WOUND HEALING AND REGENERATION

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Summary

Wound healing and regeneration are a complex series of processes involving the cooperative and coordinated efforts of many different cell types, including cells located in the wound margin and cells recruited from the circulation to direct the healing response. These cells communicate with one another via cytokine messages that bind to cellular receptors, inducing intracellular signals that culminate in a programmed response. Healing progresses in a timely and methodical manner in most instances. However, many stimuli or coexisting disease processes can inhibit healing. This leads to a high prevalence of chronic wounds. For example, in the United States, persons with chronic wounds comprise one of largest patient populations. The impact of nonhealing or inadequately healing wounds imposes a tremendous burden on the healthcare system, society, families, and individuals.

The methodical investigation of wound healing events holds much promise for the future of medicine. Wound healing involves core physiological processes that affect every tissue in the body. By understanding wound healing, researchers and clinicians hope to develop a better understanding of other disease processes to enhance our ability to improve the human condition.

1. Introduction

As common as the cold is the occurrence of the wound. Varying in degree of severity from the superficial scratch to the cavernous sacral ulcer to the necrosis of myocardial infarction, wounds eventually afflict every living thing. Amazingly, our analysis of the scratch and the molecular and cellular steps that lead to its healing may eventually lead to the ability to reverse the morbid sequelae of other disease processes, such as myocardial infarction, emphysema, diabetes mellitus, vascular insufficiency, burn injuries, and rheumatoid arthritis.

Our current understanding of wound healing is guiding the development of treatment algorithms for the management of common nonhealing wounds, such as diabetic foot ulcers, venous stasis ulcers, and pressure ulcers. In the United States alone, these three types of wounds affect more than 2% of the total population, with an annual cost of more than $3 billion in healthcare. Globally, the numbers of people afflicted and the economic impact of these and other wounds are staggering.

1.1. Repair vs. Regeneration

A wound is created by any stimulus that disrupts the physical continuity of functional tissues. Stimuli that cause wounds, either external or internal, can be physical, chemical, electrical, or thermal.

There are two opposing but simultaneous processes by which wounds resolve or heal. The process of returning the site to its original state is termed regeneration. The process of generating a scar or less functional tissue of different form and/or composition than the original is termed repair. Repair alone does not restore complete functionality. Ideally, the human response to injury would be regeneration. Unfortunately, in most tissues, the response to wounding is intermediate to regeneration and repair.

Additionally, wounds resolve at different rates, ranging from quickly to not at all, in which the wound remains in a chronic state of disrepair. Each tissue has a characteristic range of physiologic response to wounding that is considered normal. Some tissues, such as bone and serous epithelium, have a response that is slightly more regenerative than reparative. Other tissues, such as neural or cardiac types, have a response that is keenly reparative. General health status, wound care, and coexisting pathologies can shift the balance and slow the rate of resolution.

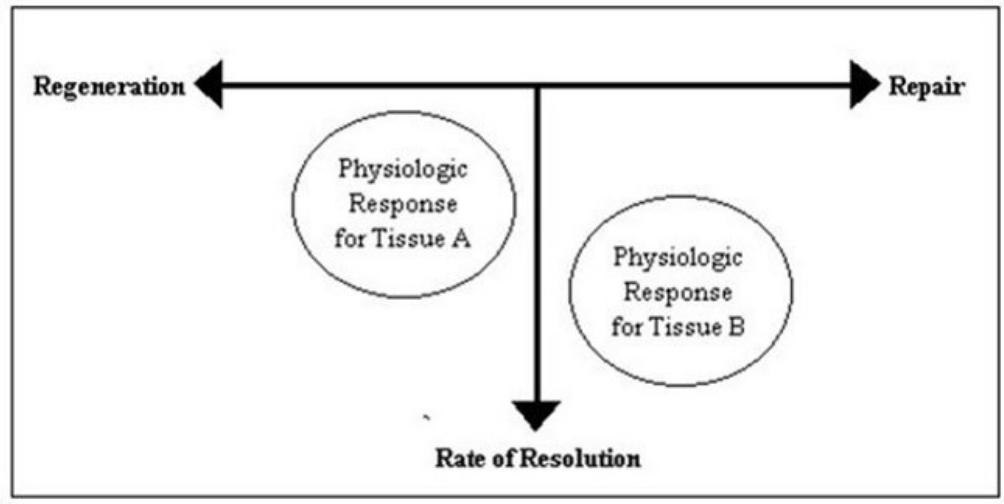

Figure 1. A continuum of tissue responses to wounding

1.2. Embryogenesis and Fetal Healing

During fetal development, cells are guided by an elaborate symphony of timed gene expressions to migrate, proliferate, and differentiate into tissues and organs. This symphony apparently does not play the same song twice, for once embryogenesis is complete, many of these gene expressions never again occur in the same pattern. The pool of undifferentiated stem cells becomes progressively depleted with time and cells may possess different receptivity to the same signals. This explains the body's inability to regenerate organs or limbs and the progressive loss of ability to heal by regeneration rather than repair. Some species maintain their ability for complete repair, like the lizard, which develops a new tail if amputated in self-defense. Furthermore, rats can rebuild liver within about a week following surgical removal.

Much research is ongoing to better understand the differences between prenatal and postnatal healing and the implications for converting reparative healing, such as fibrosis after myocardial infarction, to a regenerative process.

Fetuses possess a limited ability to regenerate some tissues after sustaining a wound, often termed scarless fetal healing. This response is most apparent in the first and second trimesters, but disappears thereafter. Research indicates that this difference in healing may be attributable to multiple factors, including the sterile amniotic fluid, which is rich in extracellular matrix components such as fibronectin and hyaluronic acid, and growth factors, especially insulin-like growth factor (IGF-I) and hyaluronic acid stimulating factor (HASF). Experiments with fetal sheep have shown that the fetal tissue oxygen tension is less than half that of adults (16 mm Hg vs. 45–60 mm Hg), an interesting point since experimental evidence has shown that healing in human adults is facilitated by enhanced tissue oxygenation. The fetal response to wounding involves a different pattern of TGF-β expression from that seen in adult wounds. The exact role of TGF-β in regenerative healing is under intense investigation.

1.3. Prototypic Wound

Although every tissue has a characteristic response to healing, all tissues, excluding perhaps the central nervous system, follow the same general pattern of resolution. This fact has enabled research conducted on a specific tissue to be broadly applied to our understanding of healing in other tissues. An enormous amount of research has been conducted using excisional skin wound models, which lend themselves easily to experimental rigors. For the purpose of this discussion, the prototypic wound is an excisional skin wound that disrupts the epidermis and dermis, leaving an open deficit with separated wound edges.

The unwounded epidermis consists of stratified epithelial cells of ectodermal origin. The surface layer of cells is continually desquamated and replaced by the mitotic activity of a single basal layer of cells. These polygonal to cuboidally shaped cells become engorged with keratin, harden, and flatten as they approach the surface, providing a waterproof defense against microbial invasion, preventing dehydration, and assisting with thermoregulation.

Deep to the epidermis is a sheet-like acellular basement membrane produced by the epidermal basal cells. It consists of collagen (types IV and VII), glycosaminoglycans (GAGs) such as heparan sulfate, and anchoring proteins such as fibronectin, vitronectin, and laminin. Anchoring proteins bind cellular adhesion molecules to tether the epidermis above and the papillary dermis below. The dermal–epidermal junction is highly convoluted—a system of rete ridges that conveys additional resistance to shearing forces.

The dermis is derived from mesenchymal mesoderm and consists of a sparsely cellular matrix of fibroblasts, collagen, elastic fibers, GAGs, proteoglycans, and other support proteins. This matrix is approximately 90% cross-linked and aggregated collagen fibers arranged in orderly bundles. There are two distinctive layers: the superficial papillary dermis and deeper reticular dermis. The thin papillary dermis abuts the basement membrane, has perpendicularly oriented collagen and elastin, and relatively more amorphous matrix. The reticular dermis is much thicker with broad prominent bundles of collagen and elastin oriented parallel to the surface. Also present in the dermis are small blood vessels, mast cells, deeply seated epithelial appendages, sensory receptors,

and nerve endings. The dermis supports the thinner epidermal layer and functions to protect the underlying tissues and support sensory reception. The dermis also conveys strength, flexibility, and elasticity to the skin.

The task of healing in this prototypic wound is to replace each of these components. The degree to which these components are regenerated versus repaired determines the degree to which full functionality is restored to the tissues.

1.4. Main Cellular Effectors

Wound healing is largely orchestrated by the concerted activity of specialized cells that circulate in the blood in readiness to respond. These cells, collectively called leukocytes, are constitutively and inducibly produced in the bone marrow. Each member of this group has a characteristic role to play in the resolution of a wound.

Neutrophils—the most important polymorphonuclear cells (PMNs)—are specialized for the clearing of pathogens and debris from a wound site. They possess a repertoire of enzymes for bacterial killing and the digestion of cellular debris. Myeloperoxidase, Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, and catalase are particularly important enzymes that use molecular oxygen and its derivatives to produce antibacterial levels of reactive oxygen species. In addition, neutrophils secrete cytokines into the wound that serve to activate other adjunct cellular responses.

Monocytes are recruited from the bloodstream to enter a tissue and become macrophages. Macrophages are specialized phagocytic cells that kill bacteria, clear debris, and release important cytokines and other products into the wound. These include antibacterial enzymes, matrix-degrading enzymes, complement components, coagulation cascade factors, nitric oxide, and eicosanoids.

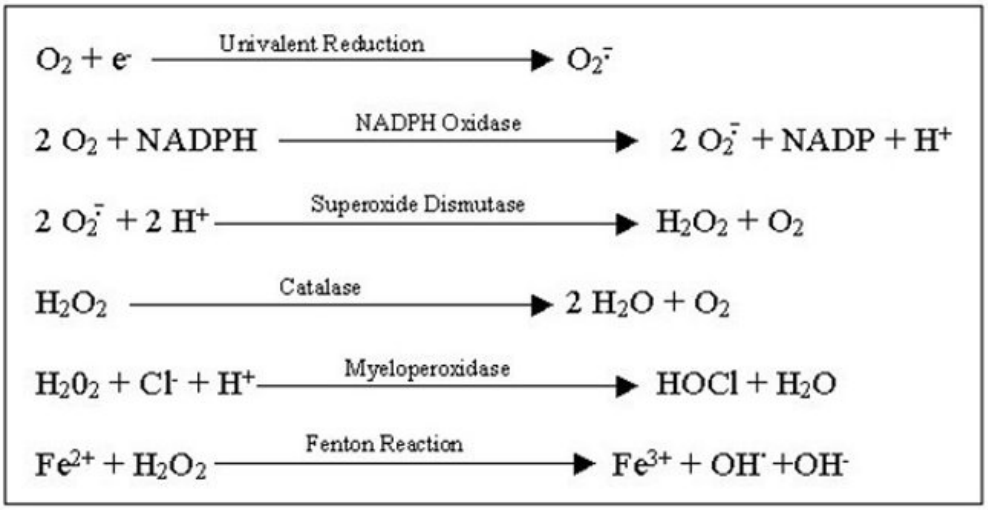

Figure 2. Several enzymatic reactions involving the formation of oxidants

Basophils—also classified as PMNs—are recruited from the bloodstream to become mast cells that reside in tissues adjacent to blood vessels. Mast cells manufacture and store heparin, histamine, leukotriene-3, and other proteoglycans in granules. Exocytosis of granules, termed degranulation, plays an important role in the early management of a wound.

Lymphocytes, including B cells, T cells, plasma cells, natural killer cells, and others, are responsible for mounting a precise, sustained, and remembered defense against invading organisms, involving antibody production and cell-mediated attacks. Although the processes of these "special forces" are important in the ultimate decontamination of a wound, and therefore wound healing, their complex activities are beyond the scope of this article.

Besides the several leukocytes briefly described above, other cells are also involved in healing. Platelets are the first cells to enter the wound and are important mediators of blood clotting. These cells are small disc-shaped anuclear cells that fragment from larger precursor cells. Platelets contain granules storing coagulation factors, serotonin, thromboxane, adenosine diphosphate (ADP), calcium ions, lysozymes, catalase, and other molecules. These agents stimulate clot formation and assist in the recruitment of other cellular effectors to the wound.

Fibroblasts are cells of mesenchymal mesoderm origin. These cells produce the proteins and GAGs of extracellular matrix. The induction of fibroblast proliferation, migration, and protein synthesis is responsible for filling a wound deficit and conveying tensile strength to the healed site. Myofibroblasts are modified fibroblasts that contain contractile elements; they are normally inconspicuous and play a role in decreasing the size of the wound.

Endothelial cells form and line the interior of blood vessels. All cells rely on local blood vessels, which may be destroyed during wounding, to refresh the interstitial fluid in which cells are bathed. Without refreshing, the interstitial fluid becomes depleted of needed metabolic fuels and resources, such as oxygen, glucose, water, and ions. The interstitial fluid also accumulates toxic levels of metabolites, such as bicarbonate, ammonium, hydrogen ions, and other molecules that require central detoxification. A critical component of wound healing is the induction of endothelial cell migration and proliferation, termed angiogenesis. Viable tissue will only be deposited in proximity to the expanding blood supply.

1.5. Cytokines and Growth Factors

The activities of cellular effectors are directed by molecular messengers called cytokines. Cytokines are polypeptides produced and secreted by cells in response to a specific stimulus. In turn, these cytokines bind with cellular receptors and transmit a preprogrammed message that effects another response. Cytokines can affect the same cell from which they were released (autocrine), stimulate responses within the cell before external release (intracrine), and stimulate cells in close proximity (paracrine) or cells located some distance away, using the vascular system as a transport highway (endocrine).

The cytokine messenger and cellular effector system can be compared to the military, where the directives are cytokines and the personnel are cells. Consider a military general who gives a broad command to the chiefs of staff. The chiefs distribute more specific commands through many ranks of officers, and finally, the platoon leaders provide directives to the actual troops who will directly maneuver and effect the results ordered. To achieve a successful, cohesive effort, the many units of troops must each receive directions, far more precise and specific than the general's, that use the specialized training and weaponry a particular unit possesses. This necessitates an increasingly specific translation of the general's command as it passes through the ranks of officers, who analyze current intelligence and determine which specialized troops to recruit for the assignment. Cytokines are the communications that pass between different types of cells, ordering the strategic performance of specific assignments that coordinate to heal the wound.

Cellular response algorithms are preprogrammed within the genetic code. Cellular responses to cytokines are elicited by receptor–cytokine binding, the orderly transduction of this signal, and subsequent intervening intracellular reactions that ultimately promote or inhibit transcription events. A specific cytokine may have broad effects on many cell types, a specific effect on only a few cell types, or an effect that varies with the cell type and the environment. Additionally, different cell types may be programmed to respond to the same cytokine at different rates. For this reason, cytokine release and effect mapping has proven challenging despite enormous research efforts. However, it is clear that, in the absence of pathology, cytokine release and cellular responses follow a precise, orderly, and sequential process in a highly repeatable pattern that eventually produces wound resolution. The further elucidation of this process and pattern is the goal in continued wound healing research.

2. Phased Healing Response

The healing process can be described in terms of inflammatory, migratory, proliferative, and late response phases. Each phase has characteristic cellular effectors and tasks to achieve. These phases tend to overlap and can be derailed by pathologic processes.

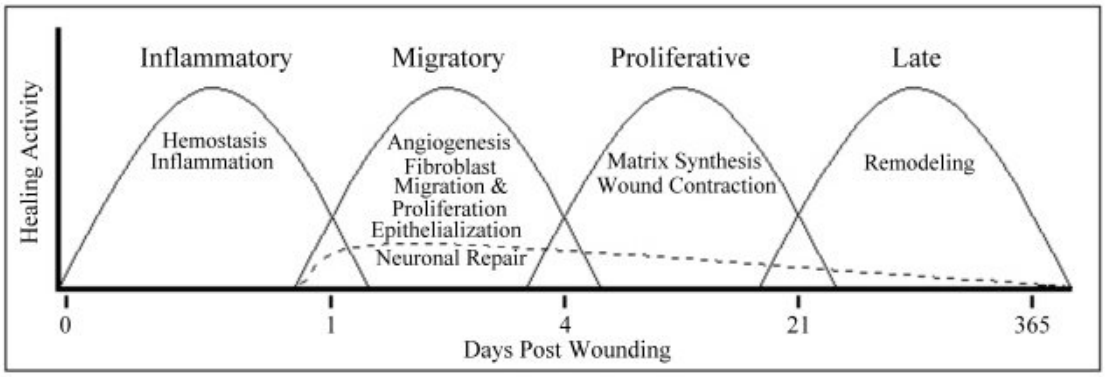

Figure 3. Phased healing response
Source: Adapted from W.T. Lawrence (1998).

2.1. Inflammatory Phase: Hemostasis and Inflammation

The earliest phase begins on wounding and continues through the first 24 hours. This is considered the acute response period. The accomplishment of definitive hemostasis and inflammation within this time frame are critical for host survival.

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Biographical Sketches

Laura M. White completed her B.Sc. degree in Nutrition at the Ohio State University in June 2000. She is training to receive a degree in Medicine from the Ohio State University College of Medicine in 2004. She is a recipient of the Dr. R. S. Hosler Memorial Educational Foundation Scholarship and the Samuel J. Roessler Memorial Research Scholarship. As a Research Assistant in the Laboratory of Molecular Medicine in the Dorothy M. Davis Heart and Lung Research Institute, Ms. White has participated in a study of the therapeutic significance of oxygen in wound healing. In June 2001, she founded The Wound Healing Journal Club—a student organization sponsored by the Department of Surgery, Office of Research at the Ohio State University Medical Center. This group focuses intensively on understanding the developing paradigm of wound physiology and evaluating empirical evidence supporting therapeutics used in wound care.

Dr. Sashwati Roy received her Ph.D. in Physiology/Environmental Sciences from the University of Kuopio in Finland in 1994. She joined the University of California at Berkeley in 1995 as a Postdoctoral Fellow, and her area of focus was redox-regulation of signaling processes in endothelial cells. Two years later, she was promoted to Assistant Research Biochemist. She continued to work in that capacity until her recruitment as Scientist to the Lawrence Berkeley National Laboratory in 1999. In September 2000, Dr. Roy joined the Ohio State University where she was appointed as a Research Scientist in the Department of Surgery and Davis Heart and Lung Research Institute. Currently she is working on the redox-aspect of inflammation and aging. She has published over 50 scientific articles and has lectured in over ten countries.

Dr. Gayle M. Gordillo graduated with a B.A. degree in Psychology from Stanford University in 1984. She received her Medical degree from the Ohio State University in 1990. She completed her residency in general surgery at Ohio State, followed by a research fellowship in the Division of Transplantation, which was completed in 1997. She was a plastic surgery resident from 1997 to 1999. Dr. Gordillo currently holds the position of Assistant Professor of Surgery in the Division of Plastic Surgery.

Dr. Loree K. Kalliainen graduated with a B.Sc. degree from Michigan Technological University in 1987. She received her medical degree from the University of Michigan in 1991 and did three years of a general surgery residency at the University of Iowa. She then transferred to the University of Michigan where she was a research fellow for two years in the Muscle Mechanics laboratory with Dr. William M. Kuzon, M.D., Ph.D. Her areas of study were muscle force losses with injury and aging, and novel methods of nerve repair. Following this, she entered the Plastic Surgery program at the University of Michigan and graduated in 1999. She did a Hand Surgery fellowship at the University of Virginia from 1999 to 2000 and currently holds a position as an Assistant Professor of Surgery at the Ohio State University. Her current research focuses on nerve recovery after injury and development of animal

models for wound healing studies. Clinically, she focuses on injuries and deformities of the hand and upper extremity as well as the management of complex wounds.

W. Scott Melvin is an Associate Professor of Surgery appointed to both the James Cancer Hospital and Solove Research Institute and the College of Medicine and Public Health of the Ohio State University in Columbus, Ohio. He also serves as Chief of General Surgery and Director of the US Surgical Center of Excellence for Minimally Invasive Surgery at the Ohio State University Medical Center. Dr. Melvin's main area of interest is gastrointestinal surgery, with special emphasis on laparoscopy and use of computer-enhanced surgical systems. He has been Principal Investigator for numerous studies, including prevention of serious infections in patients undergoing upper gastrointestinal surgery and outcomes studies of laparoscopic methods versus open surgery. He is a Fellow of the American College of Surgeons and of the International College of Surgeons and a member of the Society for Surgery of the Alimentary Tract and the Association for Surgical Education. He also serves on the Board of Governors of the Society of American Gastrointestinal and Endoscopic Surgeons. He was Elected President of the Columbus Surgical Society in 2001. With a background in biology and chemistry, Dr. Melvin earned his medical degree from the Medical College of Ohio. He went on to a general surgery residency at the University of Maryland School of Medicine and Maryland Institute for Emergency Medical Services in Baltimore, followed by a fellowship in gastrointestinal surgery at the Ohio Digestive Disease Institute of Grant Medical Center, Columbus, Ohio. As a member of the faculty at the Ohio State University College of Medicine and Public Health, Dr. Melvin has won numerous teaching awards, selected by both surgical residents and medical students alike. Dr. Melvin is married and has three children.

E. Christopher Ellison MD is a 1976 graduate of the Medical College of Wisconsin. Dr. Ellison served his internship and residency in surgery at Ohio State. During his residency he completed two years of basic research with emphasis on multiple organ failure in pancreatitis and pathophysiology of gastrointestinal peptides. He served as a clinical cancer fellow through the American Cancer Society. Joining the Ohio State University faculty in 1984 as Assistant Professor of Surgery, Dr. Ellison continued his research and surgical practice, focusing on benign and malignant disease of the pancreas, liver, and biliary system. In 1987 Dr. Ellison became Codirector of the Ohio Digestive Disease Institute and Clinical Assistant Professor of Surgery. He was a founding member of this multidisciplinary center for research, treatment, and community education related to disease of the gastrointestinal system. He was instrumental in developing and directing a fellowship for advanced training in hepatobiliary and pancreatic surgery. Dr. Ellison has directed and taught postgraduate courses in basic and advanced laparoscopic surgery. He has written chapters and peer-reviewed articles on the diagnosis and treatment of disease of the liver, pancreas, and biliary system. Dr. Ellison was appointed Robert M. Zollinger Chair and Chief of the Division of General Surgery in addition to promotion to Associate Professor at the Ohio State University in 1993. He was promoted to Professor and appointed Interim Chair of the Department of Surgery in 1999 and subsequently appointed Chair of the Department of Surgery in 2000. He was also appointed Associate Dean of Surgical Services in 2001. Dr. Ellison is past president of the Columbus Surgical Society. He has been elected to membership in the Society of University Surgeons, the Central Surgical Society, American Surgical Society, and the American Association of Endocrine Surgeons, and is a member of the Board of Governors of the American College of Surgeons.

Dr. Chandan K. Sen completed his M.Sc. in Physiology at the University of Calcutta, India in 1990. In the same year he enrolled for a Ph.D. program in Physiology at the University of Kuopio in Finland. In January 1995, he moved to the University of California at Berkeley as a postdoctoral fellow in the Department of Molecular and Cell Biology. In 1996 he was promoted to Assistant Research Biochemist in the same department. In 1998, Dr. Sen was appointed as a Staff Scientist in the Lawrence Berkeley National Laboratory in Berkeley, California. After two years in this position, he moved to his current position at the Ohio State University Medical Center as the Director of the Wound Healing Research Program and Vice Chairman of Research in Surgery. During the last six years, Dr. Sen and his associates have published over 100 research papers and several books on oxidants and antioxidants. He directs the Laboratory of Molecular Medicine and the Genetics/Microarray facility at the new Dorothy M. Davis Heart and Lung Research Institute at the Ohio State University Medical Center. Dr. Sen directs an NIH-funded research program addressing the role of oxygen in wound healing.