Utilizing Chemotactics to Stimulate Collective Cell Migration of Keratinocytes to Speed Second-degree Burn Wound Healing

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Abstract

Worldwide, severe burns cause over 180,000 fatalities every year, and millions of people with non-fatal burns undergo severe, life-long physical and psychological morbidities. In an adult organism, collective cell migration is essential for a proper immune response, wound healing, and tissue homeostasis. A cell's migration is often affected by a mechanism by which specific chemicals either attract or repel the cells of multicellular organisms, which is called chemotaxis. Various aspects of collective cell migration and chemotaxis are recommended to be utilized in the process of burn wound healing. This paper introduced a modern approach that will allow the treatment process of second-degree burn wounds to be faster and less painful. This is done by introducing chemotactics to the burn wound dressing. IFN-, TGF-, IGF-1, and FGF-7 are examples of chemotactic proteins. These chemotaxes were specially chosen based on their availability and effectiveness in stimulating the keratinocyte cell migration in the dermis. To help future validation of the idea, three types of mathematical models have been investigated: agent-based models, continuous models, and local-versus-non-local models. Agent-based modelling facilitates the transfer from describing individual models (individual cells) to describing collective behavior. Unlike agent-based models, continuous models are not constrained by a lack of computational power, which makes them suitable for idea validation. One of the main classes of continuous models is reaction–diffusion–advection (RDA) equations. The RDA equations use the local chemoattractant gradient to describe population drift. On the contrary, non-local models describe the overall behavior of the element inside its system.

I. Introduction

The establishment and preservation of a multicellular organism's appropriate organization depends critically on cell migration. From the large-scale migrations of epithelial sheets during gastrulation to the movement of individual cells during the development of the nervous system, the final form of the living organisms can be seen as an outcome of cellular locomotion. Cell migration is necessary for healthy immune response, wound healing, and tissue homeostasis in an adult organism, while abnormal cell migration is linked to several diseases. Indeed, as our understanding of migration grows, we might anticipate, for instance, reducing the spread of extremely dangerous cancer cells, delaying the invasion of white blood cells during an inflammatory response, or speeding up the healing of wounds. Cells can react to a

variety of environmental cues, and frequently these cues cause directed cell migration either toward or away from the signals they are responding to. One of the oldest and most difficult challenges in biology is how cells detect these cues and convey that information to the cytoskeletal apparatus controlling cell migration [1]. While chemotaxis, or migration toward diffusible chemical signals, has been studied for more than a century, knowledge regarding how cells react to those cues is only now starting to become available. The exact mechanism of cell translocation, the chemotaxis process and other topics are going to be further discussed in the following sections.

i. Single Cell Migration

Internal molecular actuators that move a complicated substance are what make cell migration an intrinsic mechanical process (Figure 1). Myosin motors, which enable contractile force generation and aid in cytoskeletal crosslinking; adhesion complexes, which connect the cell to its external environment and enable force transmission; and polymerizing and depolymerizing actin filaments, which propel forward protrusions and facilitate internal remodeling, make up the essential mechanical machinery. Interconnected signaling channels and feedback mechanisms, which provide cell polarity and modify cell migration modes and kinetics, control the coordination and arrangement of these mechanical components.

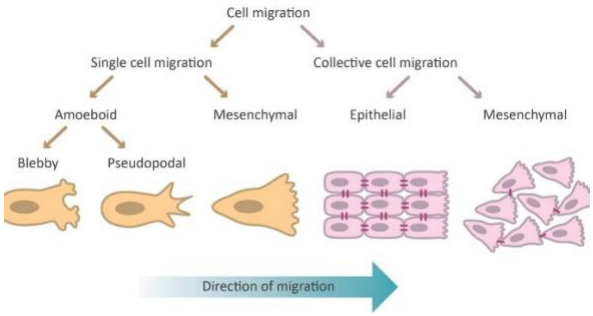

Figure (1): Cell migration process among different cells.

The fundamentals of cell migration must be understood to describe how cells move in a particular direction in response to different signals. *The four-step cycle of cell crawling* created by early pioneer in the area Michael Abercrombie may be the most prominent model for understanding cell migration[2]. He used phase contrast and interference reflection microscopy to observe migrating primary and cultured fibroblasts (In vitro, these cells have been objects of extensive study because of the ease of culturing them), which led to the development of this paradigm. The protrusion of a leading edge, which in fibroblasts is predominated by lamellipodia and filopodia, is the initial event in this scheme. The cell produces the first adhesions to the substrate after that. Through a combination of pulling from the front and squeezing from the back, these adhesions attach to the contractile apparatus of actomyosin stress fibers and cause the cell body to move forward (Figure 2). At the trailing edge, previous adhesions are finally separated from the substrate or broken down. Although textbooks define this as a stepwise cycle of sequential steps, all events of the cycle happen at once and probably have an impact on one another rather than happening one after the other.

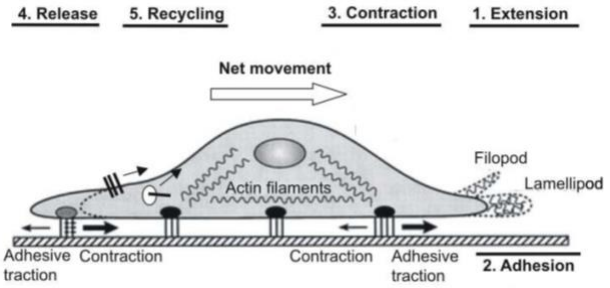

Figure (2): Mechanism of cell migration.

In order to arrange the vast quantity of data required to comprehend directed migration in response to different stimuli, a conceptual framework of four events that must take place during all types of directed migration was developed:

a. Generating the signal: A signal must be produced first for directed migration to take place. This signal could be a momentary trigger, such as a diffusible chemical signal released into the environment to drive cells for a brief burst of migration. In contrast, the signal could entail a long-lasting modification to the environment that directs cells for a long time, such the creation of physical routes.
b. Sensing the signal: Cells must be able to detect signals once they have been created. This is a straightforward process of receptor-ligand interactions in the case of chemotaxis. The sensing process for other signals, such as the substrate's mechanical compliance, is less simple and requires more complicated mechanotransduction pathways involving both surface proteins and mechanically related internal proteins.
c. Transmitting the signal: The third pillar of directed migration requires the signal to be sent from the sensor to the equipment required to move the cell.
d. Executing the signal: The production of asymmetric or polarized forces with respect to the substrate is

necessary for the translocation of cells, either individually or in groups, relative to their surroundings. There are several sources of this force generation, but a fundamental idea is that directed migration-promoting signaling biases these forces in one direction or the other in relation to environmental cues. Directed cell migration cues favor the cell polarity and migration machinery that function during regular, random cell migration rather than activating unique cell translocation processes.

ii. Collective Cell Migration

It is helpful to define collective cell migration before discussing it. When cells migrate in groups, come into contact at least occasionally, and have an impact on one another, the phenomena is said to be collective[3].

Morphogenetic motions are required for the development of multicellular animals, in which extensive cell migrations help the development of tissues and organs. In the adult, collective migration also takes place during tissue repair, angiogenesis, and wound healing, and has been linked to the spread of tumors. Understanding the molecular mechanisms behind collective migration is crucial for discovering new treatment targets to stop the metastasis and spread of tumors as well as for understanding morphogenetic processes (Figure 3).

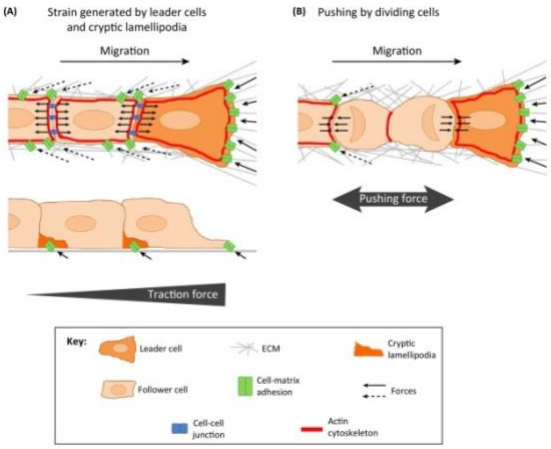

Figure (3): Mechanism of collective cell migration.

If cells can move independently, why do they move together? Collective migration has the potential to accomplish many proccesses:

a. Maintain the continuity and integrity of a tissue or structure while remodeling it;

b. Enable mobile cells to transport other, immobile cell types along;

c. Enable migrating cells to influence one another, ensuring proper cell distribution and shaping of a tissue;

d. Enable collective decisions that may be more system-supportive. This shows how multicellular organisms are not just a collection of autonomous cells but also interdependent cells that cooperate to create a whole is collective migration[1].

iii. Chemotaxis Effect on Cell Migration

Chemotaxis is the technique by which a sperm cell locates an ovum, white blood cells identify an area of inflammation or injury, or unicellular creatures find nutrition and escape from danger[4]. Chemotaxis is the process by which cells of multicellular organisms are attracted to or repelled by certain chemicals. Today, the terms "positive chemotaxis" and "negative chemotaxis" are used to describe how cells migrate toward or away from a chemical source, respectively (Figure 4). The chemical is classified as either a chemoattractant or a chemorepellent. Chemotaxis is also generally defined as any cell movement that is influenced by a chemical gradient in a way that causes net propagation up a chemoattractant gradient or down a chemorepellent [5].

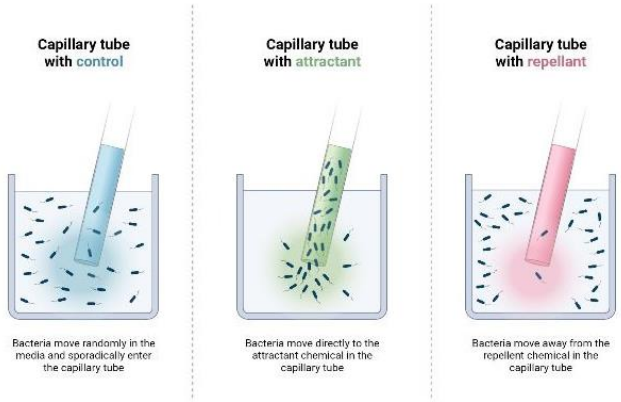

Figure (4): Positive and negative chemotaxis.

Directional sensing, cell polarization, cell adhesion, and cell motility are all necessary for the dynamic process of chemotaxis. In order to localize the reaction and transfer the signal across the plasma membrane, cells must engage with the chemoattractant. Cell movement is oriented in reference to the ligand gradient by an underlying directional sensing mechanism that works as a compass and favors the development of pseudopodia towards or away from the source of chemoattractant or repellent[5]. To achieve a polarized morphology, in which the cells have a distinct front and rear, the cell must typically reorganize the membrane and cytoskeleton to do this movement. This entails a variety of feedback

mechanisms to synchronize the actin polymerization in pseudopodia at the leading edge of the cell with the contractile forces produced by myosin motor proteins at the rear.

Studies on the social amoeba Dictyostelium discoideum and mammalian neutrophils have contributed significantly to our present understanding of chemotaxis-signaling pathways through G-protein-coupled receptors (GPCRs) (this term refers to both primary neutrophils and HL60s, a neutrophil-like cell line). To find microorganisms, dictyostelium cells use chemotaxis to move toward released metabolites like folic acid[6]. However, this organism's reaction to hunger is more drastic. The individual amoebae group together and create multicellular structures with spores that can withstand hunger through a sequence of morphogenetic adjustments and cell-fate decisions.

II. Collective Cell Migration in Wound Healing

One of the hallmarks of wound healing is collective migration[7]. A number of connected physiological developments, ranging from chemical signaling to tissue level structures in the body at subcellular to intracellular levels, come together to form the complex dynamic multicellular mechanism of wound healing[8]. The healing of a wound goes through three stages.

1- The initial stages of wound healing: include hemostasis and the activation of different inflammatory cells.
2- The intermediate stages involve the proliferation and migration of cells (Figure 5), matrix deposition, and angiogenesis.
3- The last stage of wound healing involves remodeling of extracellular matrix (ECM), resulting in scar tissue formation.

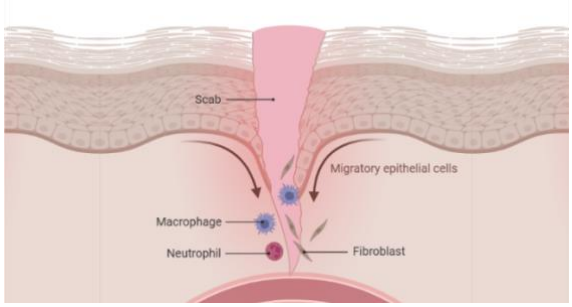

Figure (5): Collective cell migration in wound healing.

However, specialized tissues like the liver and skeletal tissues have unique kinds of regeneration and tissue repair and use different routes[8]. Moreover, depending on the nature of the wound, these stages might vary, as in the case of burn wounds.

III. Burn Wounds

Before investigating the mechanism of collective cell migration in healing burn wounds, various aspects regarding these life-threatening wounds should be discussed first. Burns are the fourth most frequent injury category after falls, vehicle accidents, and physical assault. Nationwide, severe burns cause over 180,000 mortalities every year, and millions of people with non-fatal burns endure severe, life-long physical, and psychological morbidities[9]. Due to enhanced capillary permeability, substantial plasma loss occurs in extensive burns and causes shock, whereas entire blood loss results in shock in other acute wounds. Due to the immunocompromised state of burn patients, wound infection and septicemia are the main causes of death in significant burns, despite the fact that burn wounds are initially sterile compared to the majority of other wounds.

i. Pathophysiology of Burns

Due to enhanced microvascular permeability, vasodilation, and extravascular osmotic activity, the pathophysiology of the burn wound is characterized by an inflammatory response that quickly results in oedema formation. These responses are brought on by both the chemical mediators of inflammation and the direct heat influence on the microvasculature. Histamine release frequently causes the first step of vasodilation and enhanced venous permeability.

A rapid production of prostaglandin would come from the activation of the enzymes that catalyze the hydrolysis of the prostaglandin precursor (arachidonic acid[10]. Prostaglandins inhibit the release of norepinephrine and may thus be of importance in modulating the adrenergic nervous system, which is activated in response to thermal injury.

The morphological interpretations of the changes in the functional ultrastructure of the blood lymph barrier following thermal injury seem to be an increase in the numbers of vacuoles and many open endothelial intercellular junctions. Furthermore, changes of the interstitial tissue after burn trauma are of great importance. The continuous loss of fluid from the blood circulation within the thermally damaged tissue causes increased hematocrit levels and a rapid fall in plasma volume, with decreased cardiac output and hypoperfusion at the cellular level. If the fluids are not adequately

restored, burn shock develops. Furthermore, the burn wound provides a vast area of entry of surface infection with a high risk of septic shock.

ii. Types of Burn Wounds

According to the involvement of skin and deeper tissues, burn wounds can be divided into the following categories (Figure 6):

- First-degree burns: also known as epithelial burns, cause erythema without vesication of the skin.
- Second-degree burns: involving the epidermis and varying dermal thickness. This is further classified into:
  - Second-degree superficial burns, where only the papillary dermis is affected and vesication and inflammation are visible in the skin.
  - Second-degree deep -eschar formation is seen as it involves deep reticular dermis.
- Third-degree burns, also referred to as full thickness burns since they have eschar formation.

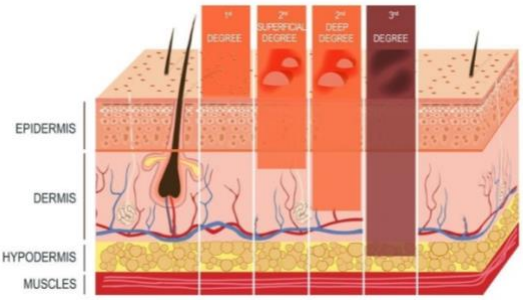

Figure (6): Degrees of burn wound

IV. Healing of Burn Wounds

Inflammatory (reactive), proliferative (reparative), and maturation (remodeling) stages of wound healing are the same for all types of wounds; the only difference in burn wounds is the length of each stage[10].

i. Phases of Burn Wound Healing

a. Phase of inflammation:

The body's inflammatory response, which contains vascular and cellular factors, starts as soon as an injury occurs.

- Vascular response: Local vasodilation and fluid extravasation in the third space occur right away after burns. Increased capillary permeability may become universal in severe burn injuries, resulting in significant plasma extravasation that need fluid replacement.
- Cellular response: The first cells to migrate to the site of inflammation are neutrophils and monocytes. Later, macrophages begin to replace neutrophils as the former start to decline. Chemotactic factors including kallikreins and fibrin peptides generated during the coagulation process, as well as chemicals released by mast cells, like tumor necrosis factor, histamine, proteases, leukotrienes, and cytokines, cause these cells to migrate. The cellular reaction aids in the removal of dead tissue and poisons from burned tissue by phagocytosis.

b. Phase of proliferation:

Re-epithelialization in partial thickness burns begins as keratinocyte migration from healthy skin appendages in the dermis a few hours after injury, and the wound is typically healed in 5-7 days. The basement membrane zone develops between the dermis and epidermis during re-epithelialization. Angiogenesis and fibrogenesis are helpful to rebuild the dermis. In deep burns, healing occurs via delayed primary intention following primary excision and grafting. After primary excision, the proliferative phase of wound healing includes taking skin grafts.

c. Phase of remodeling

Collagen and elastin, which are fibrous structural proteins, are initially laid down surrounding epithelium, endothelium, and smooth muscle during this last stage of wound healing. This extracellular matrix remodels later in the resolution phase into scar tissue, and fibroblasts take on the myofibroblast phenotype, which oversees scar contraction.

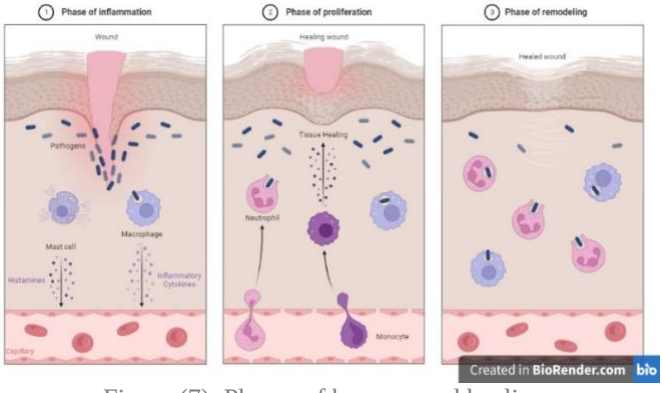

Figure (7): Phases of burn wound healing

ii. Durations of Healing of Burn Wound According to its Degree

a. First-degree burns: These burns typically heal in three to six days without leaving any scars and don't produce any blisters. When squeezed, they blanch and are painful, dry, and red.

b. Second-degree burns: Burns of the second degree usually recover in 7 to 21 days. These burns are extremely painful, blister, ooze fluid, and turn white when rubbed. They involve the top two layers of skin, hence why. The burned region may leave a scar and change color permanently in either a darker or lighter direction.

c. Third-degree burns: Without surgical intervention, such as skin transplants, these burns cannot recover. They penetrate all layers of the skin, obliterating it entirely. Usually, the burned region is painless. It might be a deep crimson, waxy white, leathery grey, or scorched black in hue[9]. Since they are very painful and require a long time to heal, the treatment of second-degree burns must be efficiently increasing the rate and speed of the healing of these burns.

d. Treatment of Second-degree Burn Wounds

Typically, a second-degree burn that doesn't cover more than 10% of the skin's surface can be treated without hospitalization. Depending on the extent of the burn, the following treatments might be used[11]:

- antibiotic ointments
- dressing changes one or two times a day depending on the severity of the burn.
- daily cleaning of the wound to remove dead skin or ointment
- possibly systemic antibiotics

An approach can be done in this treatment method in order to facilitate the healing process of burn wound. This is in a trial to allow the healing to become faster and the treatment to be more effective. This can be done by stimulating collective cell migration of keratinocytes during the proliferation phase of burn wound healing. This is because keratinocytes form the majority of the epithelial tissue in skin. This part is often in more contact with the dressing.

V. Using Chemotactics to Stimulate Collective Cell Migration to Heal Burn Wounds

To allow the treatment process of second-degree burn wounds to be faster and less painful, it is advisable to introduce chemotaxis to the burn wound dressing. When a wound occurs, various growth factors and chemokines are released at the site, which recruit neutrophils and phagocytes via chemotaxis.

Key players in the healing of burn wounds are keratinocytes. They migrate to the area of the wound and occupy the void left by the burn wound. A prior study noted the migratory response of newly isolated and cultured human keratinocytes to chemotactic stimuli for various cell types. The chemotactic effectiveness of interleukin-1 (IL-1), interferon (IFN), interleukin-8 (IL-8), and tumors necrosis factor (TNF) was examined in a modified Boydcn chamber experiment. It has been shown that IFN, IL1, and IL8 operate as chemoattractants for newly separated keratinocytes. However, only IFN was discovered to have chemotactic characteristics for cultivated cells[4].

Another researchers reported that IGF-1 and EGF, when combined with HGF, were more stimulating than either growth factor alone in enhancing migration in wound assays using fourteen distinct growth factors and cytokines. In the transmigration experiment, HGF and TGF-1 had a stronger chemokinetic effect than either growth factor by themselves. To keratinocytes, TGF-1, FGF-7, FGF-2, and AGF had chemotactic effects. Cell migration on ECM proteins was improved by EGF, TGF-, IL-1, IGF, and MGSA[12].

The chemotactics included in these previous studies were further investigated to analyze their efficiency and availabity to be integrated in the wound dressing.

- Interleukin-1 (IL-1)

Interleukin-1 (IL-1 alpha and beta), a proinflammatory cytokine released by injured keratinocytes, acts as the initial warning signal to neighboring cells. Production of extra cytokines and their receptors by keratinocytes and other skin cells is one of the effects of interleukin-1 release[13]. Monocytes, on the other hand, release biological processes linked to both types of IL-1. Significant levels of the immunoreactive IL-1 beta protein, all of which were in the 31-kD form, could be detected in keratinocyte cultures using a variety of monoclonal antibodies to IL-1 beta. These data suggest that administration of an IL-1 receptor antagonist within the perioperative period could decrease wound pain[13]. However, the molecule that triggers inflammation and promotes fever and sepsis[14].

- Interferon (IFN)

A crucial immunoregulatory protein, interferon gamma (IFN gamma) is secreted by CD4+, CD8+, and NK cells. IFN- has a significant role in the neutrophilic inflammatory response at the wound site as well as the proliferation phase of skin wound healing[15]. In accordance with earlier studies, IFN promotes keratin K17 via the activation of K16 and STAT1 transcription factors. In terms of the availability of IFN, white blood cells from the majority of people who have developed M. tuberculosis will release interferon-gamma (IFN-g) when combined with antigens obtained from M. tuberculosis.

- Interleukin-8 (IL-8)

A pro-inflammatory cytokine called interleukin (IL)-8 directly affects immune cells like polymorphonuclear cells. An abundant source of IL-8 is keratinocytes. The apparent function of IL-8 as a chemotaxis and cell migration factor points to a major role in wound healing. Indeed, it has been shown that human keratinocytes, which express IL-8Rs, may be chemotactic in response to IL-8[16]. IL-8 significantly affects keratinocytes by accelerating cell migration. Macrophages, as well as other cell types such as epithelial cells, airway smooth muscle cells, and endothelial cells, create the chemokine interleukin 8[16].

- IGF-1

Researchers have conducted studies to highlight the function of IGF-1R and the signaling components in skin. Skin dermal fibroblasts and epidermal keratinocytes both express IGF-1R, and when these cells are stimulated by IGF-1, they proliferate and become mitogenic (7, 22). IGF-I plays a significant role in wound healing through a variety of methods, including acting as a chemotactic agent for endothelial cells, inducing keratinocyte and fibroblast proliferation and migration, and strengthening the wound[15]. However, enhanced cell proliferation, skin hyperplasia, and cancer are linked to rising levels of IGF-1 or IGF-1R[17].

- EGF

At the location of the wound, platelets produce large amounts of EGF, which is a crucial component. Due to its therapeutic role in promoting skin cell development, proliferation, and migration, epidermal growth factor (EGF) has gained recognition as a superior wound healing agent. When given topically, epidermal growth factor (EGF) has been demonstrated to hasten the healing of fresh, split-thickness cutaneous lesions. However, because of its brief half-life and insufficient formulation, transdermal delivery of EGF presents a serious issue. EGF[18] can be obtained from several sources, one of which is the epidermal growth factor (EGF) that's made in bioengineered barley seeds.

- HGF

HGF is produced by stromal cells and stimulates epithelial cell proliferation, motility, morphogenesis, and angiogenesis in a variety of organs through tyrosine phosphorylation of its receptor, c-Met. Endogenous HGF is essential for the self-repair of wounded livers, kidneys, and lungs, among other things. Furthermore, HGF protects epithelial and non-epithelial organs (including the heart and brain) through anti-apoptotic and anti-inflammatory signals [19].

- TGF-1

Transforming growth factors beta 1 cytokines are widespread, versatile, and necessary for survival. They are essential for growth and development, inflammation and repair, and host immunity [22]. However, due to the complexity of its structure and functions, as well as the unpredictability of its downstream targets, its activation and mechanism of action are only partially known [21]. TGF-beta is present in many tissues, but it is highly common in bone, lung, kidney, and placental tissue. TGF-beta is generated or released by many but not all parenchymal cell types, as well as infiltrating cells such as lymphocytes, monocytes/macrophages, and platelets [20].

- FGF-7

Keratinocyte growth factor (KGF), a member of the fibroblast growth factor (FGF) family (and alternatively designated FGF-7), is a paracrine growth factor produced by mesenchymal cells and mitogenic specifically for epithelial cells. Whereas most FGFs promote various cell types' proliferation and/or differentiation, KGF appears to function solely on epithelial cells. KGF has been shown to enhance epithelial cells proliferation and migration, but it also impacts differentiation processes [23].

- FGF-2

FGF, which was discovered in pituitary extracts in 1973, is found in a variety of cells and organs. Acidic FGF (FGF1) and basic FGF (FGF2) were initially isolated from the brain and pituitary gland as fibroblast growth factors [24]. Fibroblast growth factor (FGF) 2, also called basic FGF, has the potential to accelerate wound closure by activating vascular endothelial cells and fibroblasts. bFGF is a glycoprotein that is commonly used to treat wounds and ulcers. bFGF can be simply applied to any type of wound and results in improved colour, texture, and firmness [25].

- AGF

For the identification of an angiopoietin-related growth factor (AGF), which is mostly released into the systemic circulation by the liver [26], experiments were made on mice. During those experiments, AGF expression was induced in epidermal cells, which resulted in keratinocyte proliferation and enhanced cutaneous wound healing, indicating that AGF enhances keratinocyte growth. Furthermore, it was discovered that K14-AGF mice have enhanced vascular permeability and blood vessel number, indicating that AGF possesses angiogenic activity. Based on the idea that keratinocyte angiogenesis and proliferation are key components of wound healing. AGF's biological roles in keratinocytes and vascular cells could lead to novel therapeutic techniques for wound care and epidermal regenerative medicine [27].

- MGSA

Melanoma growth stimulatory activity/gro alpha (MGSA), a member of the alpha-chemokine family, is produced by a variety of dermal and epidermal cells and can function in a paracrine and autocrine manner. However, little is known regarding the role of MGSA in wound healing [28]. MGSA is an acid and heat stable auto-stimulatory growth factor that was isolated from culture medium conditioned by the Hs294T human melanoma cell line [29].

VI. Mathematical Modeling For Cell Migration

i. Agent-Based Modeling

An agent is an individual element with characteristics and actions in a computer-based simulation. Agent-based modelling (ABMs) is a type of modelling that describes the world as a collection of agents and an environment. It also describes the agent-agent and agent-environment interactions. It is expressive enough to enable high and complex models [30].

The benefits of using Agent-based modelling can be summarized in two points. Its ability to provide a natural description, flexibility, and ability to describe emergent phenomena are the benefits of using (ABMs). Emergent phenomena result from the interactions of agents in a system. A disagreement between two people or more can be considered an emergent phenomenon as it occurs because of interaction between agents.

Agent-based modelling facilitates the transfer from describing individual models (induvial cells) to describing collective behavior (colonies). This facilitation depends mainly on the definition of Agent-based modelling (agents and environment). Despite this facilitation, the heavy dependence on computation as population size increases is one of the disadvantages of Agent-Based models.

An agent-based model can be created using any programing language. However, there have been created single purposes programming languages for the sake of creating agent-based models. For instance, NetLogo is a programming language for creating agent-based models. NetLogo, also considered an integrated development environment (IDE) for creating agent-based models. As shown in figure 7, NetLogo (IDE) and language are used to create an ecological predation model for an environment that includes wolves and sheep as agents and grass as an environment. The program then tests the results thousands of times as you like and analyses them for you.

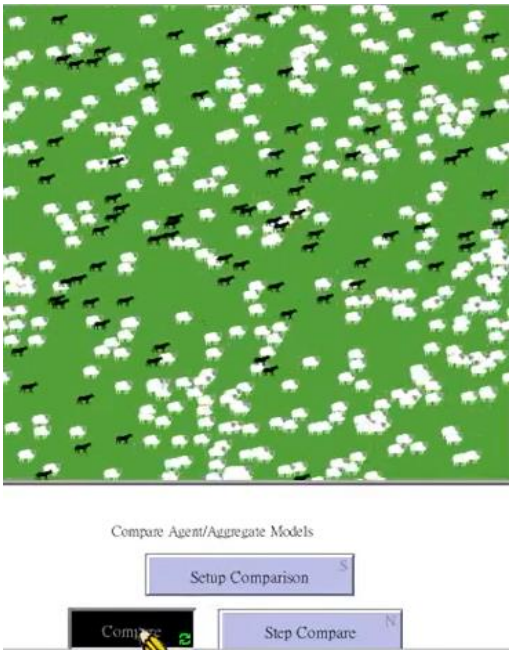

Figure (8): Wolf-sheep NetLogo predation model.

Agent-based modelling is considered a popular approach to tackle cell migration modelling as it facilitates individual-level representation. For agent-based modelling, cells are considered as point-based individuals which move with velocities determined by agent-agent interactions between cells. On the contrary, the problem with agent-based models for cell migration is the lack of analytical methods to analyze them. This leads to a high dependence on computational power as population sizes increase. However, computational resources are -inevitably- limited. Furthermore, a lack of an accurate quantitative similarity between different modelling approaches to the same problem (Cell migration). Even different implementations of the same approach can generate different results. This issue might increase with highly sophisticated and detailed models for Cell migration. In the next section, another type of modelling is presented that has a higher potential than agent-based modelling in modelling cell migration.

ii. Continuous modelling

Continuous models describe the world as a set of continuously changing variables as a continuous function of time. This function has different variables as inputs; thus, these functions are usually included in partial

differential equations. These changes can be accurately described with derivatives or integrals of other quantities via equations. These models are clearly represented in Partial Differential Equations (PDE) because they deal with functions that have more than one input [31].

Unlike agent-based models, continuous models are free of the inevitable lack of computational power. Furthermore, there have been developed analytical methods which provide important insights to researchers due to their roots in classical theory. One of the main classes of continuous models and the most famous equations in science and engineering is reaction–diffusion–advection (RDA) equations.

$$\frac{\partial u}{\partial t} = \underbrace{\nabla.\,(D(.)\nabla u)}_{diffusion} - \underbrace{\nabla.\,(a(.)u)}_{advection} + \underbrace{f(u,.)}_{reaction}$$
$$Eqution\,(1)$$

As shown in equation (1), the vector u denotes the population density at position x, x is a 3D position vector at time t. The resultant term $\left(\frac{\partial u}{\partial t}\right)$ is called temporary evolution term. The diffusion term shows how the subject diffuses in space according to a diffusing function D. The affection term shows how the position vector u is affected by the advection velocity a. Advection velocity is the average linear groundwater velocity, also known as $flux$. Advection could be active, which means done by other subjects in the same environment. Moreover, it could be passive, which means done by an environmental effect such as environmental flow. In the reaction term, f can be a wide range of reactions. It describes the subject's reaction to its surroundings [3].

The notable thing about RDA equations is that they are independent of the actual reaction done by the subject, the effect of the environment or other agents, or even the subject's diffusion method. f, a and D can be anything in the RDA equations. It is not restricted to reaction arguments. That's why it is considered one of the most famous equations in science and engineering. Models that use this equation fall into a classification called RDA models. A vast number of models fall into this classification.

To sum up, continuous models, especially RDA models, offer a more rigorous method to represent cell migration than agent-based models. Furthermore, they don't have the lack of computational power which appears in agent-based modelling (with high population sophisticated models). However, another huge issue, which is in both agent-based models and RDA models, will be presented in the next section.

iii. Local vs non-local models

Models that use RDA equations have a local nature. In other words, they describe subjects pointwise from a local perspective. For instance, the Keller–Segel model -a model for chemotaxis- is considered local as it describes chemotaxis from a local perspective because the advection term -in the RDA equations- uses the local chemoattractant gradient to describe population drift.

Local assumptions done in local models may lead to a variation between lab results and prediction done by these models. This variation can be compensated in different ways, such as mathematical models for error calculations. Proportional-Integral-Derivative (PID) and Model Predictive Control (MPC) are examples of these models [3].

On the contrary, non-local models describe the overall behavior of the element inside its system. The concerns about local models have led to the invention of a range of spatially non-local RDA models. There are three classes of spatial non-local models. There are spatial non-local models in reaction terms. Also, There are spatial non-local models in advection terms.

Non-local models are used in several diverse applications. Non-local heat conduction and machine learning are examples of them. It is used in many scientific and engineering applications. Since the non-local model is more precise and accurate than local models, they are used to accurately resolve small-scale issues that don't appear when using local models.

Scientists have been investigating a coupling strategy for the non-local and local models. Where they split the model into local factors that can be modelled using local models and non-local factors that must be solved using non-local models, this optimizing method minimizes the computation needed and the difficulty of the overall model. This method is very general. It can be applied to any non-local and local models or even to different non-local models.

iv. Mathematical Models for Collective Cell Migration in wound healing

As with cell migration generally, mathematical modelling helps in the understanding of the complex process included in wound healing. Most models in this field are continuous Models. It is a system of partially or ordinary differential equations for variables that measure different characteristics of cell migration is used to describe biological systems. These models are constructed using data deduced from laboratory results.

(ODE) Models:

Models that use ordinary differential equations (ODE) considered phenomenological, which means that they try to model the closure of the wound area or perimeter as a function of time. They determine the constants in the equation using observed data from laboratory results. Most of these models are based on linear or exponential functions. However, using ODEs has proven to be ineffective. These types of functions (Linear and exponential) are insufficient to describe the initial of the healing process, which is clearly observed in wound healing experiments [32].

(PDE) Models:

Models that use Partially differential equations (PDE) describe the dependencies of different factors involved in wound healing, so they can predict the geometric shape of the wound. This is possible in partial differential equations because it involves functions that have more than one input[32].

One of the PDE Models that also falls under the class of RDAs models is Fisher–Kolmogorov equation.

$$\frac{\partial p}{\partial t} = rp\left(1 - \frac{p}{k}\right) + \Delta\frac{\partial^2 p}{\partial x^2}$$
$$Equation\ (2)$$

As shown in Equation (2), p is the population, r is the intrinsic growth rate, k is the carrying capacity. Furthermore, it involves a logistic term for the sake of proliferation. Scientists have verified the validity of the Fisher–Kolmogorov equation in a medical context by using a scratch-wound assay. Other scientists modified the Fisher–Kolmogorov to include cell density-dependent diffusion [32].

The first PDE model to take into account the regulations of the growth factors was developed by Sherratt and Murray. The model consists of two non-linear reaction-diffusion equations. These two parts track the epithelial cell density and a chemical regulating mitosis [33].

Applications of wound healing models:

Wound healing models help us predict wound healing time. There are three commonly used methods to estimate healing time, which are Absolute Area Reduction method,

Percent Area Reduction method and Linear Parameter method. Furthermore, several originally developed models for modelling wound healing were used to model expansive growth and the migration of tumor cells, which helps cancer treatment research.

VII. Conclusion

In order to enhance burn wound healing, 11 different chemotactics have been investigated to the burn wound dressing. These chemotactics were analyzed according to their effectiveness in stimulating the cell migration in the epithelial tissue, function, and source of extraction. Among these 11 chemotactics, 5 were excluded due to their drawbacks such as cancer and inflammation. Accordingly, FGF-2, AGF, HGF,MGSA,Interferon or interleukin 8 can be used. Although these chemotactics were not experimented in real life or in vivo, some of them have proved their applicability and ability to regenerate the skin and cure the second degree burn wounds via the stimulation of the keratinocyte's collective migration. Also, mathematical modelling helps a lot in interpreting the known data about cell migration and chemotaxis and predicting the future state and behavior of the cell. A combination of local and non-local continuous models was found to be the most suitable for cell migration in wound healing modelling.

VIII. Refrences

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