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Modeling keratinocyte wound healing dynamics: Cell–cell adhesion promotes sustained collective migration

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HIGHLIGHTS

- We model the mechanism underlying collective cellular migration.
- We propose two competing hypotheses for the impact of EGF on collective migration.
- The MAPK cascade stimulates migration via increasing the pulling of leader cells.
- EGF treatment implies linear relationship for time to wound closure vs. wound area.

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ABSTRACT

The *in vitro* migration of keratinocyte cell sheets displays behavioral and biochemical similarities to the *in vivo* wound healing response of keratinocytes in animal model systems. In both cases, ligand-dependent Epidermal Growth Factor Receptor (EGFR) activation is sufficient to elicit collective cell migration into the wound. Previous mathematical modeling studies of *in vitro* wound healing assays assume that physical connections between cells have a hindering effect on cell migration, but biological literature suggests a more complicated story. By combining mathematical modeling and experimental observations of collectively migrating sheets of keratinocytes, we investigate the role of cell–cell adhesion during *in vitro* keratinocyte wound healing assays. We develop and compare two nonlinear diffusion models of the wound healing process in which cell–cell adhesion either hinders or promotes migration. Both models can accurately fit the leading edge propagation of cell sheets during wound healing when using a time-dependent rate of cell–cell adhesion strength. The model that assumes a positive role of cell–cell adhesion on migration, however, is robust to changes in the leading edge definition and yields a qualitatively accurate density profile. Using RNAi for the critical adherens junction protein, α-catenin, we demonstrate that cell sheets with wild type cell–cell adhesion expression maintain migration into the wound longer than cell sheets with decreased cell–cell adhesion expression, which fails to exhibit collective migration. Our modeling and experimental data thus suggest that cell–cell adhesion promotes sustained migration as cells pull neighboring cells into the wound during wound healing.

1. Introduction

Collective cellular migration is a critical component of many biological processes, including embryo development (McLennan et al., 2012), tissue repair (Maini et al., 2004), and tumorigenesis (Alexander, 2005). A group of cells is considered to be migrating collectively when neighboring cells adopt similar migration directions. In many cases of collective migration, neighboring cells are physically linked through some form of cell–cell junction (Friedl and Gilmour, 2009). The exact role of these cell–cell junctions has not been identified in this process, nor is it clear how temporal regulation of these junctions may influence the migration behavior of a collectively migrating group of cells. To date, cell–cell adhesion is believed to act as a component for cell–cell coupling during epithelial migration (Ilina and Friedl, 2009), but has also been shown experimentally to affect cell migration both positively (Geisbrecht and Montell, 2002; Hazan et al., 2000) and negatively (Friedl and Gilmour, 2009; Hazan et al., 2000) in different situations. However, the majority of previous mathematical

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models of cell migration assume that cell–cell adhesion affects cell migration negatively (Alexander, 2005; Anguige and Schmeiser, 2009; Johnston et al., 2012). In this study, we investigate the role of cell–cell adhesion during *in vitro* keratinocyte wound healing assays.

During the re-epithelialization phase of wound healing in mammalian skin, the migration of both fibroblasts and keratinocytes is required to reestablish the physically disrupted barrier between the organism and the surrounding environment (Clark and Henson, 1995). In this process, collective migration of layers of keratinocytes that are physically linked through adherens junctions allows for the completion of wound healing and reformation of the epidermis (Ilina and Friedl, 2009). Adherens junctions are composed of E-cadherin, α- and β-catenin, and are bound to the actin cytoskeleton through the adaptor protein vinculin, which binds α-catenin to filamentous actin (Friedl and Gilmour, 2009; Haley and Gullick, 2008; Juliano, 2002). Thus, adherens junctions serve as a bond between both the plasma membranes and actin cytoskeletons of adjacent cells.

Our experimental system of artificially constructed sheets of spontaneously immortalized human keratinocyte (HaCaT) cells (described previously in Chapnick and Liu, 2014) exhibits many similarities to *in vivo* keratinocyte behavior. These sheets of physically connected cells migrate into a wound area in response to epidermal growth factor (EGF) treatment in the same way that multiple layers of keratinocyte layers migrate during *in vivo* wound healing. Whereas fibroblasts secrete EGFR ligands *in vivo* (Werner et al., 2007), addition of exogenous EGF allows us to stimulate this keratinocyte migration into the wound. The stimulatory effect of EGF treatment on cell migration in this system is demonstrated in Fig. 1, where we have displayed the leading edge propagation of untreated (denoted as mock) and EGF-treated keratinocyte sheets. The EGF-treated cell sheets migrate more than three times as far as the mock cell sheets after 35 h.

A plethora of recent quantitative studies have analyzed *in vitro* wounding assays to investigate aspects of collective cell migration for various cell types (Arciero et al., 2011, 2013; Cai et al., 2007; Landman et al., 2007; Posta and Chou, 2010; Poujade et al., 2007; Simpson et al., 2014; Treloar et al., 2014). For example, the continuum model developed in Arciero et al. (2011) investigated how wound area, shape, and aspect ratio influence gap wound healing as a means to improve predicted wound healing times in intestinal enterocyte cells (Arciero et al., 2013). In this current study, we investigate the role of cell–cell adhesion on collective cell migration during *in vitro* wound healing assays of keratinocyte cell sheets. To do so, we develop and compare two competing mathematical models to describe how cell sheets migrate into the wound. Both models are nonlinear diffusion equations based on assumptions of how cell–cell adhesion influences the space filling response of cells to a wound. Our first model (Model H) assumes cell–cell adhesion *hinders* migration into the wound through a drag force, while the other model (Model P) assumes cell–cell adhesion *promotes* this migration with a pulling force. We simulate both models with time-dependent rates of cell–cell adhesion to accurately fit the leading edge propagation of experiments from our model system. Model P is more robust than Model H to changes in the definition of the leading edge, so we determine it to be an appropriate model of keratinocyte migration during wound healing. We also show that it can reliably predict leading edge propagation from our experimental system.

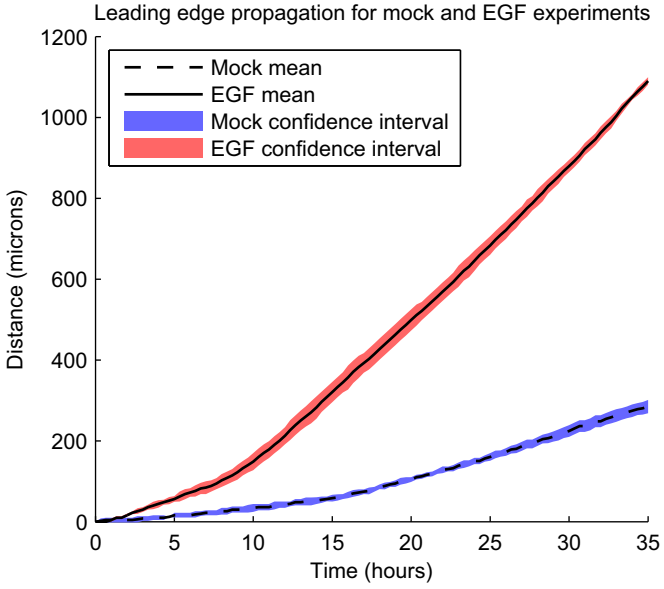

Fig. 1. Mean leading edge propagation of mock (black dashed line in blue strip) and EGF (black line in red strip) experiments between 0 and 35 h. Our leading edge computation finds where the normalized cell sheet profile reaches a certain value and is discussed in Section 2.3. The value used in this figure is 0.3. The total height of the colored strips correspond to two standard deviations of the leading edge data over time. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

Table 1
Summary of equations and assumptions relating to Models H and P.

Model features	**H**		**P**	
Cell–cell adhesion	Hinders migration		Promotes migration	
Treatment	Mock	EGF	Mock	EGF
$\Gamma(t)$	γ_1	$\gamma_1+\gamma_2(1-t/t_{final})$	γ_1	$\gamma_1+\gamma_2 t/t_{final}$
Equation	(2.4)	(2.6)	(2.5)	(2.7)

Table 2
List of parameters for the two models. Note that the fit values for different simulations are given in Tables 3 and 5.

Parameter	**Description** (μ^2/h)
D	Baseline rate of diffusion
γ_1	Baseline cell–cell adhesion rate between adjacent cells
γ_2	Response of the rate of cell–ell adhesion to EGF treatment

Performing the same experimental protocol on cell sheets with decreased α-catenin expression demonstrates that cell sheets with weakened cell–cell junctions initially enter the wound area rapidly but do not display collective migration and are unable to maintain migration into the wound. Intact adherens junctions thus allow the cell sheet to sustain the wound healing response for long periods of time through collective migration. The agreement between sheet migration behavior in wild-type sheets and model P simulations, in addition to the inability of cell sheets with decreased α-catenin expression to maintain migration, leads us to conclude that cell pulling, which is mediated by cell–cell adhesion, promotes sustained collective migration during wound healing.

In Section 2, we present our two model derivations based on different assumptions for the role of cell–cell adhesion on cell migration. In Section 3, we demonstrate how both models can fit experimental leading edge propagation data, but that Model P is robust to changes in the leading edge definition. We then use keratinocyte sheets with decreased α-catenin expression to demonstrate that cell–cell adhesion is needed to maintain a sustained wound healing response. We discuss our conclusions in Section 4 and discuss the implications of these results as well as plans for future work in Section 5.