



Electric field stimulation for tissue engineering applications

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Author(s)	Ryan, Christina N. M.;Doulgkeroglou, Meletios N.;Zeugolis, Dimitrios I.
Publication Date	2021-01-05
Publisher	BMC (part of Springer Nature)
Repository DOI	10.1186/s42490-020-00046-0

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3

4 **Authors**

5 Christina N.M. Ryan # (1, 2), Meletios N. Doulgkeroglou # (1, 2), Dimitrios I. Zeugolis * (1, 2, 3)

6

7 **Affiliations**

8 1. Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), National
9 University of Ireland Galway, Galway, Ireland

10 2. Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), National
11 University of Ireland Galway, Galway, Ireland

12 3. Regenerative, Modular & Developmental Engineering Laboratory (REMODEL), Faculty of
13 Biomedical Sciences, Università della Svizzera Italiana (USI), Lugano, Switzerland

14

15 **#Shared first authorship**

16 Christina N.M. Ryan and Meletios N. Doulgkeroglou

17

18 ***Corresponding author**

19 Dimitrios I. Zeugolis, REMODEL, NUI Galway & USI. Telephone: +41 58 666 40 00; Email:

20 dimitrios.zeugolis@usi.ch

21

22

23 **Abstract**

24 Electric fields are involved in numerous physiological processes, including directional embryonic
25 development and wound healing following injury. To study these processes *in vitro* and/or to harness
26 electric field stimulation as a biophysical environmental cue for organised tissue engineering
27 strategies various electric field stimulation systems have been developed. These systems are overall
28 similar in design and have been shown to influence morphology, orientation, migration and phenotype
29 of several different cell types. This review discusses different electric field stimulation setups and
30 their effect on cell response.

31

32 **Keywords**

33 Electric field; Galvanotaxis; Cell stimulation; Biophysical cues

34

35 **Background**

36 Endogenous electric fields (EFs) are involved in the organisation and development of tissues, as well
37 as in their regeneration following injury (1, 2). Disruption of endogenous EFs leads to abnormalities
38 (3, 4) and slows down wound healing processes (5). Physiologically speaking, for example, a
39 polarised epithelium transports ions that maintain a transepithelial potential (6). When an injury
40 occurs, the transepithelial potential is severely disrupted and an endogenic wound EF occurs that
41 drives epithelial cells to the wound for healing purposes (7). The magnitude of endogenous EFs varies
42 as a function of species, tissue, location and developmental stage [e.g. 0.02-0.04 V/cm during
43 neocortical activity in ferrets (8); 0.1-0.2 V/cm in different anatomical parts of axolotl embryos during
44 their developmental stages (9); 0.42 V/cm in wounded rat corneas (10); 0.42 V/cm in sliced tips of
45 hindlimb digit of *Notophthalmus viridescens* (11); 1.1-1.8 V/cm in wounded mouse and human skin
46 (12); 1-2 V/cm in small skin cuts of cavies (13); 20-30 mV/cm in mice brain (14)].

47 Considering the importance of EFs in physiological tissue function; disease manifestation and
48 progression; and regeneration, research efforts have been directed towards utilising EFs to study cell
49 response *in vitro* as a means to better understand the mechanism of action of EF-induced stimulation
50 and develop functional therapeutic interventions. It has now become apparent that EF stimulation *in*
51 *vitro* modulates cell morphology, orientation, migration and phenotype commitment, as well as
52 extracellular matrix (ECM) synthesis and orientation (15, 16) and *in vivo* promotes ECM synthesis
53 (17), modulates ECM deposition (18) and accelerates wound healing (19). To describe the influence
54 of EF stimulation on cell response, the theories of galvanotaxis (i.e. the process of preferential cell
55 migration towards the anode or the cathode) and galvanotropism (i.e. changes in cell morphology)
56 have been introduced (20, 21). Over the years, various EF apparatuses have been used to study the
57 influence of EF stimulation on cell response *in vitro* with variable degree of complexity and
58 efficiency, jeopardising comprehensive investigation of this *in vitro* microenvironment modulator.
59 Thus, this review provides an overview of EF setups, describes the function of their most important
60 components and discusses advancements and shortfalls in EF stimulation in controlling cell function.

61 **Main Text**

62

63 **Electric field cell stimulation setups**

64 *In vitro* EF stimulation started with a simple setup, where two electrodes were placed at the bottom
65 of a cell culture well and the cells were seeded in between (**Figure 1A**). Trial and error experiments
66 (e.g. to avoid media evaporation, avoid electrode degradation products contaminating the cells) have
67 resulted in the current setup, which includes a chamber that contains the media and the cells, with
68 agar bridges transferring the charge from the electrodes immersed into electrolytes to the cell media
69 (**Figure 1B**). In the spirit of automation and scalability, parallel setups (22) have been developed that
70 allow for multiple experiments to be conducted simultaneously (**Figure 1C**). More complex systems,
71 such as bioreactors capable of combining EF stimulation with mechanical loads (23), have also been
72 developed (**Figure 1D**). In the era of miniaturisation, compact, closed-system microfluidic devices
73 (**Figure 1E**) that provide more effective control over the uniformity of the EF, mitigate the Joule
74 heating effect, reduce the dimensionality of equipment and offer high data output have also been
75 realised (24, 25).

76 Independently of the setup, poly(methyl methacrylate) (PMMA) (26-29) and poly(dimethylsiloxane)
77 (PDMS) (30-36) are mostly used for the fabrication of galvanotaxis devices, although some devices
78 have been made from glass (33) or plastic (37). Further, all systems have a window (usually a glass
79 slide / coverslip), which allows visual assessment of cells before, during and after EF stimulation (38-
80 40). When chamber size permits, the entire chamber is placed on the stage of an inverted microscope
81 and cell behaviour is observed directly during experiments (41-46). In the subsequent sections the
82 main components of most EF cell stimulation apparatus are discussed.

83

84 **Galvanotaxis chamber**

85 Galvanotaxis chambers are constructed to allow flow of constant electric current directly over the
86 cells within a channel. An early study used a trough that was created by placing two parallel glass

87 coverslips in the centre of a petri dish. The cells were seeded in the created trough and a closed EF
88 was created by connecting the cell culture media with the agar salt bridges to the solution with the
89 electrodes (47). Due to this simple construction, similar chambers composed of glass slides or
90 coverslips separated by acetate or silicon spacers and held together with silicone grease or adhesive
91 have been fabricated (48-54). To reduce time, effort and costs associated with continuous chamber
92 fabrication, a modular chamber design comprised of parallel plates that allow glass slides or
93 coverslips plated with cells to be inserted and removed at ease without affecting the chamber structure
94 have been developed using various materials (e.g. plexiglass, polycarbonate, acrylic, graphene and
95 PMMA) (55-61). PDMS is featured in several setups either as a primary material from which
96 chambers may be excised (62) or due to its insulating properties that allow independent electrical
97 stimulation of rows of wells (63). Further, its versatility of stiffness modification (64), allows for
98 simultaneous assessment of substrate rigidity and EF stimulation on cell response. To assist cell
99 adhesion, surfaces used as channels for cell seeding are often coated with ECM proteins (e.g. laminin,
100 fibronectin, collagen) (65-70) and to improve cell motility and alignment, microgrooves are etched
101 onto glass / quartz slides (71-73).

102

103 **Electrodes**

104 Electric current is generally passed through the galvanotaxis chamber by placing electrodes into
105 phosphate buffered saline (PBS) or Steinberg's solution reservoirs, from which agarose salt bridges
106 form a conducting pathway to the chamber with the cathode connected at one side of the chamber
107 and the anode to the other (46, 47). Conductive bridges, composed of plastic or glass tubing are filled
108 with agarose (2-4 %) and can be of different lengths [from 6 cm (74, 75) to 35 cm (55, 59), although
109 most setups incorporate bridges of 15 to 20 cm (76-78)]. Some groups have even bent tissue culture
110 pipettes into U-shapes and used them as agar-salt bridges, which have the added advantage of already
111 being sterile (79). Systems with reduced size agar bridges embedded within the galvanotaxis chamber
112 (35, 36) or even setups without salt bridges, which facilitate the design of reduced size devices (80)

113 have also been reported, albeit not extensively. The bridges also act as safeguards to reduce heat
114 exposure of cells via Joule heating of the chamber (75) and prevent electrolysis products (e.g. metal
115 ions) (81) produced at the electrodes from contaminating cells within the chamber (82). Aluminium
116 (83), carbon (84, 85), copper (86, 87), platinum (60, 88) and stainless-steel (89, 90) have been used
117 as electrodes across a range of direct current (DC) EF stimulation systems, however silver-silver
118 chloride (Ag/AgCl) electrodes are the most commonly used (46, 47). These are favoured as the only
119 species involved in the electrochemical reactions at the electrode surface are chloride ions, thus
120 eliminating unwanted reactions associated with electrodes, such as platinum (91). They convert
121 electron flow to a chloride ion flow from the cathode to the anode through the conducting pathways.
122 Ag/AgCl electrodes can be fabricated from silver wire by soaking for up to 1 h in a hypochlorite /
123 bleach solution, or in 1 M HCl and then chloridised for 30 min at a current of 5-10 mA cm² (59, 91).
124 These electrodes can then be stored in distilled water or PBS for several weeks. In some setups, the
125 electrodes have been integrated into the galvanotaxis chamber by coiling them about 5 cm into
126 agarose embedded within the platform (91). This saving in size of the setup allows the platform to be
127 efficiently placed within a live cell chamber, whereby humidity, CO₂ partial pressure and temperature
128 can be controlled relatively ease.

129

130 **Power supply and electric field stimulation regimes**

131 EF stimulation utilises either DC or alternating current (AC). DC is a steady mono-flow /
132 unidirectional current, whereas AC has a sinusoidal form and constantly switches direction. As in the
133 extracellular space of plants and animals, DC signals are primarily observed (92), the vast majority
134 of EF cell stimulation studies use DC. Nonetheless, AC has also been selected to either compare its
135 effect with the frequently used DC stimulation (93), or to recreate physiological EFs, in the case of
136 the central nervous system that neurons are exposed to oscillating endogenous EFs (94, 95). Over the
137 years, numerous cell types have been exposed to different EF strengths (0-10 V/cm) and stimulation
138 duration (0-72 h) (**Table 1**). To achieve the required EF strength, DC power supplies (e.g. Keithley

139 SourceMeter®) have been used that work with DC currents of 0.0-0.3 mA and generate EFs of 0-6
140 V/cm (96, 97). Eight-channel programmable power simulators (e.g. Master-8, AMPI) (61) generating
141 EFs up to 4.5 V/cm, multi-potentiostats (e.g. CH1040A, CH Instruments) generating EFs of 0.1 V/cm
142 and currents of 0.0-0.1 mA (86) and the commonly found in laboratory setups gel electrophoresis
143 (e.g. FB600, Thermo Fisher Scientific) power sources (58, 98) have also been used with an EF range
144 of 0-10 V/cm. For the measurement and adjustment of current and field strength during a stimulation,
145 multi-meters can be positioned respectively in series and in parallel with the chamber (55). In
146 addition, current density and correlating EFs have been altered not only by adjusting applied current
147 or voltage, but also by altering resistance through the channel by varying the channel widths (0.5-3.0
148 cm) (86). For the application of AC EFs, function (waveform) generators that provide both type of
149 currents may be used (e.g. Precision 4011A, PASCO Scientific) (93) with the AC component ranging
150 less than the regimes observed in DC, usually within 0-1 V/cm (58, 99).

151

152 **Generated forces during a galvanotaxis experiment**

153 When a cell migrates in any substrate, its displacement gives rise to three-dimensional tractional
154 forces (100), which is also the case for EF assisted migration. During EF stimulation, cells are
155 exposed to forces from the EF itself and from the culture substrate. The stress can be perpendicular
156 and horizontal to the direction of the EF. Forces also develop between the surfaces of the cells, as
157 they touch each other in the restricted space of a galvanotaxis chamber during a collective migration.
158 The interaction of the cells leads to a parallel to the direction shear stress and a perpendicular to the
159 direction normal stress (101). It has been shown that by the onset of EF in a keratinocyte monolayer
160 (102), the intercellular stress component in the perpendicular axis to the EF direction increases
161 significantly in comparison to the stress component in parallel to the EF direction and that migration
162 is independent of the reorientation of the intercellular stress. In addition, the flow, which can be
163 hypothesised as laminar, applies hydrodynamic forces to the cells. These forces can be calculated by
164 the hydrodynamic equation of laminar flow mechanics.

165 The exact mechanism regarding galvanotaxis-induced motility is still unclear. In literature, different
166 hypotheses have been formulated regarding the decisive factor for cell migration during galvanotaxis.
167 These hypotheses include the effect of flow, due to hydrodynamic cell forces, on the cell membrane
168 (103); the activation of electrotaxis, owed to change of cell membrane polarity, which in turn is driven
169 by an asymmetric local concentration of ions (104); and the electrophoresis of charged membrane
170 components (e.g. proteins) (105, 106). The normally occurring hydrodynamic forces alone have not
171 been proven to contribute to directional migration, since cells were observed to move randomly in
172 the absence of an EF in almost all the reported experiments (54, 102) However, when an external
173 shear stress stimuli was applied, migration was retained in the preferential direction even without the
174 application of an EF (35). A recent work investigated the role of integrins by testing hamster ovary
175 modified cell lines that express specific human integrins and concluded that different subsets of
176 integrins may promote normal or reverse directional migration during galvanotaxis, thus highlighting
177 the importance of the intracellular domain with cell migration (107). It should be noted that the
178 strength of the EF increases the aligned directed locomotion of the cells, as it has been shown in
179 numerical simulations (108) and experimental data (109, 110). However, differences were observed
180 (83) in the time of response and the required EF intensity needed to trigger migration for clustered
181 and isolated cells. It should be noted that according to the cell type, cells may show different
182 preferences in anodal or cathodal directed migration (**Table 1**).

183

184 **Electric field stimulation *in vitro* and *in vivo***

185 Although the influence of DC and AC EFs on cell response *in vitro* and *in vivo* has been the subject
186 of many investigations (**Table 1**), it is worth noting that most studies focus on the alignment and
187 migration patterns that DC EFs induce to cells and only a few studies have assessed the influence of
188 EFs on cellular functions *in vitro* and tissue response *in vivo*. In general, subject to the cell population,
189 DC EF of up to 10 V/cm and for up to 72 h are efficient in controlling cell orientation and migration
190 (71, 72), increase cell proliferation (111, 112); and do not affect cell metabolic activity and viability

191 (113-116). Stem cell differentiation has also been studied; for example, DC EFs of 0.1-1.0 V/cm (117,
192 118) and pulsed DC EFs of 50 Hz and 6 V/cm peak-to-peak amplitude for 6 h per day (119) have
193 been shown to favour osteogenic differentiation.

194 With respect to AC EF stimulation, although it has been shown to affect cellular functions, alone has
195 not been shown consistently to result in controlled cell orientation and migration. For example, AC
196 EFs of 10 Hz and 50 Hz have been shown to sustain a more immature phenotype in porcine neural
197 progenitor cells, without promoting alignment and affecting proliferation (99). AC EF stimulation
198 (20 mV/cm, 60 kHz, 40 min per day for 20 days) has also been shown to not affect cell morphology
199 and metabolic activity in human stem cell cultures and to increase osteogenic differentiation (120).
200 Regarding differentiation, AC EFs have been used for both osteogenic (121-123) and chondrogenic
201 (124, 125) differentiation of stem cells. When mouse neural stem cells were encapsulated in alginate
202 hydrogel beads and subjected to AC EFs (0.1 to 10 Hz; 2, 4, 16 V/m; 14 and 21 days), it was reported
203 that 1 Hz frequency enhanced viability, whilst differentiation was promoted or inhibited subject to
204 culture time and EF frequency (cell morphology analysis was not conducted) (126).

205 When DC was directly compared to AC in rat neural stem/progenitor cell cultures, it was found that
206 differentiation and migration were enhanced and viability was decreased in DC EFs, whilst AC EF
207 had no effect (58). Interestingly, in human keratinocytes isolated from neonatal foreskin cultures, AC
208 led to random migration; DC alone and DC combined with AC resulted in cathodal direction; and DC
209 combined with 160 Hz AC resulted in enhanced migration in comparison to DC alone and DC
210 combined with 1.6 Hz AC (93). Other than cell morphology and migration analysis studies, more in
211 depth biological analysis studies are required to clearly illustrate whether there are any beneficial
212 effects in combining DC with AC EF stimulation.

213 In *in vivo* setting, preliminary studies advocate the use of EF stimulation. For example, the migration
214 of human peripheral blood lymphocytes was enhanced in mouse ear skin model when an external EF
215 was applied (37). EFs have also been shown to promote migration and differentiation of neural
216 progenitor cells in a rat model of chronic-phase ischemic stroke (127). In a similar manner, electrodes

217 were inserted in a rat brain and stimulated transplanted human neural progenitor cells, resulting in
218 directed migration and increased motility (128). Furthermore, transvaginal electric stimulation in
219 female mice has shown activation and proliferation of fibroblasts (129).

220 In clinical setting, electric stimulation has been used in different instances with mixed outcomes.
221 Recent studies, for example, include the use of electric stimulation to treat neurogenic bowel
222 dysfunction in patients that suffered spinal cord injuries, but without consistent results (130). On the
223 other hand, EF stimulation resulted in a reliable recovery of motor functions in patients experienced
224 a stroke (131), an improvement in visual abilities by the placement skin electrodes in patients with
225 retinitis pigmentosa (132) and accelerated wound healing (19), collectively indicating the potential
226 of EF stimulation in reparative medicine.

227

228 **Conclusions**

229 Electric field stimulation is continuously gaining pace as a means to control cell orientation, migration
230 and phenotype *in vitro* and *in vivo*. Direct current electric fields (up to 10 V/cm) are favoured among
231 investigators, as such signals are primarily encountered in the extracellular space of plants and
232 animals. Although variable in complexity galvanotaxis chambers have been used over the years, the
233 most popular setups are comprised of glass slides for cell seeding, transparent polymers that allow
234 real-time cell visualisation, Ag/AgCl electrodes that eliminate toxic electrode degradation products
235 and agarose salt bridges in phosphate buffered saline to prevent them from drying and to stabilise
236 electrode potentials. It is worth noting that despite the promising *in vitro* data, only a few studies have
237 assessed the influence of electric field stimulation *in vivo* and in clinical setting. Standardisation and
238 automation of the processes will allow more intense investigation of electric field stimulation in the
239 years to come.

240

241 **List of abbreviations**

242 AC: Alternating current; Ag/AgCl: Silver-silver chloride; DC: Direct current; EF: Electric field;
243 ECM: Extracellular matrix; PBS: Phosphate buffered saline; PDMS: Poly(dimethylsiloxane);
244 PMMA: Poly(methylmethacrylate).

245

246 **Declarations**

247

248 **Ethics approval and consent to participate**

249 Not applicable.

250

251 **Consent for publication**

252 Not applicable.

253

254 **Availability of data and materials**

255 Not applicable.

256

257 **Competing interests**

258 The authors declare that they have no competing interests.

259

260 **Funding**

261 This work has been supported by Science Foundation Ireland, Career Development Award (Grant
262 Agreement Number: 15/CDA/3629) and Science Foundation Ireland / European Regional
263 Development Fund (Grant Agreement Number: 13/RC/2073). This work has also received funding
264 from the European Research Council (ERC) under the European Union's Horizon 2020 research and
265 innovation programme, grant agreement No. 866126. The funding agencies were not involved in the
266 design of the study; in the data collection, analysis and interpretation; and in the writing of the
267 manuscript.

268

269 **Author's contributions**

270 CNMR, MND and DIZ wrote, edited and approved the manuscript. CNMR and MND equally share
271 first authorship.

272

273 **Acknowledgments**

274 Not applicable.

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Tables

Table 1: Indicative examples of the influence of electric field stimulation in various human cell types *in vitro* and *in vivo*.

Cell type	Power Supply	Electric Field Strength (V/cm)	Stimulation Duration (h)	Preferred Direction	Major Result
Chondrocytes	DC, Keithley Instruments (USA)	6	3	Bidirectional (dependent on passage of cells)	EF directed migration was influenced by passage (27)
Keratinocytes	DC & AC PASCO Scientific (USA)	0.4 at 1.6 or 160 Hz (AC) / 1 (DC)	1	Cathode	Verification of electromechanical model for migration (93)
Mammary epithelial cells	DC, Pine (USA)	0.13-1.0	6	Anode	Clustered cells were more sensitive to alignment, but migrated slower than isolated cells (83)

Osteoblasts	DC, Biometra (Germany)	0.15-0.45	7	Anode	Upregulation of ion channel gene, associating Ca ²⁺ with migration speed (133)
Peripheral blood lymphocytes	DC, Agilent Technologies (USA)	0.15-2	0.5-2.0	Cathode	Directed migration <i>in vitro</i> and <i>in vivo</i> and activated intracellular kinase pathways (37)
Neuroblastoma cells	DC, AMPI (Israel)	0.045-4.5	4	Anode	Enhancement of cell mobility (61)
Bone marrow stem cells	DC, Glassman FC (USA)	0.2-5	15	Cathode	Donor did not influence migration direction and morphological changes but affected

					response time to EF, migration speed and cell viability (22)
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Figures

Figure 1: Schematic illustration of various galvanotaxis setups. (A) The simplest setup. (B) The most common setup. (C) Parallel setup that allows multiple experiment simultaneously. (D) Multifactorial setup that allows simultaneous application of electric field stimulation and mechanical loading. (E) Miniaturised, closed system microfluidic setup.

