Research Techniques Made Simple: Analysis of Collective Cell Migration Using the Wound Healing Assay

Ayman Grada[1], Marta Otero-Vinas[1,2], Francisco Prieto-Castrillo[3], Zaidal Obagi[4] and Vincent Falanga[1,5]

Collective cell migration is a hallmark of wound repair, cancer invasion and metastasis, immune responses, angiogenesis, and embryonic morphogenesis. Wound healing is a complex cellular and biochemical process necessary to restore structurally damaged tissue. It involves dynamic interactions and crosstalk between various cell types, interaction with extracellular matrix molecules, and regulated production of soluble mediators and cytokines. In cutaneous wound healing, skin cells migrate from the wound edges into the wound to restore skin integrity. Analysis of cell migration in vitro is a useful assay to quantify alterations in cell migratory capacity in response to experimental manipulations. Although several methods exist to study cell migration (such as Boyden chamber assay, barrier assays, and microfluidics-based assays), in this short report we will explain the wound healing assay, also known as the "in vitro scratch assay" as a simple, versatile, and cost-effective method to study collective cell migration and wound healing.

Journal of Investigative Dermatology (2017) **137,** e11–e16; doi:10.1016/j.jid.2016.11.020

CME Activity Dates: January 19, 2017
Expiration Date: January 19, 2018
Estimated Time to Complete: 1 hour

Planning Committee/Speaker Disclosure: All authors, planning committee members, CME committee members and staff involved with this activity as content validation reviewers have no financial relationship(s) with commercial interests to disclose relative to the content of this CME activity.

Commercial Support Acknowledgment: This CME activity is supported by an educational grant from Lilly USA, LLC.

Description: This article, designed for dermatologists, residents, fellows, and related healthcare providers, seeks to reduce the growing divide between dermatology clinical practice and the basic science/current research methodologies on which many diagnostic and therapeutic advances are built.

Objectives: At the conclusion of this activity, learners should be better able to:

- Recognize the newest techniques in biomedical research.
- Describe how these techniques can be utilized and their limitations.
- Describe the potential impact of these techniques.

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[1]*Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts, USA;* [2]*Department of Systems Biology, The Tissue Repair and Regeneration Laboratory, Universitat de Vic–Universitat Central de Catalunya, Vic, Spain;* [3]*MIT Media Lab, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA;* [4]*College of Medicine and Life Sciences, University of Toledo, Toledo, Ohio, USA; and* [5]*Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts, USA*

Correspondence: Ayman Grada, Department of Dermatology, Boston University School of Medicine, 609 Albany Street, A600, Boston, Massachusetts 02118, USA. E-mail: Grada@bu.edu

Abbreviation: RWD, relative wound density

COLLECTIVE CELL MIGRATION

Cell migration is defined as the actual movement of individual cells, cell sheets, and clusters from one location to another. The term "cell motility" is often used interchangeably, but may technically imply a less coordinated and purposeful movement of cells. Two principal types of cell migration have been identified: single cell migration and collective cell migration. Depending on the cell type, cytoskeletal structure, and the context in which it is migrating, the cell can migrate in different morphological variants such as mesenchymal, amoeboid motility modes (Friedl and Wolf, 2003). Collective migration is

SUMMARY

Advantages:

- Relatively inexpensive and easy to perform.
- Allows observation of cell movement and morphology throughout the experiment.
- Testing conditions can be easily adjusted for different purposes.
- Creates a strong directional migratory response.
- Ability to coat assay surface with an appropriate extracellular matrix.
- Amenable to high throughput screening platforms.

Limitations:

- May not be suitable for studying specialized primary cells because a relatively large number of cells are required for the assay.
- Not suitable for chemotaxis studies or for nonadherent cells.
- Lack of standardization in its application makes it difficult to reproduce experiments.
- Scratching introduces mechanical injury to the cells, leading to release of cellular contents into the surroundings and potentially influencing the migration process.
- Cell proliferation may interfere with the measurement of cell migration. Therefore, suppression of proliferation is a recommended intervention.

the coordinated movement of a group of cells that maintain their intercellular connections and collective polarity. Depending on the anatomical and physiological context, collective migration can manifest as (i) two-dimensional locomotion across a tissue surface (also known as sheet migration) where cells migrate as flat monolayer sheets, such as epidermal keratinocytes during wound healing, or (ii) three-dimensional locomotion across a tissue scaffold where cells are organized as a network of multicellular strands (Friedl and Gilmour, 2009).

WOUND HEALING

There are four main phases in wound healing: coagulation, inflammation, migration-proliferation (including matrix deposition), and remodeling (Falanga, 2005). These phases do not represent distinct events, but rather overlap and are continuous. After tissue injury and under the influence of various growth factors and cytokines, keratinocytes at the rear of the wound margins may display a high proliferative activity. These cells then migrate forward onto the wound bed and help restore the epidermal barrier structure and function. This overall process involves cell migration, proliferation, and differentiation. In smaller wounds, the critical event is keratinocyte migration rather than proliferation (Falanga, 2005). Cell migration begins several hours after injury. Epidermal cells adjacent to the wound margin become polarized (driven by the actin cytoskeleton) and develop pseudopodium-like projections preferentially oriented outward, into the free space, and within 24 hours, the cells detach from the basal lamina and are ready for migration. Lamellipodial crawling refers to the pattern of motion that epidermal cells exhibit during migration (Ridley et al., 2003). Although we have predominantly referred to keratinocytes, one must recognize that studies of cell migration in skin processes and disease also involve other resident skin cells, including fibroblasts, microvascular endothelial cells, and melanocytes, among others.

IN VITRO WOUND HEALING ASSAY

Studying the collective migration of cells in a two-dimensional confluent monolayer in highly controlled in vitro conditions allows investigators to simulate and explore critical mechanisms of action involved in the process. A variation of this method that tracks the migration of individual cells has been described in the literature (Rodriguez et al., 2005). There is argument about whether the assay can be equated to an actual wound, which is obviously more complex, but the assay does allow modeling and testing of cell movement under well-defined conditions. This assay is suitable for cell types such as keratinocytes and skin fibroblasts that exhibit collective migration, also known as "sheet migration" (Bindschadler and McGrath, 2007). The technique involves making a linear thin scratch "wound" (creating a gap) in a confluent cell monolayer (Figure 1) and subsequently capturing at regular time intervals images of the cells filling the gap (Cory, 2011). One can then analyze the images to quantify migration. Live cell imaging using time-lapse microscopy allows recording of spatial and temporal information and allows for investigation of dynamic processes in living cells (Supplementary Movie S1 online). The measurements are generally taken for 24 hours in an attempt to limit the study to migration and minimize the contribution of cell proliferation to gap filling. However, the time frame should be adjusted according to the particular cell type to be studied. To further reduce the risk of cell proliferation confounding the study of migration, a low dose of the proliferation inhibitor mitomycin C can be used. Mitomycin C is an antitumor antibiotic that inhibits DNA synthesis. The dose needs to be carefully optimized to avoid toxic effects that may affect cell migration. Using low serum concentrations in cell medium (serum starvation) is the most common method to suppress cell proliferation in wound healing assays. However, the duration of serum starvation and the required serum concentrations need to be rigorously determined for each studied cell line. Serum starvation can elicit complex, unpredictable time-dependent, and cell-type-dependent effects (Pirkmajer and Chibalin, 2011).

APPLICATIONS OF THE WOUND HEALING ASSAY

- Quantitative and qualitative analysis of collective cell migration under different experimental conditions.
- Studying the effects of cell-matrix and cell-cell interactions on cell migration.
- High-throughput screening for genes involved in cancer cell migration (Simpson et al., 2008), small molecule screening (Yarrow et al., 2005), and drug discovery (Hulkower and Herber, 2011).