Electric Potential Across Epidermis and Its Role During Wound Healing Can Be Studied by Using an *In Vitro* Reconstructed Human Skin

Véronique J. Moulin,[1,*] Jean Dubé,[1] Olivier Rochette-Drouin,[1] Philippe Lévesque,[1] Robert Gauvin,[1] Charles J. Roberge,[1] François A. Auger,[1] Daniel Goulet,[2] Michel Bourdages,[3] Michel Plante,[4] and Lucie Germain[1]

[1]*Laboratoire d'Organogénèse Expérimentale/LOEX, Centre de Recherche (FRSQ) du CHA Universitaire de Québec and Département de Chirurgie, Université Laval, Québec, Canada.*
[2]*Hydro-Québec/Transénergie, division d'Hydro-Québec, Montréal, Québec, Canada.*
[3]*Institut de Recherche Hydro-Québec (IREQ), Varennes, Québec, Canada.*
[4]*Direction Santé et Sécurité, Hydro-Québec, Montréal, Quebec, Canada.*

Véronique J. Moulin

Submitted for publication August 10, 2011.
*Correspondence: LOEX, aile R, Centre hospitalier affilié universitaire de Québec 1401, 18e rue, Québec G1J 1Z4, Canada (e-mail: veronique.moulin@chg.ulaval.ca).

Background: After human epidermis wounding, transepithelial potential (TEP) present in nonlesional epidermis decreases and induces an endogenous direct current epithelial electric field (EEF) that could be implicated in the wound re-epithelialization. Some studies suggest that exogenous electric stimulation of wounds can stimulate healing, although the mechanisms remain to be determined.
The Problem: Little is known concerning the exact action of the EEF during healing. The mechanism responsible for TEP and EEF is unknown due to the lack of an *in vitro* model to study this phenomenon.
Basic Science Advances: We carried out studies by using a wound created in a human tissue-engineered skin and determined that TEP undergoes ascending and decreasing phases during the epithelium formation. The *in vitro* TEP measurements over time in the wound were corroborated with histological changes and with *in vivo* TEP variations during porcine skin wound healing. The expression of a crucial element implicated in Na^+ transport, Na^+/K^+ ATPase pumps, was also evaluated at the same time points during the re-epithelialization process. The ascending and decreasing TEP values were correlated with changes in the expression of these pumps. The distribution of Na^+/K^+ ATPase pumps also varied according to epidermal differentiation. Further, inhibition of the pump activity induced a significant decrease of the TEP and of the re-epithelization rate.
Clinical Care Relevance: A better comprehension of the role of EEF could have important future medical applications regarding the treatment of chronic wound healing.
Conclusion: This study brings a new perspective to understand the formation and restoration of TEP during the cutaneous wound healing process.

Abbreviations and Acronyms
ATP = adenosine tri phosphate
EEF = epithelial electric field
K^+ = potassium ion
Na^+ = sodium ion
TEP = transepithelial potential
TES = tissue-engineered skin

BACKGROUND

The epidermis of mammals has a transepithelial potential (TEP) that results from an uneven distribution of sodium ions through the skin, which causes a potential difference varying from 10 to 60 mV.[1] Although TEP is known for the past >150 years,[2] a complete understanding of this phenomenon is still lacking. When the skin is damaged, Na^+ gradient is broken, and the TEP is

DOI: 10.1089/wound.2011.0318

disrupted. While approaching the lesion site, the TEP decreases, and the electric field is enhanced. The electric current (the movement of positive charges) is oriented toward the center of the wound, from the intact skin to the edges of the wound. The current forms a loop by returning to the normal epidermis.[3] The cells in the intact part will then continue the ion transport to the basal layer of the epidermis to maintain the TEP. The strength of the electric field gradually decreases over time until the wound is covered with epithelial cells.[4] The presence of epithelial electric field (EEF) may be an important factor in skin healing,[5,6] but its mechanism of action remains unknown.

TARGET ARTICLE

Dubé J, Rochette-Drouin O, Levesque P, Gauvin R, Roberge CJ, Auger FA, Goulet D, Bourdages M, Plante M, Germain L, and Moulin VJ: Restoration of the transepithelial potential within tissue-engineered human skin *in vitro* and during the wound healing process *in vivo*. Tissue Eng 2010; **16:** 3055.

CLINICAL PROBLEM ADDRESSED

Nonhealing skin wounds are a major clinical problem, and many new therapies try to address this challenge. Stimulation of wound repair with electricity, known as electrotherapy, is used in several countries and is based on either the knowledge that an EEF is present in the wounds or on the hypothesis that EEF play a role during re-epithelialization. The clinical results of an electrotherapy can be spectacular, but can also be unsuccessful.[7–9] These differences may be explained by the fact that protocols are empirical, which prevents any prognostic of treatment. A better comprehension of the effect of endogenous electrical phenomenon on the homeostasis of skin and wound healing will be crucial to develop adequate clinical protocols using electric stimulation.

RELEVANT BASIC SCIENCE CONTEXT

Several studies carried out on cell monolayers have shown that the exposition of cells to electric fields of physiologic intensity affects cell migration, protein synthesis, cell orientation, protein distribution, and activation.[10–13] Researchers hypothesized that the transient EEF generated after wounding can similarly stimulate keratinocytes *in vivo* and can improve re-epithelialization.

In epithelial tissues, the origin of the TEP is linked to the transport of sodium through the epidermis. This transport is accomplished by both the action of the sodium channels located on the top side of the cells and the Na^+/K^+ ATPase pumps localized at the bottom of the cell.[11] From the cellular point of view, the Na^+ ions enter into the cells by the sodium channels and are then extruded by the Na^+/K^+ ATPase pumps to contribute to the maintenance of a low Na^+ level within the cell. The differential transport favors an ionic gradient with an ion concentration larger in the basal layer of the epidermis than in the upper layers of the epidermis. After wounding, the TEP decreases in the epidermis surrounding the wound.[4,14] When skin regeneration is completed, TEP is restituted. However, the mechanism responsible for its restoration is unknown.

EXPERIMENTAL MODEL OF MATERIAL: ADVANTAGES AND LIMITATIONS

In vitro wound healing model

To determine the variations in TEP during the restoration of the epidermis during wound healing, we carried out studies by using a human tissue-engineered skin (TES) model developed in our laboratory.[15,16] *In vitro* reconstituted skin was wounded by using a 6 mm punch biopsy and placed over a dermal sheet to allow physical support for keratinocyte migration that was followed during the next 14 days. The re-epithelialized surface was photographed every day until the final biopsy. Samples of wounded TES were processed for histology, staining, and immunofluorescence techniques. The advantage of this *in vitro* model is the use of human cells and the set up for the study of TEP under sterile conditions over several days. Moreover, the same cell populations can be used to reconstruct numerous TES; thus, the reproductibility of the model is very high. Although the major limitation is the absence of several other epidermal cells such as Langherans cells or immune cells, our results show that TEP obtained using only keratinocytes is very similar to those obtained *in vitro*.

In vivo wound healing model

Six wounds of $6\,mm \times 2.5\,mm$ were created on the back of three pigs. For each wound, three measurements of TEP were recorded at every other day over a week.

Measurement of TEP

TEP measurement was adapted from Barker *et al.*[4] Two mobile electrodes were prepared by using silicon tubing and 2% w/v of agar in buffered solution. Each measurement was performed with the positive electrode placed inside the culture

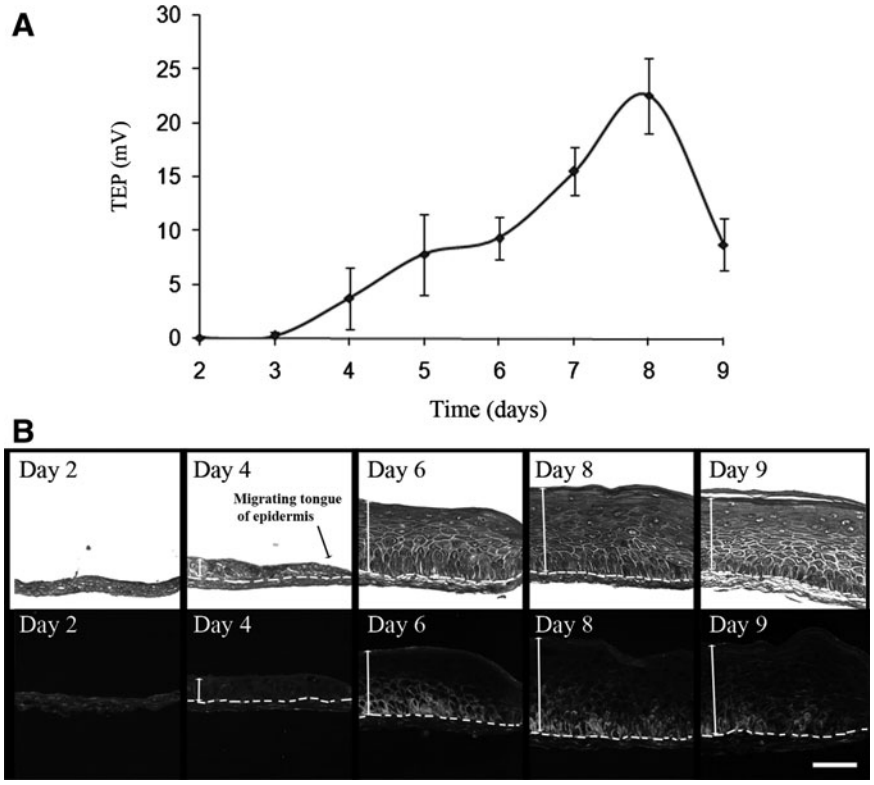


Figure 1. Re-epithelialization of TES after wounding. **(A)** Measurement of TEP according to time during re-epithelialization of wound on TES. **(B)** Masson's trichrome staining (*top*) and indirect immunofluorescence (*bottom*) for the detection of the Na^+/K^+ ATPase pumps carried out on the center of the wounded TES at day 2, 4, 6, 8, and 9 after wounding; bar 100 μm. White arrows: epidermis, white dotted line: junction between epidermis and dermis. Reprinted by permission from Dubé *et al.*[17] TES, tissue-engineered skin; TEP, transepithelial potential.

medium and the negative electrode installed at specific points on the top of the wound.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

TEP variations during *in vitro* and *in vivo* re-epithelialization of the epidermis

The re-epithelialization of the wound created on TES was followed over time using histology (Fig. 1). At day 2, migrating epithelial cells had not reached the center of the wound and only the fibroblast sheet, added as a substrate for migration, was observable in the middle. During the next 2 days, keratinocytes progressed toward the center of the wound, where a thin layer of epithelial cells was observable at day 4. Six days after creation of the wound, all the layers of the pluristratified epidermis were observed. Measurements of TEP in the wound showed a gradual increase of TEP values according to time during the whole progression of the wound closure and can be correlated with the progressive differentiation of the epidermis. The maximum value (22.6 ± 3.4 mV) was reached at day 8 when the stratum corneum appeared. A rapid TEP decrease to 8.7 ± 2.4 mV was observed at day 9, probably related to the increase of the stratum corneum thickness, increasing electrical resistivity, and, thus, reducing the measured TEP. These results suggest that an ionic polarity remains in the

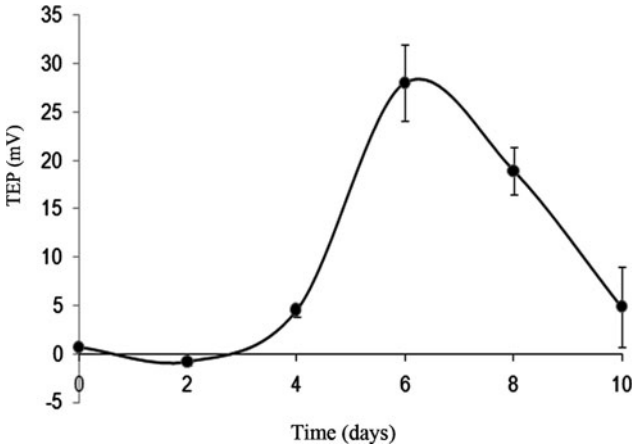


Figure 2. Measurements of TEP according to time during re-epithelialization of the skin wounds in pigs. Curve represents the average of TEP for three different wounds per pig and carried out on three different pigs. This result is representative of two independent experiments including three pigs in each. Reprinted by permission from Dubé *et al.*[17]

mature epidermis of TES and that this model can be used to study TEP formation and modulation. The maximal TEP values measured on TES during the re-epithelialization process reached 30 mV *in vitro* and are in agreement with *in vivo* measures on human skin previously reported by others.[1]

In wounds created on pig's skin, the TEP increased after wound closure followed by a fast decrease as observed with TES (Fig. 2). The maximum TEP was reached at day 6 after wounding and reached values between 25 and 40 mV for different wounds.

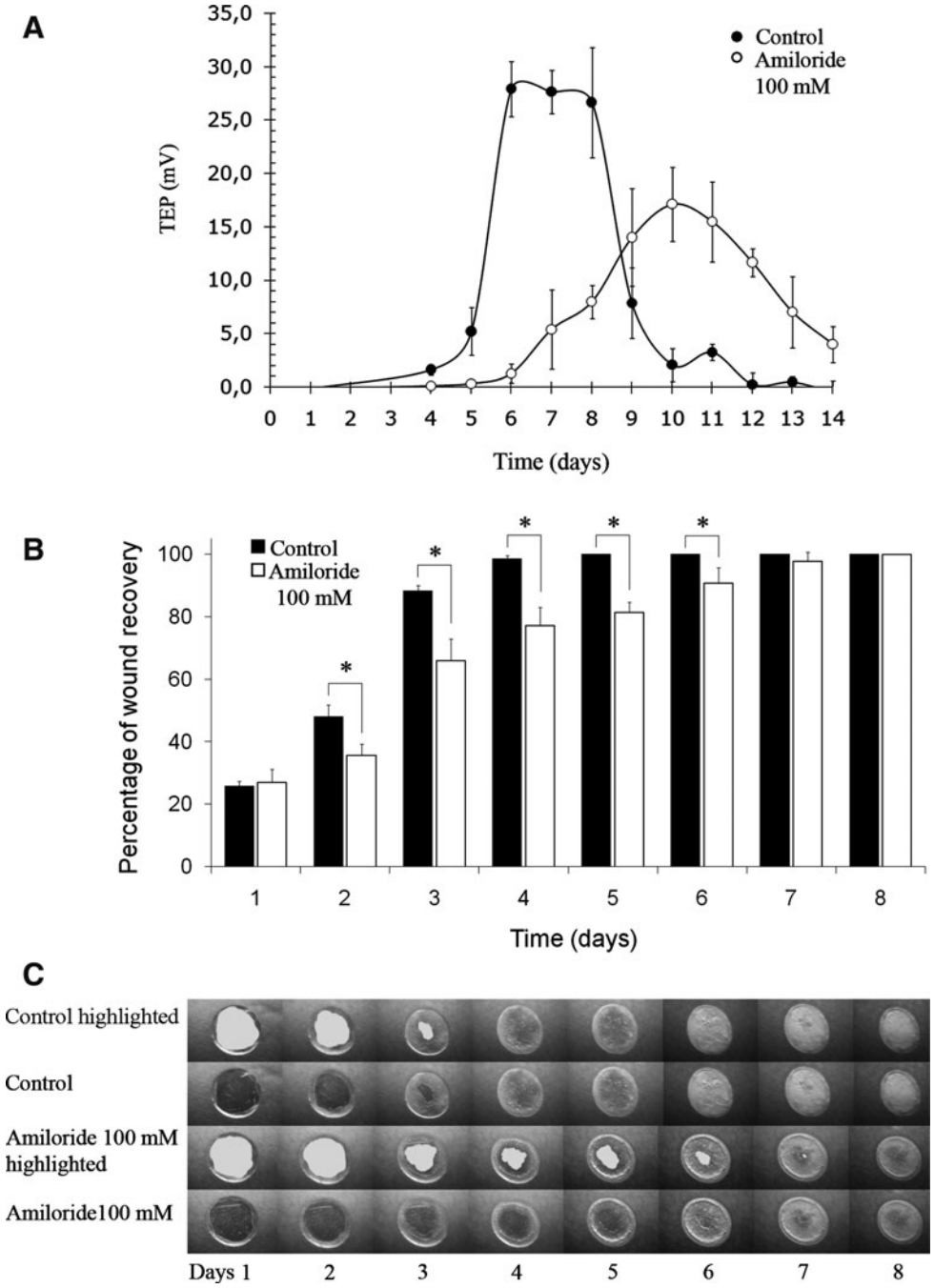


Figure 3. Measurement of TEP and the rate of re-epithelialization of the wound over time in the presence or absence of cationic transport blocker (amiloride). **(A)** Measurement of TEP according to time during the re-epithelialization of a wound; control (●), amiloride 100 μM (○). **(B)** Percentage of the surface of the wound that is re-epithelialized. Control (black), amiloride 100 μM (white). *Statistical difference between control and amiloride treatment ($p < 0.05$ using Student's test). **(C)** Macroscopic pictures of the migrating epidermal tongue according to time with amiloride 100 μM or not (control). The clear color was added on pictures to highlight non-re-epithelialized part of the wounds in different conditions. Reprinted by permission from Dubé *et al.*[17]

Action of TEP on re-epithelialization

Detection of the Na^+/K^+ ATPase pump was carried out on biopsies obtained from wounded TES for each day of TEP measurement during wound healing. Our results showed that the Na^+/K^+ ATPase pumps were re-expressed after wound closure with a staining that strongly intensify between day 4 and 6 followed by a weak reduction at day 9. The presence of a bottom-up gradient of expression of the Na^+/K^+ ATPase pumps decreasing from the basal layer of the epidermis toward the top was also detected in the restored epidermis (Fig. 1).

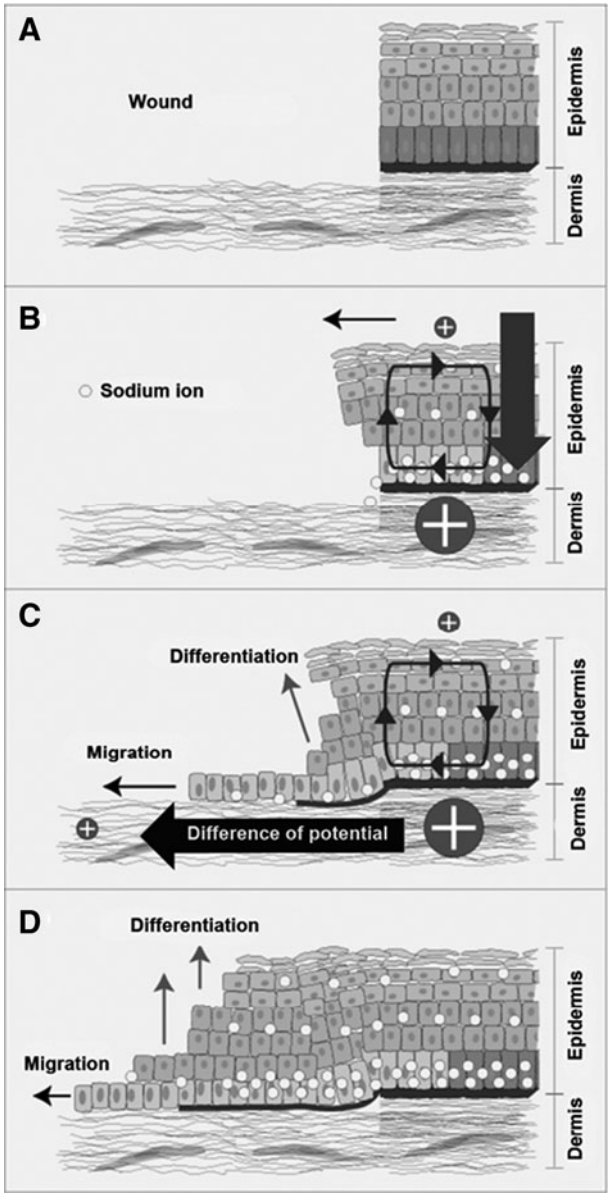
Amiloride is a blocker of cationic (including Na^+ and K^+) transport. Its action on the TEP cycle observed during the re-epithelialization of a wound was analyzed. The presence of amiloride induced a delay in the TEP increase and caused a 3-day shift in the curve when compared with the control (Fig. 3). Moreover, the TEP increase was slower in the presence of amiloride (days 7 to 14) when compared with the control (days 5 to 9). The percentage of wound re-epithelialization was calculated for each condition. When amiloride was added, a statistical delay of 4 days was noted for the closure of the wound compared with the control. The lack of effects of amiloride in a scratch test and a cell growth measurement (data not shown) carried out on monolayer cultured human keratinocytes excluded that amiloride may affect the speed of migration. Taken together, these results suggest that the delay induced by amiloride on re-epithelialization was mediated by disruption of the cationic transport which was present in the epidermis but not on cells cultured in the monolayer. This provides evidence that the cationic transport influences the re-epithelialization process during skin wound healing.

INNOVATION

This study showed for the first time that TEP can be detected *in vitro* by using an *in vitro* reconstructed human skin. Further, we have demonstrated that TEP was restored during re-epithelialization. TEP development seems to be linked to the ionic active transport that is correlated with the differentiation status of epithelial cells and their Na^+/K^+ ATPase pump expression. A better understanding of the effect of endogenous electrical phenomenon on skin homeostasis and wound healing will be crucial for the development of adequate clinical protocols using electric stimulation.

SUMMARY ILLUSTRATION

Schematic model of the wound re-epithelialization including the concept of epithelial electric field (EEF). **(A)** The skin is damaged and completely removed with part of the dermis, creating a wound. A few minutes after the injury, the upper layers of the epidermis are moving to cover a portion of the edge of the wound, and the release of sodium ions create an EEF loop (white circles and thin arrows) **(B)**. The EEF activates keratinocytes of the basal layer and induces an increase in intracellular calcium. The poorly differentiated keratinocytes begin to migrate to the wound bed (surface dermal or granulation tissue). The EEF influences the orientation of the keratinocytes to promote the convergence of cells toward the center of the wound **(C)**. The accumulation of fluid (blood or water) and the leakage of



sodium ions into the center of the wound leads to a difference of potential parallel to the skin and amplifies the section of the EEF loop. When wound re-epithelialization progresses, the maturation of the epidermis takes place behind the migration front **(D)**. Suprabasal layers of cells are derived from undifferentiated cells that have migrated previously in the wound. Once the wound area is covered by epithelial cells, the different layers of the epidermis are restored and trans-epithelial potential is restored.

CAUTION, CRITICAL REMARKS, AND RECOMMENDATIONS

The evidence supports effectiveness of electrotherapy, but results highly depend on the skill of the practitioner. When used badly, this treatment is a waste of time and money. Normal skin healing is a complex process with numerous players (keratinocytes, growth factors, matrix …). TEP and the appearance of EEF after wounding are only one of the several parameters that can play a role during healing. The recovering of a nonhealing wound by epidermis could, thus, be obtained by combining all these parameters together to improve the re-epithelialization rate by epidermal cells. As a result, the parameters of electrotherapy have to be better defined and standardized in the pathological context of nonhealing of wounds. Further research into TEP and EEF is, thus, needed to better guide clinicians in their practice when using electrotherapy.

FUTURE DEVELOPMENT OF INTEREST

Numerous results demonstrate that electrotherapy can stimulate *in vivo* healing but without a possibility to tip the result. An *in vitro* demonstration of a positive effect of electrotherapy on a reconstructed skin will increase the possibility of studying the parameters necessary to stimulate *in vivo* re-epithelialization.

TAKE-HOME MESSAGE

Basic science advances

The action of an electrical stimulation on cells is usually studied by using cells cultured in a monolayer. This model impedes any demonstration of the EEF action that needs several differentiated layers of epithelial cells. Before our demonstration, the only means to study EEF action was by using animal models. We have demonstrated that human skin reconstructed *in vitro* by using a tissue-engineering method has similar TEP than *in vivo* skin and, thus, this model can be used to analyze EEF phenomenon. Using this model, we have shown the role of Na^+/K^+ ATPase pumps during re-epithelialization of skin wounds. Further, we have determined that the modulation of TEP using a pharmacological agent can modulate the re-epithelialization rate.

Clinical science advances

Before using electrotherapy to treat nonhealing wounds in patients, a better demonstration of the effects of the various parameters have to be done. We have demonstrated that the *in vitro* reconstructed human skin developed in our laboratory is a good model to understand the mechanisms related to the action of TEP on re-epithelialization.

Relevance to clinical care

Management of nonhealing wounds represents a major challenge. Electrotherapy should provide a valuable mean to stimulate re-epithelialization to close wounds. However, a better definition of the treatment parameters (type of electric field, intensity, time of treatment, …) has to be done, and parameters have to be defined. Comprehension of the mechanisms at the origin of the TEP will help to do this.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This work was supported by grants from Hydro-Québec and the Canadian Institutes of Health Research (CIHR). Lucie Germain holds a Canadian Research Chair on Stem Cells and Tissue Engineering from CIHR. Véronique Moulin was supported by a scholarship from the Fonds de Recherche en Santé du Québec (FRSQ). Jean Dubé was supported by studentships from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQNRT).

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. No ghostwriters were used to write this article.

REFERENCES

1. Foulds IS and Barker AT: Human skin battery potentials and their possible role in wound healing. Br J Dermatol 1983; **109:** 515.
2. DuBois Reymond E: Vorla ä ufiger Abriss einer Untersuchung uber den sogenannten Froschstrom und die electomotorischen Fische. Ann Phy U Chem 1843; **58:** 1.
3. Nuccitelli R: Endogenous ionic currents and DC electric fields in multicellular animal tissues. Bioelectromagnetics 1992; **13 Suppl 1:** 147.
4. Barker AT, Jaffe LF, and Vanable JW, Jr.: The glabrous epidermis of cavies contains a powerful battery. Am J Physiol 1982; **242:** R358.
5. Altizer AM, Stewart SG, Albertson BK, and Borgens RB: Skin flaps inhibit both the current of injury at the amputation surface and regeneration of that limb in newts. J Exp Zool 2002; **293:** 467.
6. Jenkins LS, Duerstock BS, and Borgens RB: Reduction of the current of injury leaving the

amputation inhibits limb regeneration in the red spotted newt. Dev Biol 1996; **178:** 251.

7. Carley PJ and Wainapel SF: Electrotherapy for acceleration of wound healing: low intensity direct current. Arch Phys Med Rehabil 1985; **6:** 443.
8. Wolcott LE, Wheeler PC, Hardwicke HM, and Rowley BA: Accelerated healing of skin ulcer by electrotherapy: preliminary clinical results. South Med J 1969; **62:** 795.
9. Gault WR and Gatens PF, Jr.: Use of low intensity direct current in management of ischemic skin ulcers. Phys Ther 1976; **56:** 265.
10. Kloth LC: Electrical stimulation for wound healing: a review of evidence from *in vitro* studies, animal experiments, and clinical trials. Int J Low Extrem Wounds 2005; **4:** 23.
11. Nuccitelli R: A role for endogenous electric fields in wound healing. Curr Top Dev Biol 2003; **58:** 1.
12. McCaig CD, Rajnicek AM, Song B, and Zhao M: Controlling cell behavior electrically: current views and future potential. Physiol Rev 2005; **85:** 943.
13. Zhao M, Song B, Pu J, Wada T, Reid B, *et al.*: Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature 2006; **442:** 457.
14. Vanable JW: *Integumentary Potentials and Wound Healing.* New York: Alan R. Liss, Inc., 1989, pp. 171–224.
15. Michel M, L'Heureux N, Pouliot R, Xu W, Auger FA, and Germain L: Characterization of a new tissue-engineered human skin equivalent with hair. In Vitro Cell Dev Biol Anim 1999; **35:** 318.
16. Laplante AF, Germain L, Auger FA, and Moulin V: Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. FASEB J 2001; **15:** 2377.
17. Dubé J, Rochette-Drouin O, Levesque P, Gauvin R, Roberge CJ, Auger FA, Goulet D, Bourdages M, Plante M, Germain L, and Moulin VJ: Restoration of the transepithelial potential within tissue engineered human skin *in vitro* and during the wound Healing process *in vivo*. Tissue Eng 2010; **16:** 3055.