discovered that there is an optimum of intensity (5<>100 microamperes), over and below which the treatment is either harmful (causing cell necrosis) or neutral (bearing no effect), respectively; earlier, an upper threshold of either 20 microamperes [37] or 30 μA of DC [38] had been established. [50] discovered that ES in osteoblasts induces genes for Transforming Growth Factor-beta (TGF-β), among which the ones for ALP (Alkaline Phosphatase), Bone Morphogenetic Protein (BMP), and Collagen type-1, and [51] observed that different polarities caused different osteo-neogenitive effects: bone formation was much more intense around the cathode. Despite such evidence, a meta-analysis of 2008 [52] maintained that no advantage in healing of long bone fractures could be attributed to ES.

The rationale for the use of microcurrent treatment for soft connective tissue damage, especially for tendons and ligaments [53] is based theoretically on the fact that the extracellular matrix (ECM) in such tissue is formed by diversified fibroblasts, the latter positively observedto migrate, proliferate and increase synthesis of ECM proteins under electric stimuli [1]. DC currents seem to be the usual choice [54,55], but PEMF has been used, too [56].

The abovementioned applications also fell within the sports injuries field, where electricity has received a warm welcome since drugs are dealt with under a light of suspicion. In this field, muscle injury [21] and, far more usual, muscle soreness due to intense exercise or overtraining are the commonest cases [20,22]. Although practically a different subject, the rehabilitation of muscular dysfunction due to irradiation [24] or other reason is similar to treating sports injuries. In both cases, the metabolism of the muscle must be up-regulated, to achieve growth and development; to implement this, an increase in oxygenation and blood supply is needed. It is important to remember that the first re-use of the electrotherapy was during WWII, to prevent muscle loss of long immobilized wounded troops, while in 1855 G. Duchenne, the “Father of Electrotherapy” was using it for muscle contraction, for which he declared that AC was preferable to DC as the former caused no thermal injury and was more adjustable to the treated muscle's status [11,25].

The up-regulating effect of ES in different cell types makes ambiguous its use at or near actual and even suspected tumors. The optimistic view maintains that electrostimulation of μA-level normalizes cell growth: this means ES actually inhibits cell division if abnormally accelerated (which implies an innate tumor confinement or suppression application); stimulates cell division after injury and does not affect it when in a proper state of equilibrium [57]. This is on top of previous results with implanted electrodes so as to enclose a tumor and to electrocute it with higher voltages [11,58,59]. The pessimistic view is that the tumor may benefit more from the stimulating effect than immune cells and thus its progress will be accelerated. The authors of the present review think that there is a possibility to make the tumor grow too fast to be supported by existing-and even expanding- vessels and thus starve it to necrosis, but the prospect requires accurate fine-tuning which may be unattainable as a generalized prerequisite.

The analgesic effect of electricity is widely used in different modalities. Transcutaneous Electrical Nerve Stimulation (TENS) was cumbersome. It produced electrically induced nerve fiber signalsto block the body's ability to perceive the pain that is being treated, possibly by increasing production of endorphins. The main effect of TENS is believed to stimulate A-beta pain-suppressing nerve fibers to overwhelm chronic pain-carrying C-fibers [16,17]. PENS (Percutaneous Electrical Nerve Stimulation) was the merger of acupuncture and TENS, using the specifics of Low Frequency TENS; that is <10Hz and high intensity. On the contrary, HF-TENS (High Frequency TENS) uses low amplitude and high frequency, >50Hz. The nervous system, though, gradually accommodates to this high level of current, causing tolerance similar to that ofchemical analgesics [11]. Micro Current Electrical Therapy (MET) is faster and lasts longer using lower intensities for longer time (microAmpere range, *i.e.* 10^{-3} of the TENS') and below sensation threshold [14,60,61].

4.2.2 Contraindications

The ES modalities were considered relatively free of contraindications during the 90s: Mercola and Kirsch, writing about Micro Electrotherapy (MET), suggested caution during pregnancy because electrical stimulation might affect the endocrine control systems and cause miscarriage; the ultimate contraindication was the use of demand-type cardiac pacemakers. Other than these two conditions, there were no known significant adverse side effects to MET at the time [14]. These grew to no less than six (adjacent tumors [62], use of metal-containing powders, any electric or electric-sensitive device, metal implants, neuroexcitatory syndromes, presence of active infection/inflammation) in 2013 [11,63]. The possibility that ES would induce cancer is not as believable and alarming as the notion of upregulating the already increased metabolism rate of cancerous cells, possibly assisting in the progress of the disease [62]. Although [57] suggests rather a normalization of the cell growth, which argues against actually causing cancer, the precise impact in already established tumors is a very different subject, as tumor-specific kinetics may be implicated. Additionally, although ES seems to exert antimicrobial effect on the open wound [64], it is due to fully blown infections that some of the patients in [63] discontinued their treatment, and the issue of the exact dynamics and kinetics relative not only to microbial species but to growth status (phase and cell number), is always a source for concern. Next comes the subject of electromagnetic compatibility. Although the term usually refers to interference between/among different electric devices-such as the ES modality and a pacemaker in the present study, the concept is wider: the presence of metal implants or metal-containing powders and colloids might case dislocation with or without consecutive injury and local acute raise of temperature resulting in lesions and/or burns [65]. Lastly, there is the issue of interference with the nervous system. The truth is that ES modalities had been used for neurological ailments very early on; its use for such cases has never been completely discontinued, despite the very negative aura it always carried. In the present day the very issue of electroceuticals is to a considerable extend focused on nerve-related ailments, might that be pain management or neurological disorders such as Parkinson's disease [9].

4.3 The Electrode Factor

Moreover, different material for the electrodes, not to mention different kinds of electrodes, may affect not only infection and burn risk and pain/discomfort management, but, possibly, the outcome of the treatment proper. An interesting classification is achieved by Meng*et al*[28], according to whom electrodes used in ES of cultures fall in one of 4 main categories, which are: (i) Metallic (Copper, stainless steel, Silver, Platinum). They are the baseline electrodes and available in many forms (needle, wire, coil) but they often cause burns, as their temperature rises when charged. Their contact with live human tissue might be painful or irritating and they tend to chemicallyinteract with current and chemicals, releasing ions at the points of application and also producing possibly noxious products, as are Reactive Oxygen Species [1,4,14]; (ii) Salt bridges, on the other hand, are basically metal electrodes immersed in gauges or other permeable material soaked in chemically benign electrolytes so as not to contaminate the culture/tissue with noxious agents produced electrolytically; the use of membranes [21] might be added in this category. (iii) Carbon Nanotube (CNT). Such electrodes present excellent physical (thermal stability, mechanical adaptability, conductivity) and chemical properties (stability), as they supposedly produce no free radicals or any drastic moiety; still, they have proved toxic for different cell types, and are thus deemed unsuitable for therapeutic use. On the contrary, the last category, (iv) Conductive Polymers (CP), seem to entail multiple advantages for *in vivo* use. Conductive polymers are mechanically adaptable and flexible, while they do not create noxious agents during use. Moreover, they are better tolerated by living tissue than metal pieces and the interface between organism and circuitry is much smoother, making them thus suitable for implantation. Although their formation results in mediocre homogeneity among the conductive and other, inert parts, intensive research has already produced two alternatives to the PPy (polypyrrole) which is currently the preferred CP: PT (polythiopine), which currently shows unsatisfactory conductivity and PANi (polyaniline) which is rather unstable in terms of electrochemistry. Further research might alleviate the drawbacks by modification, by developing other alternatives and, most important, by changing the requirements: for example, as lower intensities seem to become the preferred choice, low conductivity may be tolerated.

4.4 The Microbial Dimension

Another issue is the interaction of electrostimulation with microflora. Microorganisms of economic interest (but of clinical interest as well) have been tested adequately -though not extensively- with a number of electrification formats to test stimulative or prohibitive effect and possible in-between boundaries and factors. Generally, microbes show galvanophilia in culture or in bioreactors, under certain conditions, whereas the effect is reversed in traumas as shown with *Staphylococcus aureus*-infected wounds [66,67]; similar results were obtained with *Pseudomonas* and *Proteus*[14, 68] still, fully-blown infections are considered contraindications for trauma treatment, as the dynamics of electrostimulation versus microbial flora remain unexplored. The observation that *in vivo* microbial inhibition in wounds is preferably accomplished by negative currents, while wound healing with positive [14], explains in part one advantage of AC formats. On the other hand, it continuous μA-level currents show better antimicrobial activity compared to pulsed currents [69]. The interaction between electrostimulation and antibiotics is -more or less - terra incognita; possible synergistic effect, or even additive effect [40], might revolutionize wound care, and exert significant impact in infectious diseases as well.

The antibiotic effect of electric fields is documented since the 60s [70] for Alternate Current fields; Direct Current pulsed fields were successfully studied in the 90s [71], while static fields furnished inconclusive results [72], as do magnetic and electromagnetic fields [73]. Actual current transfer, though, is another issue altogether. There are many studies, differing in the type of current (AC, DC or other- [41, 69, 74]) or the type, design, material and polarity of the electrodes used [4,75] and, of course, dosage [39]. A number of studies describe positive [76] and another, considerable number, negative effects in microbial growth [41,69,72]. The Gram status of a bacterium might well be of some importance [4,41,75]. The matter clearly remains open and undecided [73], but the bulk of the published work is performed using contact electrodes onto unicellular organisms; mostly bacteria [77], but occasionally some yeasts as well [76,78]. A conclusion reached by the authors thanks to the exhaustive and cumulative presentation of relevant studies [4] is that DC current of more than 5 μA for 20 min or more have bacteriostatic (and sometimes bactericidal) effect if delivered by a wide range of metallic electrodes (Platinum, stainless steel, Copper), with silver-made ones being more effective.

5. WIRELESS MICROCURRENT STIMULATION (WMCS): THE NEW APPROACH INES

ES is traditionally performed by applying on the tissue pin or dressing electrodes connected by cables to a current-generating device. In contrast, Wireless Micro Current Stimulationtechnology (WMCS) is an innovative, simple, noninvasive, pain-free method to transfer current wirelessly to the wound [63]; it disposes of contact electrodes and thus solves the major issue of infection risk during therapeutic sessions in extended and deep wounds and ulcers, while also tackling the just as important one of pain/discomfort. The WMCS is a subcase of a wider methodology, the Non-Contact Current Transfer (NCCT), which has broader applications. The WMCS turns atmospheric gases (eitherOxygen orNitrogen,depending on the device) to ions and sprays themonto the receiving tissue, while an adjustable flexible bracelet (neutral electrode), worn around a healthy wrist or ankle of the subject, closes the circuit. The subject is sitting or lying on an insulated bed/chair and thus the sprayed ion load is driven back to the device by a cable attached to the bracelet. As there is no contact electrode in the vicinity of the ulcer/wound, there is no pain or irritation, no electrochemical byproducts, no risk of burn or allergy and the risk of infection is minimal and focused at airborne pathogens transmitted to the wound by the spraying procedure.The distance between target tissue and the device is less than 15 cm, in standard conditions the risk is negligible, as all such treatments imply [79]. With no physical contact to the wound thanks to its spray effect, it offers a radical advantage compared to other currently used ES techniques. Obvious improvementof chronic wounds and a significant reduction of pain (even within 1-2 weeks since the first application) has been observedwhen using the WMCS method. Up to now, the WMCS has been successfully tested in ulcers [64,80], in burns ([63,81] and Poulas *et al*, unpublished results), while extensive testing in bed sores, sports injuries and muscle soreness is under way. The WMCS therapy thus entails multiple advantages in comparison to previous methods of ES and other, non-ES, common methods in the treatment of chronic wounds [63,79]although further testing and comparative controlled studies are necessary to confirm this.

A robust design is required to determine whether the WMCS has a positive or negative effect on microbial growth; such knowledge is of paramount importance for biotechnological applications, as non-medical cultures could be affected in a desirable way; the increase of yield, in terms ofeither biomass or metabolites (the latter being vital for the development and production of new pharmaceutical compounds)is one;, the reduction of harvesting time or the decrease on incubation temperatures (bothbeing especially lucrative perspectives) is another such way. Alternatively, negative effects might imply potential for sterilizing, antiseptic or decontaminating use, bypassing adverse effects of ionizing irradiation and environmental chemical/pharmaceutical burden. Both dynamic and kinetic studies in different *in vivo* backgrounds should be carried out for representative type strains in the presence of a wide range of antibiotics, to evaluate possible generalization of the encouraging findings of [40].

The observation that all formats exhibiting antimicrobial effect *in vitro* use currents of 5 μA or more [4] brings about the question of what happens within the 0.5-4 μA range, where newer modalities –such as the Wetling 200 WMCS device, shown in Fig. 2- work due to the lower risk of ohmic damage and greater similarity to innate bioelectricity; it is more than possible that microorganisms show a more or less bell-shaped curve in intensity/growth diagram and the optimum in terms of growth for most bacteria is to the left of the 5 μA mark.

Fig. 2. The W-200 device for wireless micro-electrostimulation applications

6. CONCLUSION

The different formats of electrical stimulation and the extent of their accumulative therapeutic application are not exhaustively presented in this work. The intent of it is more to give an idea of the heterogeneity of such approaches, the width and depth (historical depth included) of its use, the advancements of technology which make it more user-friendly and suitable for generalized use inboth in- and out-patient groups. But the most important issue is to understand that the therapeutic use of electricity, may be very sophisticated in terms of administering and thus must be described, in terms of electrodynamics and electrokinetics, just as biochemical compounds are, so as to properly integrate it in modern therapeutic schemes either as a supplement [40] or as an alternative [64] to biochemical compounds. Ample evidence supports a curve in dosage-therapeutic progress relationship [39], where very low dosage does not meet therapeutic thresholds and excessive dosage is counterproductive [1,31,49], thus substantiating the notion of electrodynamics. On the other hand, serial,theantithesis of multi-session treatment reachinga plateau in the improvement attained when treatment sessions are individually extended over a time threshold, to progress continuing with more sessions (accumulative extension) offers a robust indication of a kinetic constituent present in the electrotherapeutic phenomena. The increase in healing function observed when specifically timed changes of the polarity of the administered current are implemented (compared to steady polarity or very frequent changes) [82,83] clearly demonstrate the preponderance of the electrokinetics aspect.

ACKNOWLEDGEMENTS

This work was funded by an FP-7 Research Potential Program/REGPOT SEEDRUG of University of Patras (Grant Number: EU FP7 REGPOT CT-2011-285950).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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