Available online at www.sciencedirect.com

ScienceDirect

Biomedical Journal

journal homepage: www.elsevier.com/locate/bj

Original Article

Quantifying cell behaviors in negative-pressure induced monolayer cell movement

Shu-Er Chow [a], Carl Pai-Chu Chen [b], Chih-Chin Hsu [c,d,*], Wen-Chung Tsai [e], Jong-Shyan Wang [f], Ning-Chun Hsu [c]

[a] Department of Nature Science, Center for General Studies, Chang Gung University, Taoyuan, Taiwan
[b] Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Taipei, Taipei, Taiwan
[c] Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Keelung, Keelung, Taiwan
[d] School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[e] Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan
[f] Institute of Rehabilitation Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Received 12 April 2015
Accepted 17 August 2015
Available online 28 March 2016

Keywords:
Cell movement
Cytoskeleton
Intercellular junctions
Negative-pressure wound therapy
Wound healing

ABSTRACT

Background: Negative-pressure of 125 mmHg (NP) has been shown to accelerate wound healing. Effects of NP on human keratinocyte behaviors during wound healing process were highlighted in this study.

Methods: An NP incubator incorporating the electric cell–substrate impedance sensing (ECIS) technique has been built to quantify monolayer keratinocytes movement in serum-free media at the ambient pressure (AP) and NP for 12 h. Monolayer cell motions were continuously recorded by ECIS in the frequency range of 22.5–64 kHz. Membrane capacitance (C_m), cell–substratum resistance (α), and cell–cell junction resistance (R_b) were evaluated in cells at the different pressures.

Results: A greater monolayer cell migration distance was found in cells at NP. Decreased cell–substratum adhesion reflected in the significantly low α (AP:NP = ~5 $\Omega^{0.5}$:~3 $\Omega^{0.5}$·cm), decreased integrin expression, and increased cell–substratum distance were seen in cells at NP. A significantly increased C_m (AP:NP = ~4:~8 $\mu F/cm^2$) in association with increased membrane ruffling and microtubule filaments were observed early in the monolayer cell movement at NP. A progressive drop in the R_b from 1.2 $\Omega \cdot cm^2$ to 0.8 $\Omega \cdot cm^2$ corresponding to the gradually decreased E-cadherin expressions were observed 6 h after wound closure after NP treatment.

Conclusion: A quick membrane ruffling formation, an early cell–substratum separation, and an ensuing decrease in the cellular interaction occur in cells at NP. These specific monolayer cell behaviors at NP have been quantified and possibly accelerate wound healing.

* *Corresponding author*. Department of Physical Medicine and Rehabilitation, Chang Gung Memorial Hospital at Keelung, 222, Maijin Rd., Keelung, Taiwan. Tel.: +886 2 24329292 ext. 2725; fax: +886 2 27933328.
E-mail address: steele0618@gmail.com (C.-C. Hsu).

Peer review under responsibility of Chang Gung University.
http://dx.doi.org/10.1016/j.bj.2015.08.005

At a glance commentary

Scientific background on the subject

We have scientifically documented morphological adaptations, including increased membrane ruffling, increased cell–substratum separation, and decreased cell–cell adhesion, in keratinocytes at a negative-pressure of 125 mmHg (NP). Cytoskeleton re-organizations, altered adherens junction, and integrin proteins may play important roles in NP-induced accelerated wound healing process.

What this study adds to the field

We have established a wound healing model with better O_2 and CO_2 tension controls for observing cell behaviors at NP. The results will contribute to the understanding of cell behaviors in NP, aid in refining the contemporary treatment modality, and encourage the development of new facilities in the future.

Changing environmental pressures have been applied to preserve food and treat human diseases in modern science. Hypobaric storage significantly inhibits the respiratory intensity and extends the storage life by up to 50 days for green asparagus [1]. Hyperbaric oxygen (HBO) therapy is defined as a treatment in which patients intermittently breathe 100% oxygen in a chamber of the pressure greater than sea level and is widely accepted as the treatment of chronic wounds [2]. In endothelial cells at the HBO environment for 5 h, 19 genes involved in adhesion, angiogenesis, inflammation, and oxidative stress were down-regulated, and only angiogenin gene expression was up-regulated [3].

In contrast to HBO therapy, negative-pressure wound therapy (NPWT), creating a negative-pressure gradient of 125 mmHg (NP) to accelerate wound healing, has gained popularity in current wound cares [4]. Basic sciences for this therapy have been proposed as creating a moist wound healing environment, enhancing angiogenesis at the wound bed, and reducing bacterial loads [5]. Decreased E-cadherin expression and enhanced cell locomotion have also been shown in several studies [5–9]. Although, all of the above studies have provided valuable information on the effects of NP on wound healing at tissue, cell, and even molecular levels, they have reported the end results rather than a process of the group cells migration.

Cutaneous wound healing is a dynamic biological process involved in keratinocytes re-epithelialization which is majorly associated with migration of keratinocytes at the wound edge. Keratinocytes migrate both individually and as a cellular sheet over the denuded dermis to form a new epidermis [10]. Therefore, keratinocytes become the target in the study. Monolayer cell movement, which refers to two or more cells moving together coupled by cell–cell junctions, is a well-orchestrated multi-steps process that is involved in cutaneous wound healing [11]. The cell group behavior retains a single cell migration cycle, including protrusions of the cell leading edge and disassembly of the cell rear adhesion, and preserve intercellular adhesions [12]. A monolayer epithelial cell model for cell locomotion in wound healing has shown that the force of the lamellipodia, the cell–stratum attachment, and the cell–cell adhesion are the primary interactions governing the monolayer movement [13]. The results were derived by the serial time-lapse images instead of uninterrupted recordings of cell layers at ambient pressure (AP). To observe cell movement of wounded keratinocytes in NPWT, an investigation which is capable of continuously monitoring monolayer cell movement without interruption of the applied NP is indicated.

Electric cell–substrate impedance sensing (ECIS) is a method of obtaining information regarding changes in cell motions and of cell morphology. The cell motion can be continuously recorded with high reproducibility and the resolution order for the cell motion is nanometers [14]. Cell parameters such as cell–substratum resistance (α), transmembrane capacitance (C_m), and intercellular resistance (R_b) of cell monolayers can be calculated by the developed cell–electrode model [15]. The doubling time of HaCaT cells is 21 h [16]. In order to focus on cell movement and eliminate the cell proliferation confounding factor, cells were treated with different pressures for 12 h [5]. Thus, effects of NP on cell proliferation can be neglected for the healing model of wounded monolayer keratinocytes in the study. Our hypothesis in this study was that compared with cells at AP, cells at NP for 12 h would display reduced cell–substratum, and cell–cell adhesions with a significant cell deformation. Quantification of cell behaviors at NP for 12 h is indicated to testify this hypothesis. The results will contribute to the understanding of cell behaviors in NP, aid in refining the contemporary treatment modality, and encourage the development of new facilities in the future.

Materials and methods

Cell culture

Human skin keratinocytes (HaCaT cell line), kindly provided by Dr. Weng-Hung Chung (Department of Dermatology, Chang Gung Memorial Hospital, Linkou, Taiwan), were cultured in DMEM/F12 (Sigma–Aldrich Corporation, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS) and 100 μg/mL streptomycin–penicillin.

Cell viability

Keratinocytes (3×10^4) were seeded in each well of two different eight-well cell culture clusters (Corning Incorporated, Corning, NY, USA) and were incubated in DMEM/F12 solution with 10% FBS overnight. The attached cells were then respectively placed at AP and the NP in a NPI (NPI1500, Linston Advanced Technology Corporation, Longtan, Taoyuan, Taiwan, China) for 12 h. The cells were washed with phosphate buffer saline (PBS) for 5 times to simulate the serum-free condition and were then incubated at AP and NP for 12 h. The relative cell viability (%) related to the cells incubated with 10% FBS at AP was calculated ($n = 8$ in each condition) as our previous protocol [5].