Review

The role of biophysical properties of provisional matrix proteins in wound repair

Daniel Chester and Ashley C. Brown

Joint Department of Biomedical Engineering, *North Carolina State University and University of North Carolina at Chapel-Hill, Raleigh, NC 27695, United States*
Comparative Medicine Institute, *North Carolina State University, Raleigh, NC 27607, United States*

Correspondence to Ashley C. Brown: *Joint Department of Biomedical Engineering, North Carolina State University and University of North Carolina at Chapel-Hill, 911 Oval Drive, Suite 4204B, Raleigh, NC 27695, United States.*
aecarso2@ncsu.edu
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Abstract

Wound healing is a complex, dynamic process required for maintaining homeostasis in an organism. Along with being controlled biochemically, wound healing is also controlled through the transduction of biophysical stimuli through cell interactions with the extracellular matrix (ECM). This review provides an overview of the ECM's role in the wound healing process and subsequently expands on the variety of roles biophysical phenomenon play.

Introduction

Cells are capable of interacting with and responding to their environment in several different ways, with one of the most well studied pathways involving biochemical molecules such as cytokines and growth hormones. Biophysical stimuli also play an important role in modulating many important cellular activities and the biophysics of cellular processes are a growing area of research [1]. Cells are able to transduce biophysical stimuli through their extracellular matrix (ECM), therefore understanding the biophysics of the ECM and how ECM biophysics modulate cellular processes is a pivotal component of cellular biophysics research. This review details the role of provisional ECM biophysics in cutaneous wound repair.

The wound healing process is a complex and highly regulated biological process that begins with the disruption of the homeostatic structure and/or function of an organism's cells or tissues. Wound healing concludes with the restoration of the structural integrity of the tissue and is the result of specific interactions between cells, cytokines, and ECM components. Key cell types involved in cutaneous wound healing include platelets, leukocytes, fibroblasts, keratinocytes, and endothelial cells [2]; key cytokines include transforming growth factor beta (TGF-β), platelet derived growth factor (PDGF), tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) [3]; and key ECM components, include fibrinogen/fibrin, fibronectin, proteoglycans, and collagen [4]. The cytokines mentioned above are responsible for regulating cellular interactions with the ECM as well as creating and regulating the inflammatory response, acting as chemotactic cues for cells [5], and controlling cell proliferation, differentiation, and ECM production [6].

Wound healing occurs in four temporally overlapping phases – the hemostatic phase, the inflammatory phase, the proliferation stage, and the remodeling stage – and can result in either normal tissue repair, excessive tissue repair, or deficient tissue repair. The ECM components mentioned above play a significant role in each stage of the wound healing process beginning with the formation of a provisional matrix that acts as a scaffold for promoting cellular interactions with the environment [7,8]. The ECM components of this provisional matrix temporally modulate specific actions of the wound

healing process including directing initial platelet adhesion at the wound site during hemostasis, directing immune cell infiltration during inflammation, and promoting fibroblast proliferation and ECM production during the proliferation and remodeling stages [9].

Cell-ECM interactions are predominantly mediated via transmembrane cell surface receptors known as integrins that physically bind to ECM components [10]. Cells are able to sense changes in their mechanical microenvironment, such as changes in ECM stiffness, through integrin-ECM connections. Intracellularly, integrins complex with focal adhesion proteins, including vinculin and paxillin, that in turn bind the actin cytoskeleton. Through these focal adhesion contacts, contractile cells are able to exert forces onto the provisional matrix in order to assess its stiffness [11], unfold proteins to reveal cryptic integrin binding sites [12,13], and migrate through the provision matrix [14,15]. Importantly, such biophysical cues from the ECM play a role in how effectively wound healing occurs. Recent studies have found that integrin-specific interactions with the wound-associated ECM components fibrin, fibronectin, and collagen all directly control specific actions in the wound healing process through biophysically mediated processes. As an example, during the early stages of wound repair, platelets contract, adhere, and spread differentially based on the mechanical properties of the fibrin rich provision matrix [16,17]. Additionally, fibronectin unfolding has been shown to modulate integrin specific binding and thereby control specific cellular responses such as differentiation [12,13,18]. Lastly, the stiffness of collagen has been shown to influence fibroblast proliferation and migration [11,19]. Furthermore, integrins have been shown to regulate TGF-β activation, which plays a role in fibrotic diseases where there is an overproduction of connective tissue and ECM components [20–24]. Here, we discuss the wound healing process and how biophysical phenomena of the provisional ECM and its constituents modulate the process.

Wound healing overview

Wound healing begins with the disruption of the homeostatic structure and/or function of an organism's cells or tissues. Once an injury has occurred, four overlapping phases of the wound healing cascade occur in order for equilibrium to be reestablished [2]. These four phases are: the hemostatic phase, the inflammatory phase, the proliferation stage, and the remodeling stage diagramed in Fig. 1. The overarching goal of the wound healing cascade is to reestablish homeostasis by replacing the damaged tissue with tissue that is structurally and mechanically similar.

Hemostatic phase

Immediately following tissue damage, the hemostatic phase of wound repair begins with the initiation of the coagulation cascade, resulting in the accumulation of platelets at the wound site and stemming of blood flow [25]. These platelets become embedded in a provisional matrix composed of cross-linked fibrin, fibrinogen, and fibronectin [26]. Fibrin is the primary component of this early provisional matrix and is derived from its precursor fibrinogen. Fibrinogen is a circulating glycoprotein produced by hepatocytes and has a normal blood concentration of 9 μM. However, during periods of inflammation, fibrinogen synthesis increases dramatically [27]. This drastic increase in the production of fibrinogen is important in creating the provisional fibrin network. Fibrinogen is cleaved and converted into fibrin monomers by α-thrombin, and spontaneously forms insoluble fibrin polymers creating a stable fibrin clot [27]. Overtime fibrin fibers are crosslinked by factor XIIIa, introducing additional clot stability and mediating red blood cell retention in the clot [28]. The resulting clot stems blood loss and provides a scaffold for subsequent tissue repair through interactions with, platelets, monocytes, fibroblasts, epithelial cells, keratinocytes [15,29], and the growth factors FGF, PDGF, TGF-β, and VEGF that bind fibrinogen/fibrin [14,30]. In the presence of factor XIIIa, soluble fibronectin can be covalently incorporated into the fibrin network. Fibronectin binds platelets, fibroblasts, epithelial cells, keratinocytes [15], as well as growth factors such as TNF-α [31] and TGF-β [14], therefore the incorporation of fibronectin into the provisional fibrin network provides additional cues for directing cell fate during the early wound repair process. Fibronectin exists as a soluble dimer in the blood, but can also be found as a fibrous component of the ECM. The structure of fibrinogen and fibronectin is described in detail in subsequent sections.

In addition to fibrinogen/fibrin and fibronectin, the proteoglycans hyaluronan, or hyaluronic acid (HA), and heparin sulfate (HS) are components of the early provisional matrix. Proteoglycans are protein-carbohydrate complexes that are distinguished by the numerous glycosaminoglycan (GAG) side chains bonded to a core protein strand. The GAG chains can have as few as ten or as many as 20,000 disaccharide molecules and are not rigid in structure, however, their flexibility does depend on the nature of the sugars that comprise the chain. HA is a linear polymer of glucuronic acid *N*-acetyl glucosamine-glucuronic acid disaccharides [32]. HA is a major component of early granulation tissue with HA production increasing greatly during days 1–5 of the wound healing process, decreasing 5–10 days following injury, and then remaining fairly constant from day 10 onward. HA is able to bind to fibrinogen,