Eur Biophys J (2009) 39:185–189
DOI 10.1007/s00249-009-0447-8

TRIBUTE

# Discovery of "punch-through" or membrane electrical breakdown and electroporation

**Hans Gerard L. Coster**

Received: 4 February 2009 / Revised: 9 March 2009 / Accepted: 16 March 2009 / Published online: 3 April 2009


**Abstract** As part of a detailed study in Alex Hope's laboratory of the V–I characteristics of the membrane of the giant cells of *Chara corallina*, it was discovered that at a well defined potential difference of around 500 mV (depending on the temperature), the cell membrane undergoes a reversible electrical breakdown. The author coined the word "punchthrough" to describe this electrical breakdown phenomenon. Detailed studies followed on the nature of this electrical breakdown phenomenon, in various cells, aimed at elucidating the physical mechanism(s) involved. The applications and the significance of the phenomenon that were subsequently developed in later years were not foreseen at that time. Electrical breakdown/electroporation is now a commonplace procedure and has entered into the mainstream biological vocabulary. Here we trace its humble beginnings to experiments carried out in Alex Hope's laboratory and review briefly some of the aspects of this phenomenon and its applications that were developed much later by others as well as the author. The discovery of membrane electrical breakdown described below took whilst the author was his student of Alex Hope but whilst Alex was away on sabbatical leave in the UK. Because this occurred in his absence, Alex Hope elected to not put his name on the paper that described the discovery in 1965.

"Proteins, membranes and cells: the structure–function nexus". Contribution from a special symposium in honour of Professor Alex Hope of Flinders University, South Australia held during the annual scientific meeting of the Australian Society for Biophysics, Canberra, ACT, Australia, September 28 to October 1, 2008.

H. G. L. Coster (✉)
School of Chemical and Biomolecular Engineering,
The University of Sydney, Sydney, NSW 2006, Australia
e-mail: hcoster@usyd.edu.au

**Keywords** Electrical breakdown · Electroporation · Punchthrough · Cell Electrofusion · V–I characteristics · Membranes

## Introduction

It is now long established that cell membranes contain functional protein modules imbedded in a bimolecular lipid leaflet or "bilayer", although that picture was not clear at the time we were investigating the electrical characteristics of the plasma membrane of the giant cells of *Chara corallina* in Alex Hope's laboratory at the University of Sydney in the early and mid 1960 s. The overall thickness of the plasma membrane of *Chara*, in common with other living cells is around 6 nm, depending on the nature of the proteins imbedded in and extrinsic to the membrane, see Fig. 1.

The membrane potential (difference) in living cells ranges from a modest 10 mV (in human erythrocytes) up to ~240 mV in *C. corallina.* A typical electric field strength in cell membranes $E = V/d$ is therefore of the order of $10^7$ V/m; a very intense field indeed. Furthermore, the electric field is not expected to be uniform and hence the peak field is likely to be much greater than this. Many bulk materials undergo electrical breakdown when subjected to such intense fields.

## Electrical characteristics of the plasma membrane

The electrical properties of single cells of *C. corallina* were investigated using intracellular electrodes using what has become a fairly standard electrophysiological apparatus. The voltage–current characteristics were then obtained by scanning the current. A typical V–I characteristic so

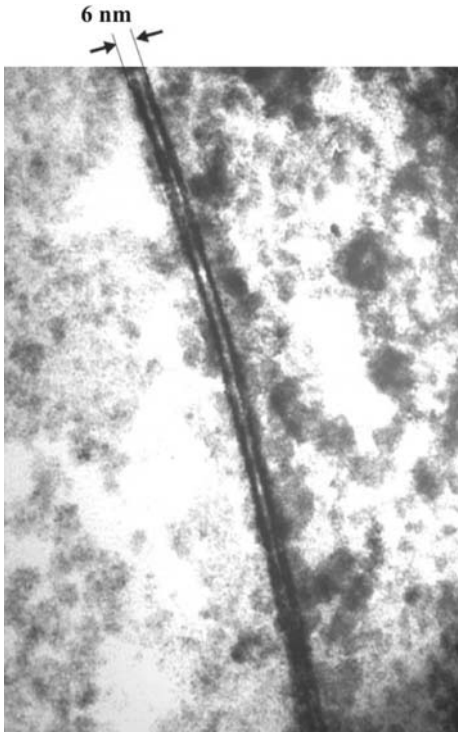


**Fig. 1** Electron micrograph of the plasmalemma of *Chara corallina* (fixed with $OsO_4$) (extended image from Coster 1973)

obtained is shown in Fig. 2. For membrane potentials from $\sim -300$ mV (hyperpolarized) to $-50$ mV (depolarized) the membrane shows very strong rectification. However, when the reverse bias (hyperpolarized) approaches $\sim -400$ mV the current suddenly increases very rapidly with potential (Coster 1965). The viability of the cell, however, is not affected by this provided the current at that point is maintained for only short periods and the

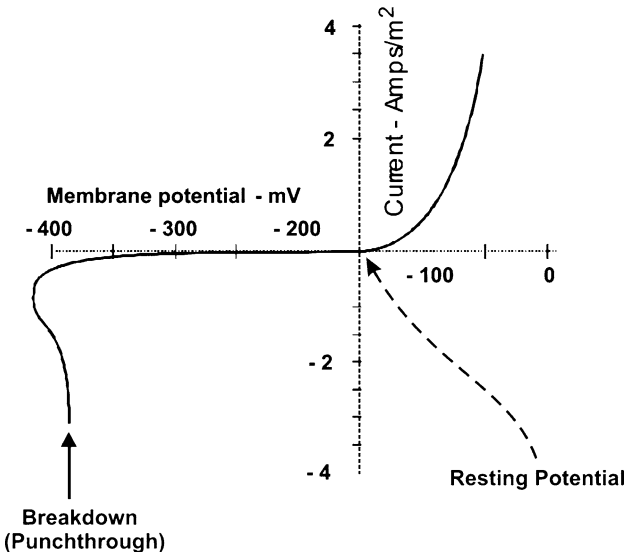


**Fig. 2** The V–I characteristics for the plasmalemma of *C. corallina* using current scans (based on Coster 1965)

entire V–I curve can be obtained reproducibly with repetitive current scans. The author coined the term "Punchthrough" to describe the rapid increase in current at these hyperpolarizing potentials. The term was borrowed from a phenomenon that displays similar characteristics in thin p–n junction semiconductor devices and, indeed, a model was developed to account for the V–I characteristics, including "punchthrough" based on the presence of double fixed charge membrane modules. Experiments with other cells revealed similar electrical breakdown or "punchthrough" phenomena. Studies were also undertaken to identify ionic movements during electrical breakdown (e.g. Coster and Hope 1968). Soon after, related phenomena on the effects of intense electric field pulses applied to suspensions of cells were also reported (Sale and Hamilton 1967). Further, more indirect observations using flow counters ("Coulter Counters") (Zimmermann et al. 1974) also implied electrical breakdown in cells such as erythrocytes at critical transmembrane potentials.

## Electrical breakdown

Further experiments showed that at the "punchthrough" or electrical breakdown potential the membrane displays instabilities. This is clearly visible in the oscilloscope traces shown in Fig. 3. Here square current pulses of increasing magnitude were injected into the cell (upper trace). The potential response shows the classical charging effect due to the membrane capacitance and then levels off. When these pulses were large enough to take the membrane to the electrical breakdown potential, the membrane potential showed instabilities (lower two traces) and would sometimes go into oscillation as long as the current was maintained. Subsequent investigations with cells of *Valonia utricularis*, using pulsed currents, also revealed electrical breakdown at well defined, critical, membrane potentials (Coster and Zimmermann 1974, 1975a, b). This is illustrated in Fig. 4.

An electrostriction model of the phenomenon was proposed (Coster and Zimmermann 1975a, b) in which the geometrical dimensions of modules imbedded in the membrane are determined by the effects of electrostrictive compression, $P_e$ forces and elastic mechanical restoring forces, $P_m$. Using a linear extrapolation these are given by:

$$P_e = -\frac{dW}{d\delta} = \frac{1}{2}\frac{\varepsilon\varepsilon_0}{\delta^2}V_1^2$$

$$P_m = \int_{x=\delta_0}^{x=\delta} \frac{dx}{x} = Y \ln\frac{\delta}{\delta_0}$$

where $Y$ is the Young's modulus of the membrane module, $\varepsilon$ is the dielectric constant and $\varepsilon_0$ the permittivity of free

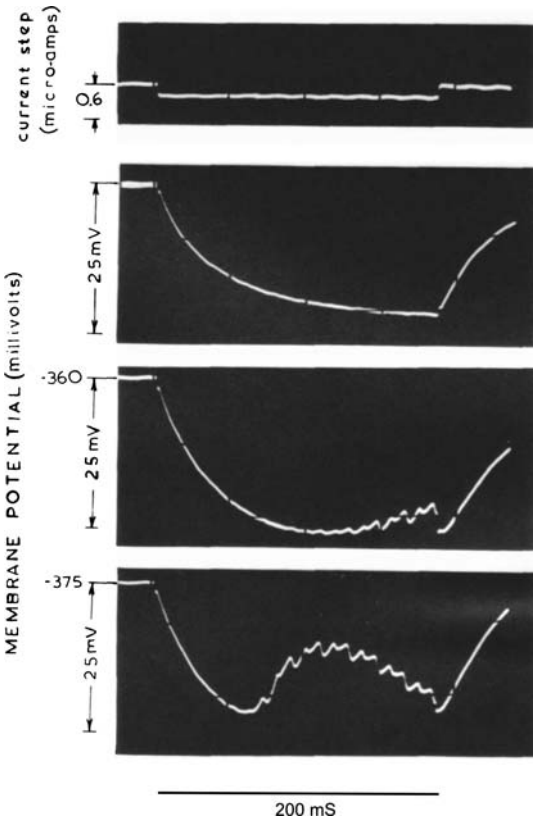


**Fig. 3** The response of the plasma membrane potential in *Chara corallina* to increasing, square, current pulses injected into the cell via an intracellular electrode. Note the capacitative charging and discharging at the start and finish of the current pulse. As the membrane potential approached ~385 mV (*third trace*), instabilities start to appear. Increasing the current pulse further (*lower trace*) enhanced these instabilities. The electrical breakdown or punch-through potential for this cell determined from V–I scans as shown in Fig. 2, was −385 mV (from Coster 1966)

space ($8.85 \times 10^{-12}$ F/m), $\delta$ the membrane thickness and $\delta_0$ the thickness at zero potential. Electrical breakdown on this model occurs when the electrostrictive forces increase more rapidly with potential than the elastic restring forces, that is, when:

$$\frac{\partial P_e}{\partial \delta} = -\frac{\partial P_m}{\partial \delta}.$$

This leads to the following expression for the critical breakdown voltage at which point

$$V_C = \left[\frac{0.3679\, Y\, \delta_0^2}{\varepsilon\varepsilon_0}\right]^{\frac{1}{2}}.$$

This rather simple model fitted the experimental data rather well and could readily account for the dependence of the breakdown potential on temperature through a temperature dependence of the elastic modulus ($Y$). This is illustrated in Fig. 5.

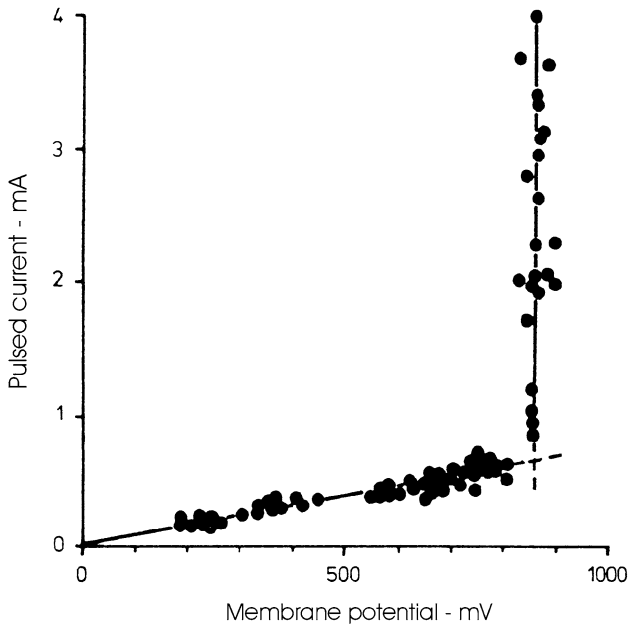


**Fig. 4** The V–I characteristics for a cell of *Valonia utricularis* using ~1 mS current pulses. At a membrane potential of ~850 mV, the current increases very sharply with membrane potential. The electrical breakdown potential is thus well defined. The *points* shown were obtained in random order (varying between super-critical to sub-critical regimes) (from Coster and Zimmermann 1974)

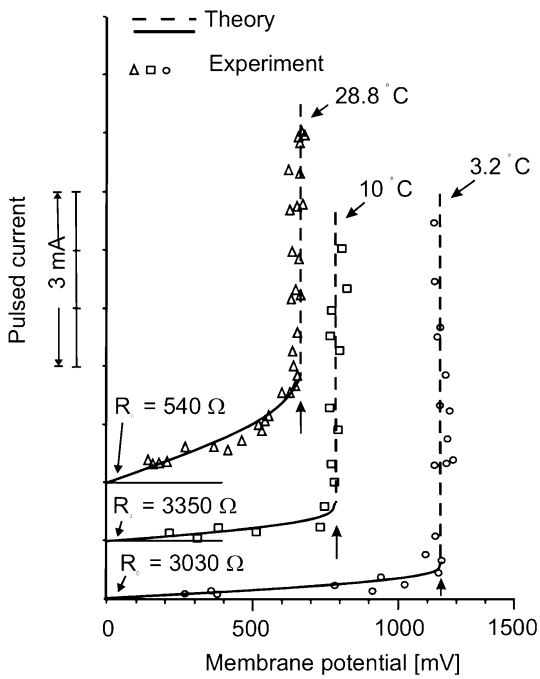


**Fig. 5** The V–I characteristics using ~1 mS current pulses for three different temperatures. Note the strong dependence of the critical breakdown potential on temperature. The *lines* drawn are a theoretical plot derived from the electrostriction model. For the fits, the initial slope of the V–I curves was used to fix some of the parameters. The fit, including the predicted breakdown were then obtained without adjusting any other parameters (Coster and Zimmermann 1975a)

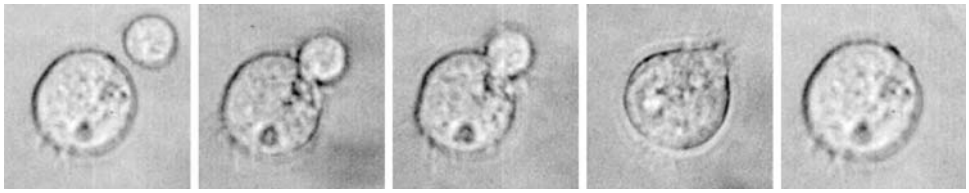

**Fig. 6** A sequence of micrographs showing electrofusion between a human B lymphocyte and a cell from the K562 cell line (human). The micrographs were taken at ~10 s intervals (Coster and Monaghan 2002)

## Electrical breakdown and electroporation

Electrical breakdown of the membrane leads to the formation of transient "defects" or pores in the membrane and as a result, the membrane becomes transiently permeable to relatively large molecules with molecular weights of up to hundreds of kilo Daltons (e.g. see Vieken et al. 1978). The formation of these transient "pores" as a result of electrical breakdown is referred to as "electroporation". The enhanced permeability following electrical breakdown can persist for extended periods, up to minutes after being electroporated. Electroporation can also be induced using extra-cellular electrodes, for instance using plate electrodes in a cuvette in which a suspension of the cells are placed. This usually requires larger current pulses because the current in such an arrangement flows around the cells rather than through them. Electroporation using larger current pulses, particularly under hypotonic conditions, can lead to cell lysis (e.g. see Sale and Hamilton 1967) and this could have possible diagnostic applications (e.g. for determination of haemolytic cell fragility; Goncharenko and Katkov 1985; Oliver and Coster 2003). Electroporation is now widely used for transfecting cells with DNA in genetic engineering and specialized equipment to perform electroporation are manufactured by a number of companies. A recent review of electroporation and its applications has been given by Katkov (2002)

## Electrical cell fusion

During electroporation, small (probably tens of nanometre) "pores" are created in the cell membrane. Subsequent to the electroporation these electrically induced pores reseal. If electroporation is induced in two cells held in contact (either mechanically or through dielectrophoresis), the membranes of the juxtaposed cells may fuse, thereby joining the two cells topologically and only a single pore remains that provides a connection between the cytoplasm of the two cells. Figure 6 shows a sequence of micrographs of the fusion of a human B lymphocyte with a cell from the K562 cell line. In this case the cells were drawn and held together using dielectrophoresis induced by the application of a 500-kHz signal applied to remote electrodes in the solution.

A large current pulse[1] was imposed just before micrograph 2 was taken. This pulse caused electroporation in the membranes of both cells at their point of contact. In the following micrographs, taken approximately at 10 s intervals, the cells can be seen to merge and the hybrid cell can be seen to round up. This pore then gradually enlarges and the cytoplasm of the two cells mix as the resulting cell slowly rounds up. Cell electrofusion has become an alternative to using polyethylene glycol to achieve cell fusion (see for example Sowers 1986; Coster and Monaghan 2002). Electrofusion is also an integral step in the procedures used in cloning animals.

## Conclusion

Electrical breakdown of cell membranes that manifests in electroporation is now a commonly used procedure to effect cell lysis, for transfecting large molecules into cells and vesicles, for cell fusion to create hybrid cell lines and as an integral step in animal cloning protocols. These applications grew out of systematic fundamental studies by many researchers of the electrical characteristics of cell membranes but had its beginning in curiosity driven research whilst the author was a Ph.D. student of Alex Hope in his Biophysics Laboratory at the University of Sydney.

## References

Coster HGL (1965) A quantitative analysis of the voltage–current relationships of fixed charge membranes and the associated property of "punchthrough". Biophys J 5:669–686. doi: 10.1016/S0006-3495(65)86745-5

Coster HGL (1966) Ionic relations and the electrical properties of the membranes of giant algal cells. Ph.D. thesis, University of Sydney

[1] In this case the current pulse was chosen so that the major Fourier component ensured that the current distribution created a very strong field near the point of contact of the two cells. The field strength used to induce fusion was ~300 kV/m. This could be achieved by applying a pulse of ~100 V to the wire electrodes in the solution.

Coster HGL (1973) The double fixed charge membrane model: an hypothesis concerning the structure and morphogenesis of cell membranes. Biophys J 13:1119

Coster HGL, Hope AB (1968) Ionic relations of cells of *Chara australis*: XI chloride fluxes. Aust J Biol Sci 21:243–254

Coster HGL, Monaghan D (2002) Human hybridoma technology for the production of monoclonal antibodies. Innov Pharm Technol 1:76–82

Coster HGL, Zimmermann U (1974) Direct demonstration of dielectric breakdown of the membranes of *Valonia utricularis*. Z Naturforsch 30:77–79

Coster HGL, Zimmermann U (1975a) The mechanism of electrical breakdown in the membranes of *Valonia utricularis*. J Membr Biol 22:73–90. doi:10.1007/BF01868164

Coster HGL, Zimmermann U (1975b) Dielectric breakdown in the membranes of *Valonia utricularis*: the role of energy dissipation. Biochim Biophys Acta 382:410–418. doi:10.1016/0005-2736(75)90281-3

Goncharenko MS, Katkov II (1985) Effect of cholesterol on the stability of human erythrocytes membranes to electrical breakdown. Biofizika 30:115–441

Katkov II (2002) Electroporation of cells in applications to cryobiology: summary of 20-year experience. Probl Cryobiol 2:3–8

Oliver LD, Coster HGL (2003) Electrical breakdown of human erythrocytes: a technique for the study of electro-haemolysis. Bioelectrochemistry 61:9–19. doi:10.1016/S1567-5394(03)00050-1

Sale AJH, Hamilton WA (1967) Effects of high electric fields on micro-organisms. I Killing of bacteria and yeasts. Biochim Biophys Acta 148:781–788

Sowers AE (1986) A long-lived fusogenic state is induced in erythrocyte ghosts by electric pulses. J Cell Biol 102:1358–1362. doi:10.1083/jcb.102.4.1358

Vieken J, Jelstsch E, Zimmermann U (1978) Penetration and entrapment of large particles in erythrocytes by electrical breakdown techniques. Cytobiologie 17:182–196

Zimmermann U, Pilwat G, Riemann F (1974) Dielectric breakdown in cell membranes. Biophys J 14:881–899. doi:10.1016/S0006-3495(74)85956-4