1 Wound Healing

1.1 The Biology of Wound Healing

Wound healing generally goes through the following consecutive phases, with the predominant cells and the growth factors they secrete, changing as wound healing progresses:

Phase	Effects	Cells	Growth Factors
Inflammatory	Phagocytosis Debridement	Platelets Neutrophils Macrophages	TGF-ß PDGF bFGF
Proliferative	Granulation tissue Angiogenesis	Fibroblasts Endothelial cells	bFGF VEGF
Maturation	Collagen production Epithelialization Wound contraction	Fibroblasts Keratinocytes Myofibroblasts	IGF-1 KGF

Table 1 TGF-ß = tissue growth factor beta
PDGF = platelet derived growth factor
bFGF = basic fibroblast growth factor
VEGF = vascular endothelial growth factor
IGF-1 = insulin like growth factor
KGF = keratinocyte growth factor

The table above is useful in terms of a broad understanding of wound healing, although it oversimplifies a complex biological process. The diagrams below better illustrates

1. the relative importance of different cell types over time

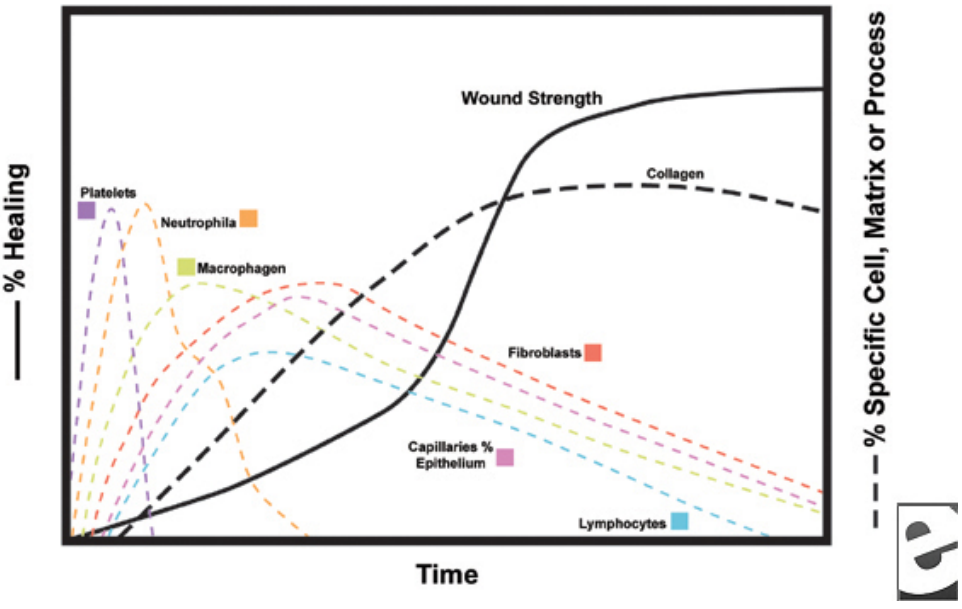

Fig 1 From Torre et al, Wound healing, chronic woun eMedicine, 2008

2. the complexity and interactions between cells, growth factors and stage of healing

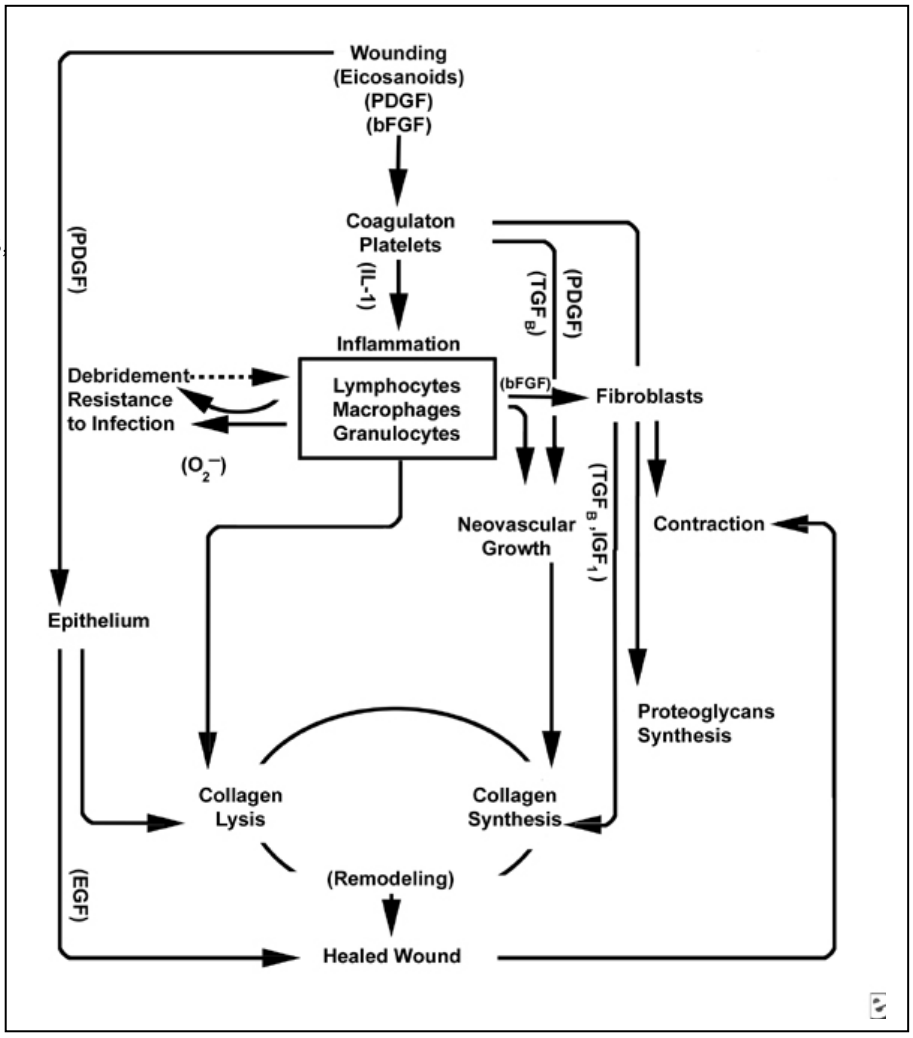

Fig 2 From Torre et al, Wound healing, chronic wounds, eMedicine, 2008

References

The following two articles provide concise and up to date overviews of the wound healing process.

1. Torre et al, Wound healing, chronic wounds, eMedicine, 2008

2. Robert F. Diegelmann [1], and Melissa C. Evans Frontiers in Bioscience 9, 283-289, January 1, 2004

1.2 The Skin Battery

Measurable transepithelial potentials (TEPs) have been found in the intact skin of mammals, including humans. These voltages occur as a result of Na+ channels in the apical membrane of the skin's cells that allow extracellular Na+ to diffuse to the inside of epidermal cells .

Schematic representation of the generation of a transepithelial potential (TEP) in human skin. Selective, directional ion transport across the intact epithelium gives rise to a TEP that can be measured directly across the epithelium (70 mV in this case).

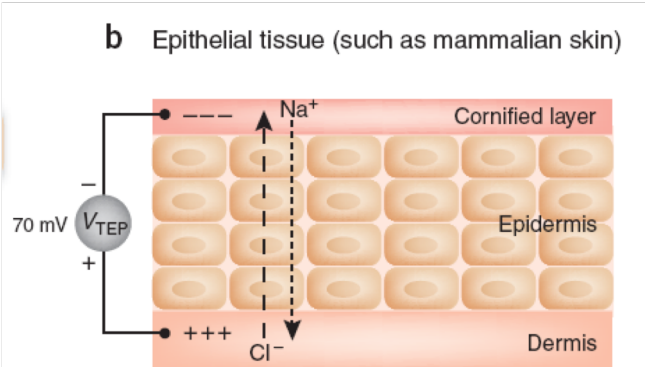

Fig 3 From Vanhaesebroeck B,
TEPs of human skin have been measured all over the body with values ranging from 10mV to 60 mV. This skin battery voltage effect is primarily produced by electrical activity in exocrine sweat glands.

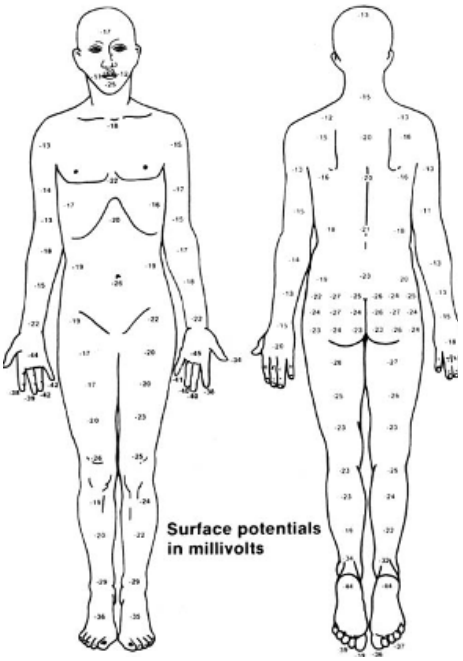

Fig 4 From Foulds L, Barker A.
Human skin battery potentials and their possible role in wound healing. Br J Dermatol. 1983.

References

3. Vanhaesebroeck B, Charging the batteries to heal wounds through PI3K, Nature Chemical Biology. 2006;2:453-455.

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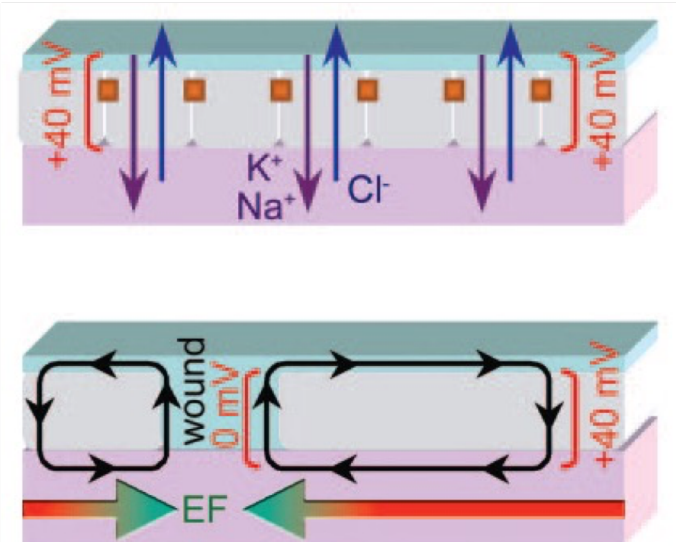
1.3 Current of Injury

When human skin is cut or wounded, the normal transepithelial potential difference is short-circuited, inducing current to flow out of the lesion from underneath the wounded epithelium and giving rise to a steady electric field at the wound edge.



Tight junctions between epithelial cells (red squares) create physical connections between cells, providing high electrical resistance and so maintaining the normal TEP in intact skin.

Inward transport of Na+ also results in a substantial TEP, which establishes an injury current (black arrows) in a wound and an electric field in the subepithelial tissues (horizontal arrow).

Fig 4 From McGaig CD Controlling Cell Behavior Electrically: Current Views and Future Potential. Physiol Rev, 2005

The term 'Current of Injury' has been introduced in 1960 by a US physician, Dr Becker. These injury potentials were discovered, however, as long ago as 1843 by the German physiologist Emil Du-Bois Reymond (the founder of modern electrophysiology), who used a galvanometer to measure microcurrent flowing out of a cut in his own finger.

Further clinical evidence was provided more recently when up to 35 µA/cm2 were recorded from the amputated fingers of children.

References

5. McCaig CD et al. Controlling Cell Behavior Electrically: Current Views and Future Potential Physiol Rev 85:943-978, 2005.

6. Illingworth CM et al. Measurement of electrical currents emerging during the regeneration of amputated finger tips in children Clin. Phys. Physiol. Meas 1 87-89, 1980.

1.4 BioElectric signals orchestrate wound healing

Further research on this endogenous bioelectric current in wounds revealed some fascinating and clinically important aspects:

A. the endogenous current rapidly falls away within a few millimeters from the wound edge. In effect it is limited to the wound surface area.

B. the endogenous electric field persists until the migrating epithelium reseals the wound, and establishes the normal high resistance, when the current drops to zero.

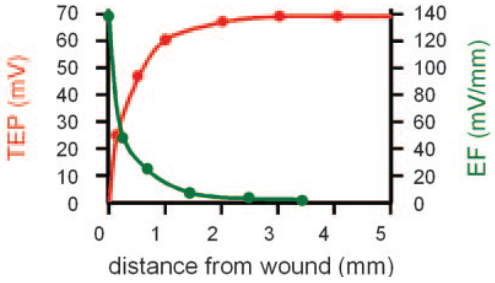


Fig 5 Redrawn from Vanable JW Electric Fields in Vertebrate Repair . 1989

This in turn means all cellular activities in a wound take place within a gradient of voltage, and as stated later in this document, explains why bioelectric signals have such a diverse and wide-ranging effect on the many different cells and growth factors responsible for wound healing.

Some activities directly controlled by bioelectric signals are cellular migration (macrophages, fibroblasts, keratinocytes) , fibroblast activation and proliferation and neo-angiogenesis.

C. the endogenous current ceases when the wound becomes dry, but is maintained when moist dressings are used. This would explain in part why occlusive dressings work : they allow bioelectrical conduction to continue, and so support the regeneration process.

1.5 Bioelectric signals activate specific genes and pathways

Another important chapter in the bioelectric wound healing story was completed recently when researchers discovered its molecular biology secret - which signaling pathways and genes are responsible for kick starting this amazing biological phenomenon. In a recent Nature publication, Zhao et al identified genes that are essential for electrical-signal-induced wound healing and also showed which genes (PI(3)K γ and PTEN) control the migration of cells under the influence of bioelectric signals.

References

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8. Cheng K,Tarjan P, Oliveira-GandiaM, et al. An occlusive dressing can sustain natural electrical potential of wounds. J Invest Dermatol 104(4):662-5, 1995.

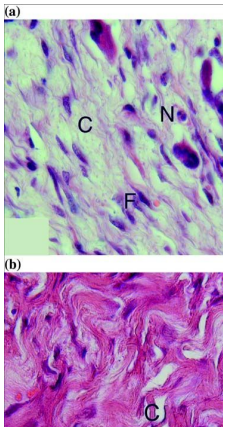
9. Zhao M, Song B, Pu J et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase- γ and PTEN. Nature 442, 457-460.

1.6 Stimulation of fibroblasts

The fibroblast cell plays a crucial role in wound healing, particularly in the proliferative and maturing phases. It not only produces the new matrix needed to restore structure and function to injured tissue, but also collagen to provide strength and stability. In addition, fibroblasts are co-responsible for neovascularization through the secretion of bFGF (basic Fibroblast Growth Factor).
Several different effects of M.E.T. (Microcurrent Electro Therapy) on fibroblast function have been reported in the literature:

- In vitro
  - enhanced DNA and protein (collagen) synthesis,
    an increase in Ca^{++} uptake [Bourguignon 1987]
  - upregulation of TGF- ß receptors
    (important in granulation tissue formation) [Falanga 1987]

- In vivo
  - fibroblast proliferation [Taskan 97]
  - increased collagen deposition [Canseven 96]
  - improved collagen fiber alignment [Bayat 2006]
  - increased tensile strength [Taskan 97, Bayat 2006]



From Bayat 2006
b) more mature fibroblasts and connective tissue fibers after M.E.T. treatment

All in all, there is a clear picture : M.E.T. stimulates fibroblasts to proliferate and become metabolically active, with a resultant increase in their function, especially granulation tissue formation and collagen production.

Several authors reported on the senescence (early death) of fibroblasts as a significant factor in chronic or non-healing wounds.

References

10. Bourguignon GJ. Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. J Cell Physiol 140(2):379-85, 1989.

11. Falanga M, Song B, Pu J et al. Electrical stimulation increases expression of fibroblast receptors for TGF- ß. J Invest Dermatol 1987;88:488-92.

12. Taskan A comparative study of the effect of ultrasound and electrostimulation on wound healing in rats. Plast Reconstr Surg. 1997;100(4):966-72.

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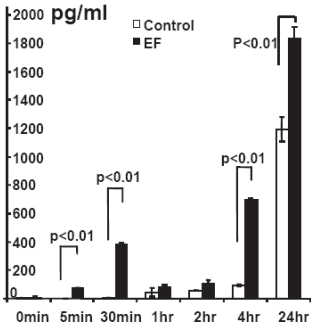
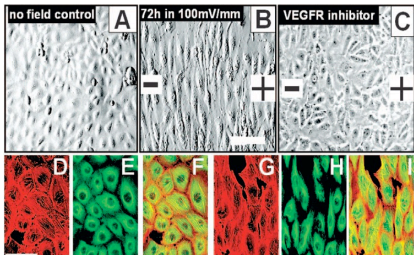
1.7 Angiogenesis

Neovascularization is a key event in wound healing, particularly during the proliferative phase. In vitro experiments have shown that M.E.T. induces a distinctive pre-angiogenic response by directing the movement of human endothelial cells, as well as fibroblast and vascular smooth muscle cells [Bai 2004].

In addition, M.E.T. stimulates VEGF production by endothelial cells, and this growth factor directly promotes neoangiogenesis, as elegantly demonstrated by Zhao [Zhao 2004]



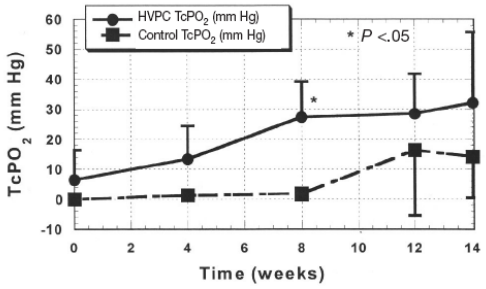
VEGF production
white = control
black = with electric field

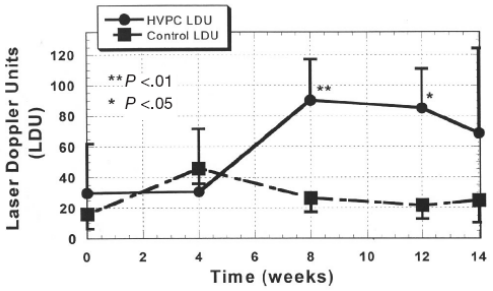


cultured endothelial cells
A = control
B= with EF, demonstrating early blood vessel formation
C = small EF but with VEGF inhibitor
G, H = EF causes elongation and reorientation of actin (red) and tubules (green)

These in vitro effects provide an explanation for what have been observed in animal studies. Greenberg, for example, reported prominent neovascularity in burn wounds and earlier epitheliazation [Greenberg 2000].

$TcPO_2$ (transcutaneous oxygen pressure) and laser Doppler flow [Goldman 2004].





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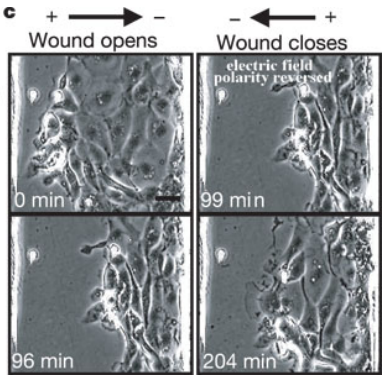
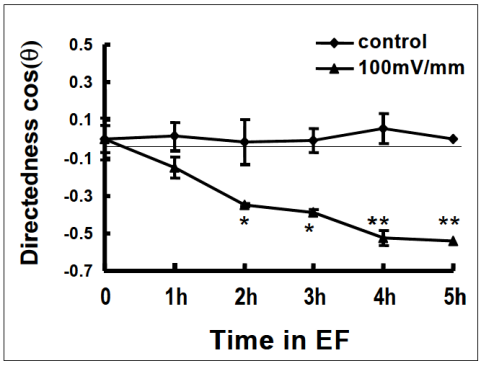
1.8 Cellular migration (electrotaxis)

The directed movement of cells is a key mechanism in wound healing, with different cells active in the different stages of wound healing.
** All the different cells depicted in this table have been shown to migrate in healing wounds under the influence of bioelectric signals [Kloth 2005]

Phase	Effects	Cells**
Inflammatory	Phagocytosis Debridement	Neutrophils Macrophages
Proliferative	Granulation tissue Angiogenesis	Fibroblasts Endothelial cells
Maturation	Collagen production Epithelialization Wound contraction	Fibroblasts Keratinocytes Myofibroblasts

In a recent Nature article, the mechanisms underlying electrotaxis have been elegantly explained [Zhao 2006]





Small electric fields (same magnitude as endogenous bioelectric current) cause asymmetric recruitment of key molecules responsible for activating cell movement

BioElectric signals direct cell migration in wound healing, as shown by movement of epithelial cells (left) and fibroblasts (right) [Zhao 2006].

In vivo studies have showed increased neutrophil counts in human skin exudates [Eberhardt 1986]. as well as faster epitheliazation [Mertz 1993].

In conclusion, bioelectric signals are responsible for the directed movement of different cells throughout the phases wound healing. It is also one of the explanations why externally applied current (M.E.T.) augments wound healing.

References

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1.9 Antibacterial effects

Several authors have reported inhibitory effects of M.E.T. on bacterial cell populations, as the summary below shows:

Reference	Study	Pathogens	Polarity	Effect
Szuminsky et al, 1994	In vitro	Staph aureus Pseudomonas aeruginosa	Both poles	Bacteriostatic
Thibodeau et al, 1978	In vivo	Oral bacteria	Anode	Bacteriostatic
Laatsch et al, 1995	In vitro	Gram positive bacteria	Both poles	Bacteriostatic
Ong et al, 1994	In vitro	Staph aureus Pseudomonas aeruginosa	Anode	Bacteriostatic
Barranco et al, 1974	In vitro	Staph aureus	Anode	Bacteriostatic

Most publications refer to in vitro studies, whilst definitive clinical evidence is still lacking.
Conclusion : In addition to its direct wound healing effects, M.E.T. can also play a useful antibacterial role. It should, however, NOT be seen as a replacement for standard therapy such as antibiotic treatment.

References

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24. Laatsch L et al. In vitro effects of silver electrodes on select wound pathogens. J Clin Electrophysiol 1995;7(1):10-5.

25. Ong P et al. Antibacterial effects of microamperage direct current in vitro. J Clin Electrophysiol 1994;6(1):14-8.

26. Barranco S, Spadero J, Berger T, et al. In vitro effect of weak direct current on Staphylococcus aureus.ClinOrthop 1974;100:250-5.

Summary of M.E.T.'s Mechanisms of Action in Wound Healing				
stage	1^0 mediators	Cellular Migration	Angiogenesis	Fibroblast Activation
Inflammatory	TGF-ß PDGF	Neutrophils Macrophages		
Proliferative	bFGF VEGF	Fibroblasts Endothelial cells	Fibroblasts Endothelial cells	Fibroblasts
Maturation	IGF-1 KGF	Fibroblasts Epithelial cells		Fibroblasts Myofibroblasts

TGF-ß = tissue growth factor beta
PDGF = platelet derived growth factor
bFGF = basic fibroblast growth factor
VEGF = vascular endothelial growth factor
IGF-1 = insulin like growth factor
KGF = keratinocyte growth factor