THE PHYSICS AND PATHOLOGY OF WOUNDS

PART 1: THE WOUND AS A SYSTEM AND A CONTROLLED MACHINE

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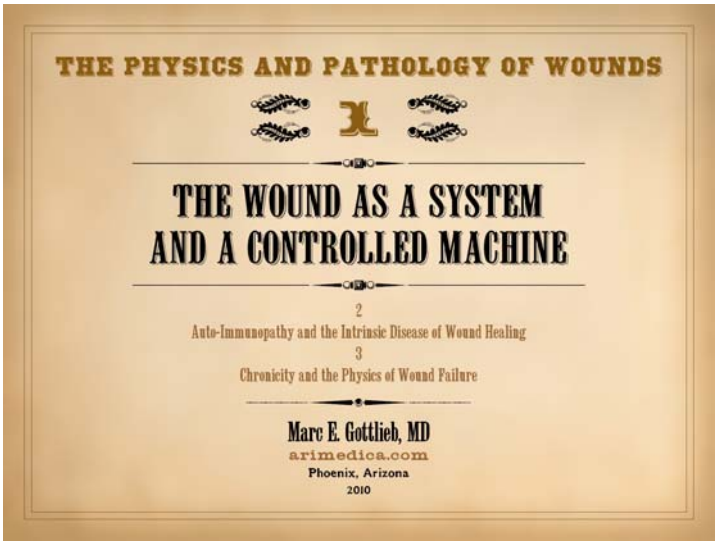
Preamble
This is the first of a series of three presentations that will explore the origins of intrinsic chronicity and wound healing failure in chronic and pathological wounds. This is **Part 1**, The Wound as a System and a Controlled Machine. It will explain the wound as a complex system, including principles of control and non-linearity. It will explain the wound as a system in the language and science of systems – Physics. It will explain why control is not only the basis for all wound dynamics, but why this is crucial to the functions of the healthy wound and why pathological wounds misbehave. In **Part 2**, Auto-Immunopathy and the Intrinsic Disease of Wound Healing, we will go from a physics-engineering perspective to a clinical-pathological one. The general stroma, the auto-immune connective tissue disorders, and the chronic wound will all equated through the principle of sustained chronic inflammation leading to immune sensitization against stromal elements. In **Part 3**, Chronicity and the Intrinsic Disease of Wound Healing, we will bring together the engineering aspects of the wound as a controlled process and the clinico-pathological aspects of intrinsic auto-immune wound chronicity to understand why chronic wounds fail to heal.



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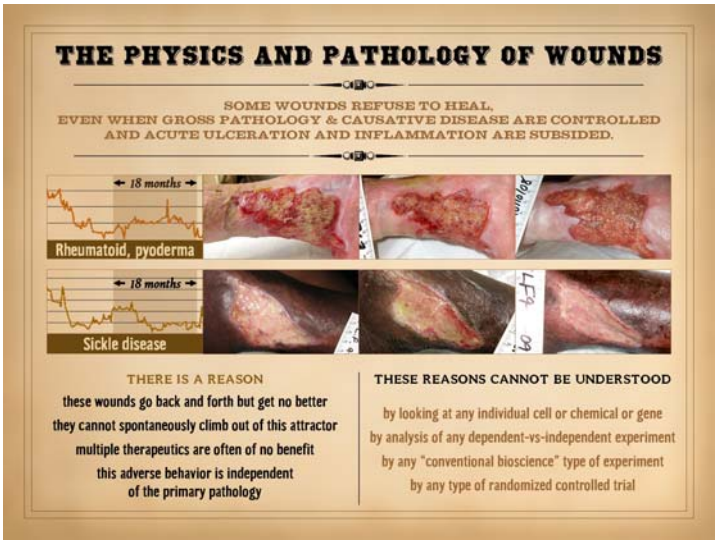
The Physics and Pathology of Wounds
Part 1: The Wound as a System and a Controlled Machine
Part 2: Auto-Immunopathy and the Intrinsic Disease of Wound Healing
Part 3: Chronicity and the Physics of Wound Failure

These presentations are titled "The Physics and Pathology of Wounds". Physics and Pathology might seem like an odd juxtaposition, but it gets more peculiar. What does auto-immunity have to do with dynamical chaos? What does wound failure have to do with controlled machines? If these associations seem incongruous and "anti-biological", it is only because conventional biosciences tend to focus on classical biology, cell biology, biochemistry, and the discrete interactions between paired elements within biosystems. Understanding and analyzing complex systems, as engineers would do, is generally a foreign concept in biology. Yet all biology, large scale and small scale, is a conglomeration of complex systems. Understanding how the many elements in a complex system inter-operate to determine the timewise or "dynamical" behavior of that system depends on the science of systems and complexity, and that science is within the domain of physics.

The overall purpose of these presentations is to explain a theory and a set of observations and hypotheses about why certain chronic wounds will not heal. The thesis concerns the "intrinsification" of the wound due to the onset of stromal auto-immunization and the appearance of chronic lymphoid inflammation. This pathological state of chronic auto-immunization against the wound must be understood in part by the conventional bioscience discoveries of the relevant cells, chemicals, and other players on the stage of injury, inflammation, and wound healing. However, conventional biosciences cannot readily explain the persistent failures or incompetencies of these wounds, even after all primary disease and injury have been relieved, and even after wound healing promotional therapies have all been tried. The true understanding of these impaired wounds and systems depends on a knowledge of complexity, non-linear dynamics, population dynamics, and self-organization – i.e. physics. To understand how this all goes wrong, we will start by looking at what happens in the complex wound system when it is healing properly. As we will now see, there is an orderly set of dynamics that governs the healing process. It is a reference-driven, feedback-regulated process – a control system – that ensures the proper behavior and output of the repair process. Control is common and deliberate in technological and human engineered systems, and hence it is easy to liken a control system to a machine. However, control is also an inherent, innate, and obligatory state of most biological systems, and without control, most biological systems would extinguish. This is not only true for wound healing, but the control loop of wound repair is easy to discern, define, and describe. It is the basis for understanding the physics of chaos, populations, and self-organization which will be necessary to understand why lymphoid intrinsification of the wound is so ill behaved.



There is a generic approach to wound treatment that is the same as for any other condition in medicine where there are acute and chronic phases of the illness. The first duty of the clinician is to get active disease under control, usually with a fairly standard set of therapies, to prevent its progression, avert jeopardy to life and limb, and alleviate symptoms. Once acute disease is controlled, you then begin managing subacute or long term aspects of the illness, to either cure the problem or make it manageable for the patient, usually with many discretionary choices to be made based on patients' individual needs. So, for instance, the ruptured colon gets a laparotomy and colostomy, and when the patient is recovered, you make all of the other necessary choices based on the primary diagnosis (diverticulitis versus trauma, etc). For patients in diabetic ketoacidosis and hyperosmolar coma, they get insulin and fluid and electrolyte management, and when they are recovered, you can start to plan their long term dietary and pharmacological management.

For wounds, we control acute disease, injury, thrombosis, necrosis, inflammation, bioburden, edema, ischemia, etc., until the wound no longer threatens the patient. The physiology of wound healing is such that it isn't going to heal anyway until these things are controlled. Once they are controlled, you then begin to pick and choose treatments that will get the wound healed, or get it healed faster, or make it easier to live with. The basic model of medical care is the same for wounds as it is for anything else. For the colostomy patient above, the discretionary planning is simple enough, based on primary disease and patient status and wishes. Teaching him how to use a colostomy bag or else restoring the continuity of the colon are easy enough, and barring the occasional complication, they are predictably effective. The diabetic patient will have a variety of choices to make about diet, weight, exercise, and medications. If the patient is incompetent or non-compliant, treatment will fail, but the disease itself remains inherently responsive to such therapies, and good diabetic management is the norm for a cooperative patient. So, for wounds, we apply the same principles of care, and what happens?

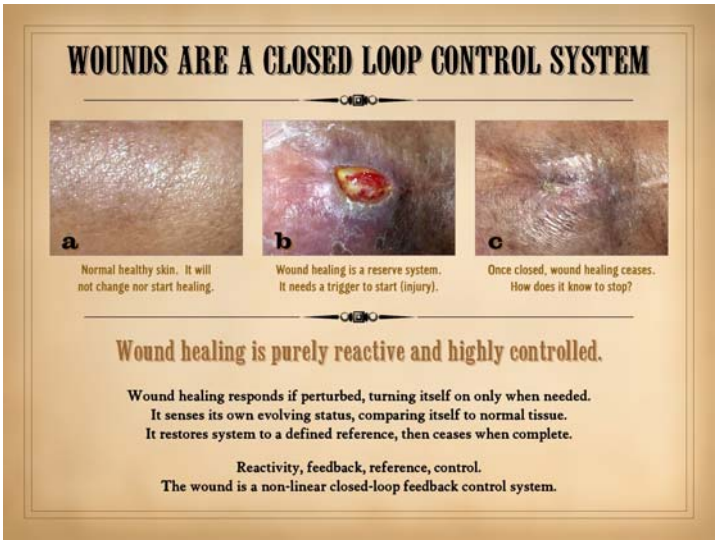
The first answer is that, yes, we certainly have lots of successes. However, anyone who deals with chronic and pathological wounds knows that, while the principles are sound, actually getting some wounds to heal is not so easy. For some wounds, in spite of all due diligence and wile, in spite of 82 different therapies you have tried, ranging from voodoo and shamanism through expensive operations, and everything in between, some of those wounds just will not budge. They are stuck where they are, mocking every feeble effort you make to show them who's boss. They refuse to heal even when gross pathology and causative disease are fully controlled and acute active ulceration and inflammation are fully subsided.

Case 1, upper: This is a 76 year old woman with long standing rheumatoid arthritis and a refractory ankle ulcer. The three images are over an interval of 18 months. The graphic curves show wound size. An initial improvement early in the course of treatment is typical of most patients – we can almost always make improvements by instituting good care or doing some surgery. However, after those early gains, progress and wound size level off. Month-to-month there will be slight variations in size and appearance, but no net gain over long intervals. During this period of time, many technology based modalities and surgical procedures were tried.

Case 2, lower: This a 35 year old woman with sickle disease and a refractory ankle ulcer. The three images are also over an interval of 18 months. All of the comments about the first case apply here. Despite persistent care and multi-modality approaches to care, the wound just will not cooperate. Yet in both cases, they look mostly like they should be healing. The wound surfaces are somewhat altered from normal, but there is no gross inflammation, i.e. no edema, erythema, active ulceration. The periwound is healthy. Why would these wounds remain so refractory when all of the features and history, natural and therapeutic, suggest that they should have healed months ago?

It is the explicit purpose of this series of three lectures to show that there is a reason for this frustrating and confounding behavior. It is a real world physical reason. However, the answer does not lie with any single gene or protein or receptor or cell or organelle or whatever. It is a reason that relates to the inter-operations of all of them. Understanding them means understanding the generic principles of how inter-operating systems work, and that means understanding their relevant physics;

In considering these problematic wounds, there is a reason: these wounds go back and forth but get no better; they cannot spontaneously climb out of this attractor; multiple therapeutics are often of no benefit; this adverse behavior is independent of the primary pathology. Furthermore, these reasons cannot be understood: by looking at any individual cell or chemical or gene, nor by analysis of any dependent-vs-independent experiment, nor by any other "conventional bioscience" type of experiment, nor by any type of randomized or other clinical controlled trial. Remember, we are not talking about wounds that are sick and getting worse due to active disease. When a wound is sick, you control the underlying disease, and things then either heal or at least remain stable. No, we are talking about those in which you have controlled all adverse conditions, and it should be healing, and it even looks perfectly healthy as though it should be healing, and you are frustrated because you cannot discern any reason why it isn't healing – but it isn't. Over the course of these lectures, the reasons will be elucidated. We start by looking at the most central physics concept that governs these behaviors: non-linearity and control.



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Wounds are a closed loop control system. For the wound which otherwise seems healthy and as though it should be healing, this concept is the starting point for understanding all the other aspects of its apparent misbehavior. We start by looking at an example which at first might seem trivial and obvious to the point of absurdity, but once you genuinely understand it, everything else follows.

The **middle** photo shows a wound. On the **right** it is healed, the same wound. The **left** image is from the same patient, at the same time. It is normal skin. It is not healing. "Of course it's not! . . . So? . . ." you might say. But think about it. The wound started to heal. How did the wound know when to start healing? How is it that it stays confined to the injury, rather than triggering a process of angiogenesis and fibroplasia throughout the body? Why doesn't the normal skin in the left just start healing for no good reason? "Simple", you say, "because it wasn't injured." So, it is a reserve system, but how is that injury tells it to start healing, but otherwise it knows enough to stay asleep? Once it starts healing, how does it know when to stop? The obvious is not necessarily so trivial, and profound principles come in the simplest of observations.

Biological systems almost all have promoters and inhibitors. Some agent tries to make you to do something, and a counter-agent puts the brakes on. If biological systems did not have such promoter-inhibitor balances, they would get out of bounds, racing ahead, exceeding their capacity, overwhelming themselves or their contingent systems, or else extinguishing, failing to achieve or sustain necessary metabolic functions. Biological systems and life are entirely dependent on the ability of promoter-inhibitor agents to keep a system within bounds, to keep it from over-reacting or under-reacting. The ability to react and counter-react, to find the healthy "center", to avoid overwhelming the system or dying out, that is what is meant by control. Nearly all biological processes, and nearly all healthy "homeostatic" states of those processes are being regulated, controlled, to maintain their desired state. Of course, when we say "desired", we are ascribing teleological intentions to the system. In reality these systems find their "centers" because of dynamical and thermodynamical principles that govern how all complex chemical and cellular processes must behave.

Conventional bioscientists are used to thinking about all of the biochemicals and cell structures which are the tangible promoters and inhibitors of such systems. However, when it comes time to understand how all of these chemicals get together and find their balance, it is not possible to do so by chemistry principles alone. Physics and engineering principles are required to understand the dynamics of regulated systems, how they behave and react over time and under the influence of many mutual inter-operating factors. Once you understand the principles by which regulated control systems operate, it is easy to see how the normal wound heals, but also how it goes wrong and how it might be treated when it is wrong.

As a system, the following are crucial properties of the wound: Wound healing is purely reactive and highly controlled. Wound healing responds if perturbed, turning on only when needed. It senses its own evolving status, comparing itself to a reference – normal tissue. It restores the perturbed system to that defined reference, then ceases when complete. The "nuts-and-bolts" of how it senses these things and actuates a response is where conventional biology comes in, but the dynamical responses and behaviors of the system are "device-independent", unconcerned with the details of each molecule or organelle. The reactivity and responses of the system depend on feedback, a reference, and control. These conditions qualify the system as "non-linear" meaning that it is self-dependent, its future value or state being a function of its current value or state (unlike a "linear" function as defined by algebra and calculus where the system value would be a function of an independent parameter). In short, the wound is a non-linear closed-loop feedback control system.

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To understand the dynamics of misbehaving wounds, here are examples of variances or faults – unregulated, broken, atypical attractor – in the operations of the wound healing "machine".

Upper left: No healing. A 6 month buttock ulcer in a 44 year old woman with severe rheumatoid arthritis on cyclophosphamide. There is no evidence of wound healing. There is a slight blush of angiogenesis, but the yellow color of the adipose is still obvious, and the lobular architecture of the fat is 100% unaltered because there is no fibroplasia whatsoever. Likewise there is no epithelialization. Yet there is also no edema, erythema, induration, active ulceration, etc. The wound is not acutely pathological, but it is not healing. This is one of the most dramatic examples of an arrested wound module that you will ever see.

Upper right: No healing. This image shows the tail end of a wound closure or reconstruction using a collagen-gag regenerative matrix (Integra® dermal regeneration matrix). The epidermis on top has not yet grown to confluence, leaving behind open areas of the regenerated dermis. In the clinical or vernacular sense, this is still a wound, and yes, it is healing. The intent here is not to "split hairs", but from a physiological and dynamical point of view, this is not the wound module functioning. Angiocytes and fibroblasts are reassembling a dermal analogue according to the events of embryonic dermatogenesis, and thus they are using a set

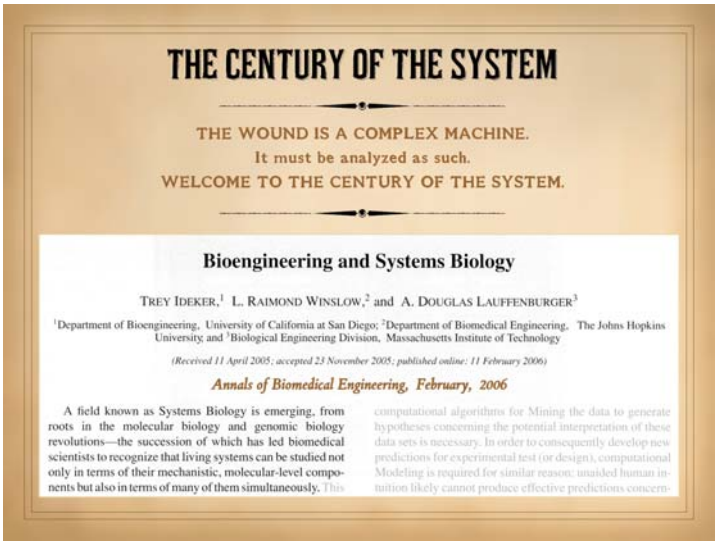
of dynamics and self-assembly sequences which share certain features yet are distinctly different than normal healing. Clinically, this "wound" has a happy favorable status, but it is nonetheless on an alternate or atypical attractor compared to normal wound healing.

Lower left: Improper healing. This is a pyogenic granuloma (more about this on slide 28). It represents healthy and qualitatively normal wound healing gone to quantitative excess. The problem is one of unregulated proliferation because the normal controls or regulators in the system have gone awry. Specifically, the system load (angiocytes and "granulation tissue") is being spoofed by an interloping controller (macrophages in the bandages) operating in parallel to the normal controller (macrophages in the wound).

Lower right: Excess healing. This is a typical keloid, a pathological form of scar hypertrophy that occurs for non-physiological reasons. Compare this to hypertrophic and contracted scar that occurs in response to the mechanical forces applied (tension, such as after an injury across a flexion surface of a joint). Such reactive contractures are clinically problematic, but they are a physiologically correct response to the mechanical forces applied – from a fibroblast's point of view, a proper response to an improper event. Reactive hypertrophy from mechanical metaplasia is also a fully regulated, closed-loop controlled process, just like normal healing, and it will cease when forces in the tissues are balanced. In comparison, the keloid has no discernible correct reason for being the way that it is. It is a condition of unregulated fibroplasia, in which the fibroblasts are either unresponsive to controls or else they are being controlled by abnormal stimuli that mask or overwhelm the normal controllers.

Right: Chaotic. This case is comparable to those shown on slide 2. Over a 1 month and then a 4 month interval, there is no net change. It is in a chaotic "orbit", a state that has a technical meaning from mathematics and physics, which will be explained further in this Part and especially in Part 3 of this series. The whole purpose of this series of presentations is to explain why wounds which behave this way have found a state of relative dynamical and thermodynamical stability from which they cannot easily escape.

From a clinical point of view, most of these behaviors are undesirable and contrary to health or normal unfettered life, From a biological point of view, these examples would generally be seen as pathological variances from normal physiology. This is where a classical bioscience perspective diverges from a mathematics and physical sciences point of view: as unnatural or undesirable as these states are from the medical perspective, they are nonetheless the standard permissible behaviors (dynamics) of controlled and complex non-linear systems, including the wound.


THE CENTURY OF THE SYSTEM

THE WOUND IS A COMPLEX MACHINE.
It must be analyzed as such.
WELCOME TO THE CENTURY OF THE SYSTEM.

Bioengineering and Systems Biology

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A field known as Systems Biology is emerging, from roots in the molecular biology and genomic biology revolutions—the succession of which has led biomedical scientists to recognize that living systems can be studied not only in terms of their mechanistic, molecular-level components but also in terms of many of them simultaneously. This

computational algorithms for Mining the data to generate hypotheses concerning the potential interpretation of these data sets is necessary. In order to consequently develop new predictions for experimental test (or design), computational Modeling is required for similar reason: unaided human intuition likely cannot produce effective predictions concern-

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Within the past decade, at the very turn of the 21st century, the human genome was first sequenced. Before and much more ever since, the complete DNA sequence for many species has been read. There was a general sense – very naive – that all things in human pathology and clinical medicine would now be understood and fixed by some gene therapy. That of course makes no more sense than the pundits who a century earlier thought that all disease was caused by a microbe and could be cured by their elimination. The fact that only about 30,000 genes were found in the human genome was also a surprise – way too few to explain the myriad and countless elements of biology that are already well understood. There are many points of extraordinary naivete in the initial premise of "discover all the genes and all will be revealed". First, it turns out that there is extra non-gene DNA in the genome, and that does something too, so regulators on genes as much as genes themselves might have something to do with the actual living biology. Then, the genes only encode for a few structural proteins (e.g. collagen), and the rest (enzymes) are catalysts and process regulators for conventional chemistry. Gene expression is through additional self-assembling intermediaries (e.g. RNA), and the results of proteomic activity are the many thousands of small biochemicals that float in the system. After that, interactions between chemicals makes other chemicals, and don't forget their degradation products which also have bioactivity. Add to that mix the effects of simple ionic, acid-base, and inorganic chemistry, and by the time you are done, the number of unique molecular species in the body with a biologically relevant role numbers in at least the hundreds of thousands, and more likely the millions. Just knowing the names and spelling of 30,000 genes is necessary but remotely far from sufficient to understand the workings and failings of the human body.

When you look at how 30 thousand or 30 million agents inter-operate, you are looking at a "complex system". In fact a system of just 1 or 2 agents can have complex behaviors, and from the point of view of mathematics and physics, any system of 3 or more agents is ipso facto complex. What actually do we mean by "complexity"? There is no exact definition. In part, complexity has the same meaning that it does in vernacular speech – intricate, detailed, and highly textured, structured, and interconnected. Technically, it also means that a system is non-analytical, i.e. it cannot be described by the functional relationships of algebra and calculus. The study of complexity and complex systems is rolled up in the physics subtopic of non-linear dynamics. Some historical perspective is needed here. Until the 21st century, biosciences were wrapped up in the very important (and well-funded) explorations-on-the-frontier of biological chemistry (including cell biology). These explorations focused on characterizing the structure and core chemistry of biological chemicals, and characterizing their simple one-versus-another reactions and interactions in isolated laboratory circumstances. Evaluating complex in vivo activities, especially those involving multiple interacting elements that cannot be isolated into one-versus-another experiments were deprecated as unworthy of serious study. There was the doctrine in the biosciences that all studies must be conducted as one-versus-another with everything else held static. To be fair, there was a doctrine in all of the natural sciences that all events must be characterized as a properly defined differential equation of one variable versus another. To do otherwise, to "solve" problems with iterations, interpolations, and piecewise approximations were the cheap floozies of the mathematical sciences, prostitutes of necessity to be indulged by engineers who needed their services, but not the kinds of algebraically respectable women you might take home to your mom the math professor.

These comments are in no way a derogation of the science of the 20th century – obviously extraordinary amazing mind-boggling discoveries and applications were achieved. But many questions about the natural world could not be solved with 20th century science. Those who derided any effort to study complex system-wide interactions were simply acknowledging the fact that there were no tools at the time to study such systems, so why waste your time? Trying to solve or model the overall integrated operations of interconnected complex systems was beyond the analytical or computational means of those times. Systems science was simply not possible until the 1970's or 1980's. What changed was the advent of theoretical foundations for non-linearity, the creation of computational technologies that can implement the theories, foundational concepts in complex modeling as well as the acceptance of sophisticated piecewise approximators such as the finite element method, and the education of a new generation of scientists not shackled to old doctrine. The history and psychology behind this is explored further on slide 18. Sadly, systems science since the 1980's has caught up slowly – barely at all – in the biological sciences. Now that we have elucidated the genome, we still know hardly anything about how those 30,000 genes do their business and lead to disease. Some of those answers will come from the methods of conventional 20th century biology, studying each gene per se or its immediate protein product. However, many answers will not come unless the complex interconnected multi-factorial systems that these genes encode are studied as such – complex systems – using the mathematics and science of complexity and systems. The study of systems and complexity has come to the biosciences more slowly than for the physical sciences, but it is starting: *"A field known as Systems Biology is emerging, from roots in the molecular biology and genomic biology revolutions – the succession of which has led biomedical scientists to recognize that living systems can be studied not only in terms of their mechanistic, molecular-level components but also in terms of many of them simultaneously."* (Ideker T, Winslow LR, Lauffenburger DA. Bioengineering and systems biology. Ann Biomed Eng. 34(2):257-64 and 34(7):1226-33, 2006.)

So, welcome to the Century of the System. For the past 150 years, we have been trying to explain everything based on biochemistry and 19th concepts of science. Now we are in the 21st century with new methods and new discoveries to answer both new questions and the unanswered questions of prior eras. **Old science:** Physiology of the 20th century, an age of biochemical discovery, was grounded in chemistry. It focused on one-on-one reactions and kinetics between any two chemicals or bio-parameters. It promulgated "homeostasis", predicated on chemical concepts of reaction equilibrium. Physiology research depended on linear models of dependent-versus-independent parameters in an otherwise invariant environment. For wounds, this classic style of research has characterized hundreds or thousands of cellular and chemical interactions. But biological systems do not really work that way. They are built from many simple elements of that nature, but they have MANY levels of inter-operation, defining them as non-linear and multi-control. The mutual integrated behavior of such systems cannot be assessed analytically by simple balanced equations. **New science:** How do 30,000 gene products and a bazillion derivative chemicals interoperate to make Life? To understand complex systems, principles are needed from the sciences of complexity, the principles of non-linear dynamics and control science. The wound is a paradigm of a complex n-body non-linear multi-control system. Its physiological behaviors and pathological misbehaviors can be readily explained when wounds are understood as a System, rather than as just a collection of dual-element linear interactions. Accurate understanding of its many behaviors must start with a meaningful model of the whole machine, the System, and not just its chemistry-oriented individual components. As a controlled system, the quintessential intrinsic machinery of wound healing – the "Wound Module" of proliferative post-inflammatory wound repair – functions as just a single control loop. This presentation, Part 1, will introduce basic concepts of closed loop control and then characterize the Main Control Loop of normal physiological wound repair.

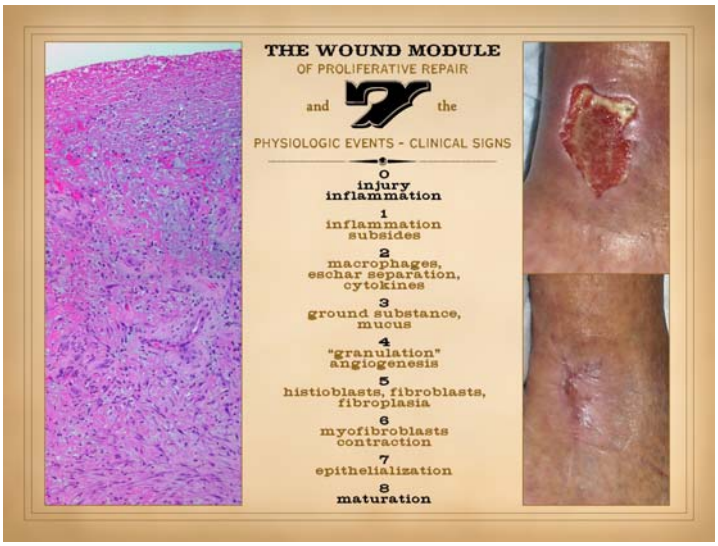
The wound is a dynamical system – meaning that it evolves in time. That is what defines a dynamical system – its dependence on and evolution in time. Many problems in physiology and pathology are dynamical, even if bioscientists are not used to thinking of them that way. However, every time you invoke the word "pathogenesis" you are making that implication. Of course, for the sake of daily medical practice, many diseases need not be thought of that way. For many clinical problems, an issue may seem static without variance, or else it may evolve in time in a trivial one-way smooth slide from here (healthy) to there (sick) without any irregularity. For example, hyperparathyroidism could be seen as being a relatively static hyperactivity of parathyroid cells and elevation of parathormone. The progressive state or evolution of the parathyroid glands or cells per se is not of much relevance to its complications and clinical care. How hyperparathyroidism then leads to bone dystrophy and possible renal failure is much more clearly a dynamical event that evolves in time. However, if untreated, the states of bone and kidney are likely to get progressively worse in a generally "linear" or smooth profile going steadily from good to bad to worse, with no back-tracking nor ups-and-downs. If a parathyroid adenoma is removed, then the problem is resolved, and further timewise misbehavior of the system will not occur.

Compare this to a patient who has congestive heart failure, aortic stenosis, coronary artery disease, low ejection fraction, emphysema, pulmonary hypertension, systemic hypertension, and hypertensive nephropathy with less than half of normal glomerular filtration, who then gets pneumonia or a broken hip or acute diverticulitis. It is the perfect recipe for mortal disaster, yet such a patient can be managed and with good outcome. Good care and outcome is contingent on the intensive care unit where moment-by-moment monitoring and treatment are implemented. We do the constant monitoring and treatments because the state of that patient can vary from moment-to-moment. The dynamical nature of the problem is intrinsically understood by all, even if most doctors have never explicitly studied that situation in such engineering or mathematical terms. Each time we take a set of clinical measurements, we are assessing the state of the system. We "feedback" this information into the system by comparing current values to target values, then calculating corrections. Each time we then implement a therapeutic correction, we are controlling some vital component of the system, which hopefully responds by bringing the system back toward the target values. What we have just described is a feedback-regulated closed-loop control system. While this may sound like engineering terminology, something designed into a machine, the reality is that human engineered systems are simply doing what biological systems do naturally – trying to regulate a system at a desired value.

In normal healthy biology, ALL systems are feedback regulated and controlled around some defined value. This is the origin of traditional concepts of "homeostasis". The reality is that biological systems, from the folding and resonance of a single peptide through the integrated functions of the cardiopulmonary system MUST stay within certain physiological parameters if the host is to be healthy. If systems or parameters tend to wander out of allowable bounds, or if they get kicked out by some perturbation, then something else senses the variance and reacts to bring things back within bounds. Thus, parathormone, calcitonin, calcium, and phosphorus levels are all sensed, and parathyroid, bone, and calcium metabolism all stay within healthy bounds. In contrast, the parathyroid adenoma has become unresponsive to controls, ramping the rest of the system out of bounds, an unhealthy state of pathology. Every physiological system in the body, from control of heart and respiratory rate, to maintaining the proper balance and trigger point of the plasma protein thrombosis system, to generating the correct density of capillaries within a tissue, to tracking an object with your eyeball and pressing the correct button on your remote control without a tremor or a miss – EVERY embryological and EVERY

physiological system is feedback regulated and controlled, and physiological parameters stay within healthy bounds. When the body can no longer regulate, when parameters get out of bounds, that is pathology, that is illness.

For the sake of this whole discussion, when we say "wound", we are referring to the physiological process of repair, i.e. the wound healing system. Of course, "wound" can mean many things, from the injury to the defect to the repair process – one term for various interrelated concepts. The intended meaning usually should be clear from the context, but to be explicit, much of the use here of the word "wound" will refer to the wound process, the wound physiology, the repair system that puts things back together after an injury or disease makes a defect or triggers an inflammation-repair response. That said, the wound is a system. When healthy, it operates to correct an injury or defect in the body. It gets back to a normal tissue architecture by following the pathway of a closed-loop reference-driven feedback-regulated control system. When it is healthy and in bounds, it stays in bounds with little activity or energy. When rocked out of bounds by some perturbation (injury), it responds to drive the system back to a stable restored tissue. This process is a sequence of integrated events that occurs over time, and thus the wound is a dynamical system. Time, dynamics, control, stability – all of these terms and concepts are mutually contingent and intertwined in a properly functioning or healthy system, regardless whether we are talking about the flight controls on a rocket, the regulation of heart rate and blood pressure in the patient described above, or . . . the healing of a wound.



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In order to draw the connections between the wound as a biological entity and the wound as a physical system, we must first have a basic descriptive explanation of the architecture-anatomy and sequence of events in normal wound healing. In the photographs on the right, a normal healthy wound goes through the natural process of healing until it is closed, i.e. epithelialized. Histologically, on the left, all of the reparative events taking place in the wound have a well organized and recognizable anatomy, and each of the features seen microscopically correlates with something that is happening or can be observed grossly. What is that anatomy and organization? What is the sequence, and how do we recognize these events?

The basic biology of wound healing can be epitomized in one concept, the **Wound Module** of post-inflammatory proliferative repair. This term was coined by Dr. Thomas K. Hunt, San Francisco surgeon-scientist and pre-eminent wound researcher of the latter 20th century. It is the core anatomy and physiology of wound healing, the same as bronchi and alveoli to the lung doctor, the same as the myocardium and valves to the heart doctor. What you observe on physical examination of the wound correlates with some distinctive event or element in the cellular physiology of wound healing. It is the orderly appearance, interaction, and assembly of these elements that constitutes the wound module. While the whole process has bazillions of individual chemicals and interactions (the stuff of everyday laboratory wound research across the globe), the process is conceptually quite streamlined and easy to abstract. In this short discussion, the process will be reduced to 7 key items, 7 physiological events with 7 clinically observable correlates, the quintessential "seven clinical signs of wound healing".

0 – Injury and inflammation: Wound healing is a reserve physiology, the wound module an ad hoc organ. They appear when injury disrupts the integrity of the body. The body's response to any injury is inflammation. Inflammation is the protective and destructive response that defends the body during injury, then cleans up the debris, then initiates the healing process. Without an initial injury and then inflammation, wound healing is not there. However, the process is complex, because while inflammation triggers the healing process, sustained inflammation also suppresses healing. This is a way to ensure that resources are not wasted, by delaying repair and not permitting it to run fully until the field is sufficiently stabilized and cleaned up. Recrudescence of injury and acute neutrophilic inflammation will put wound healing down again. Injury and inflammation are the predicates to healing. They get the process going, but only as they themselves are leaving. If significant inflammation is present, grossly or histologically, the wound remains in acute phases, and healing does not appear.

1 – Inflammation subsides: The first sign of wound healing is that inflammation subsides. As an inhibitor of the wound module, high levels of inflammation must wane before the wound module will accelerate. Clinically, there will be subsidence of erythema, edema, warmth and hyperemia, pain and tenderness, drainage, necrosis, and other markers of injury and acute response. If this does not happen, the wound module will not progress. If these changes do subside, that is the harbinger of proliferative repair events.

2 – Macrophages, eschar separation, and cytokines: Macrophages arrive in the wound as blood borne monocytes. Inflammatory mediators such as pdgf transform these cells into the macrophage. As acute inflammation and other leukocytes clear out of the wound, these cells remain to do the keystone job in the integrated inflammation-repair process. Macrophages actually have two major roles in the wound. Their **afferent** task is as phagocytic cells to remove debris. Whatever is dead or damaged and needs to be cleared, they do it. (An ancillary role in this regard is to present antigen to lymphocytes as part of immune recognition and defense against xeno-pathogens, stuff that they find as they mop up. This function is tangential or irrelevant to the wound module and normal wound healing per se. However, in chronic pathological wounds, this becomes the basis of the auto-immunization which perpetuates wound chronicity, which will be discussed at length in later sections.) Clinically, the afferent function of the macrophage is recognized by eschar separation – dead stuff is cleaved from the living stuff, and the dead stuff bit by bit falls off and disappears. Their **efferent** task is to initiate the repair process. The local repair cells need something to flip the switch to "on", and it is the transformative and stimulatory cytokines and growth factors made by the macrophages which do this. They include bfgf, pdgf, vegf, igf, and others, all of which act to stimulate local vascular and fibrous cells. Clinically, the efferent effect of macrophage wound stimulation is recognized because all of the subsequent items on this list begin to appear.

3 – Ground substance and mucus: The purpose of wound healing and the wound module is to reconstitute a basic stroma that holds the body together and provides a foundation for epithelial growth. Native stroma and repaired stroma have collagen and other connective proteins as the structural matrix. However, early cells in the wound need a place to live and do their thing as they make the new connective matrix. Architects and builders must create some form of staging on which construction workers can stand, so that they can lay the bricks and mortar, the stones and steel of some new building. Plasma proteins constitute the topmost layer of the wound, where acute inflammatory cells do their work. Below that is a zone of glycosaminoglycans (gag's), ground substance, where the early repair cells, angiocytes and fibroblasts, can live and do their job. The aminoglycan layer is the construction staging. The gag's are created by inflammatory and arriving mesenchymal cells. One of the earliest signs that the wound is entering the proliferative phase, clinically it is recognized by mucus and light reflex on the wound.

4 – "Granulation tissue" and angiogenesis: This is the most obvious positive wound finding to naïve observers, the red pebbly carpet of new blood vessels that appears, eventually covering the entire surface in any wound that is properly healing. This tissue is new blood vessels forming in the aminoglycan matrix. The angiocytes that make the new vessels are being attracted from old vessels below by angiogenic cytokines made by macrophages above. Vascular density is much higher than in normal tissues, hence why it is so red. Once these new vessels are established, they create the favorable environment in which fibroplasia can then occur.

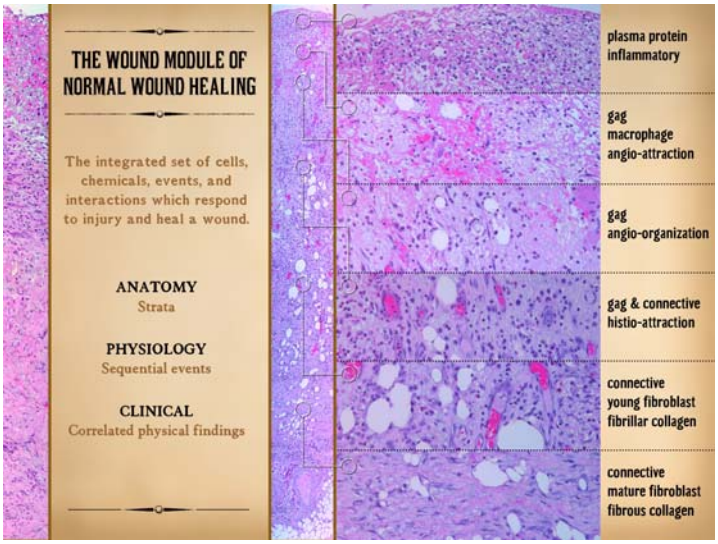
5 – Histioblasts, fibroblasts, and fibroplasia: Once angiocytes have formed vessels within the aminoglycan layer, there is now an environment hospitable to other cells. The other cell which has a restorative function is the histioblast-fibroblast. In this presentation, "histioblasts" will refer to the earlier incarnation of these cells, the uncommitted pluripotent stem or reserve cell line that will spawn new fibroblasts when needed. The "fibroblast" is the more mature version, making and embedding itself into the new connective protein matrix. The matrix starts as amorphous fibrillar collagen, and as it becomes denser and more mature, it becomes more fibrous with its characteristic mechanical properties. Clinically, thus us observed as stiffness in the wound, less mechanical compliance.

6 – Myofibroblasts and contraction: Wound closure ultimately is defined by the restoration of an epithelial boundary which sequesters the mesenchyme from the ambient world. However, to lighten the load on the epithelium, nature has another trick, wound contraction, which reduces the size of the wound. To do this, some fibroblasts develop muscle proteins and become contractile. The function of these myofibroblasts is to ratchet the wound together: tug with the muscle proteins, then cement with the connective proteins, then tug with the muscle proteins, then cement with the connective proteins . . . Clinically, this is recognized by in-curling of the wound edges, smoothing of the wound contours, and progressive reduction in wound width and size.

7 – Epithelialization: Epithelialization that separates insides from outsides is the final step. For epithelium to grow across the wound, all other components of the wound module must be in place. Epithelium will only start to grow where "granulation tissue" is in contact with the wound edges. Once the process starts, thin new epidermis (or any epithelium) outgrows across the surface until the whole thing has been "painted", a process very easy to observe clinically.

8 – Maturation: The seven events and clinical signs of wound healing and the wound module have now been witnessed: inflammation subsides >> macrophages & eschar separation >> ground substance & mucus >> angiogenesis & granulation tissue >> fibroblasts & fibroplasia >> myofibroblasts & contraction >> epithelialization. The wound is now nominally closed. However, wound healing is not over. The newly restored stroma is excessively dense with new connective proteins and vessels, and the mechanics of the tissue and functions of the epithelium are far from mature. Over a period of months or years, the new scar will be reworked and remodeled back to something akin to natural dermis or fascia. Those slow changes also have their clinical observations, mainly improved color and compliance.

This slide presented the general functions of the wound module and what you will see clinically that correlates with these events. The next seven slides will focus on wound anatomy, what you will see under the microscope, which likewise directly correlates with wound module events and the 7 clinical signs of active wound healing. These following slides are an abbreviated version of a larger presentation on normal wound healing. You can read more and get the thorough story on the Arimedica website:
http://www.arimedica.com/content/integra%20histogenesis_gottlieb-me_v2003.htm
http://www.arimedica.com/content/arimedica_integra%20histogenesis_gottlieb-me_v2003.pdf



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On this slide, we will describe the anatomy of the wound. The wound module, with its constituent cells, chemicals, structures, and interactions, is not just a jumbled mix, not a tossed salad of neutrophils and macrophages, and stromal cells. It is highly structured, and each aspect of that structure means something important to the health or morbidity of the wound. Notice the order of discussion: the last slide described wound module physiology and the correlated clinical and physical exam features, and now this slide describes anatomy. This is backwards compared to how biology and medicine are traditionally taught. For example, it is much easier to fathom cardiac function once you understand the structure of the chambers and valves. Why backwards for the wound? Because this is a dynamical system. Time defines a sequence of events, and those events in turn define the resulting anatomy. In a sense, every active wound is an embryological event in which the wound module is born, grows, and matures as it fulfills its functions. It is much easier to understand the anatomy once you understand the events which formed them.

The wound is structured vertically. Observed histologically, there are distinctive strata, going from the surface down to the layer where all of these events and effects give way to normal virgin native anatomy. This vertical anatomy of the wound reflects timewise events and sequences. The surface is happening now. The fibroplasia layer deeper down started so many days ago. The various strata in between reflect the timewise events described on slide 6. Maintenance of these strata, and the separation of cells and populations (acute inflammation and wound module) by time and vertical zone are a crucial part of healthy wound physiology. When cells and strata start to become intermixed, that is both cause and consequence of prolonged injury, delayed healing, pathological events, chronicity, and refractoriness in the wound. Remember: in the normal healthy wound, the physiology is a set of sequential events which leads to an anatomy of vertical strata, all of which have correlated clinical findings, and all of which becomes pathological when events and strata start to become chronic and intermixed. On the left of this slide are two long vertical images, two prototypical examples of completely healthy wounds healing properly (seen with basic hematoxylin and eosin stain). The center one is shown in detail via slices representing 6 major strata of the healthy wound. All of this is taking place within a depth of just a few millimeters (the depth will vary, greater or lesser, with location and the circumstances of each wound).

Zone 1 – Inflammatory or plasma protein layer: This is constituted of plasma proteins, leaked from vessels underneath, serving as the substance and environment in which acute leukocytic inflammatory cells muster to defend the host. This zone varies with the degree to which topical care and hygiene have controlled desiccation, injury, bioburden, etc. With scrupulously good care it can become rather negligible (and the opposite with no care). There is also platelet aggregation here, and this is the zone in which platelet-derived and other transformative cytokines convert blood-borne monocytes into tissue macrophages.

Zone 2 – GAG and angio-attraction layer: This is the upper part of the aminoglycan layer, at the boundary of the topmost plasma protein layer. Cell density is relatively sparse, and there are no connective proteins here whatsoever. There are still neutrophils here (acute inflammation), but not nearly in the numbers as above. There are three distinctive key elements at this level. (1) The "space" is all glycosaminoglycans, made by inflammatory and stromal cells, serving as the "ether" in which the other cells operate until they can make an actual fibrous matrix. (2) Large mononuclear cells can be found here, monocytes and macrophages, making the proliferative cytokines which induce the local repair cells. (3) "Planktonic" or migratory angiocytes, generally individualized and spindle shaped as they stream from established vessels below toward the source of chemotactic stimulation above. They can also be seen starting to reorganize, becoming ovoid again as they start to reassemble with others of their kind.

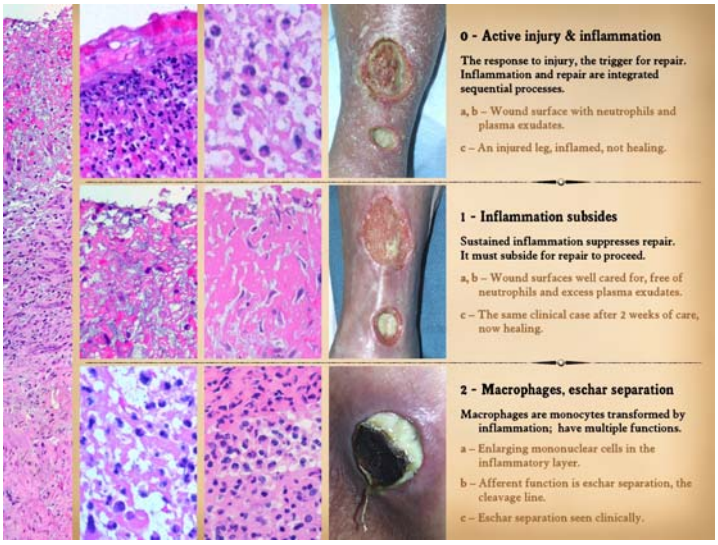
Zone 3 – GAG and angio-organization layer: This is the deeper part of the aminoglycan layer. Neutrophils can still be found here, but mostly in scant numbers, representing inflammatory chemoattraction and migration rather than any type of injury or assault. Connective proteins are still missing. The distinctive feature of this level are the angiogenic cords, reflecting angio-organization and the reformation of tubular blood vessels. The angioid cells and their cohesion are still a bit loose and immature, the cells still big and unsettled, but they have found their positions, conducting channels are open, and erythrocytes are present in the lumens. The new vessels have a distinctive look of long radial or vertical cords traversing the gag layer. This establishes the environment in which other cells can appear and do their functions.

Zone 4 – GAG-connective histio-attraction layer: This is the layer where collagen and matrigenesis begin. Aminoglycans and new vessels are still the dominant anatomy, but young fibroblasts are now present, and they are beginning to make young collagen. Neutrophils are completely absent, meaning that all afferent wound events are gone and the focus is exclusively on repair. Vessels are better organized, some mature, and some are of greater diameter, indicating that they are now supplying a downstream angiosome of vessels organizing in the upper layers. Histioblasts, i.e. progenitor fibroblasts have been stimulated into activity from mature vessels underneath, and young fibroblasts have appeared and are proliferating. They appear as small round uniform cells scattered between the nurturing angiogenic cords and young vessels. They are migratory, and they have little or no organization, yet to be trapped in the collagen they will make. However, they are starting to make young fibrillar collagen, which at this point is relatively non-descript – amorphous, pasty, and homogeneous.

Zone 5 – Amorphous collagen histio-organization layer: In zone 4, young fibroblasts appeared. In this layer, young fibroblasts are getting denser and making denser collagen, enough that connective proteins, while still young and amorphous, have nonetheless become the dominant substance of the medium. There are no neutrophils. Vessels are mature, some of greater diameter and mural thickness reflecting a mature hemo-conducting network. Fibroblasts have become very numerous and dense. They are no longer migratory, and some are becoming trapped, but they are still

more young and round rather than mature and flattened. Young collagen fills most of the space, the aminoglycans having been almost completely displaced. The collagen matrix is starting to look more fibrous, but it is still immature. This can be considered young scar, but the overall architecture is still more wound than scar.

Zone 6 – Fibrous collagen layer: Collagen has become not only dense, but highly fibrous and well organized into lamellae or sheaf-like bundles. Fibroblasts are mature, trapped and flattened, settling in for a lifetime of collagen turnover and remodeling. Arteries, veins, and lymphatics can all be discriminated. This layer can be considered real scar, and the end of the mesenchymal component of wound healing.



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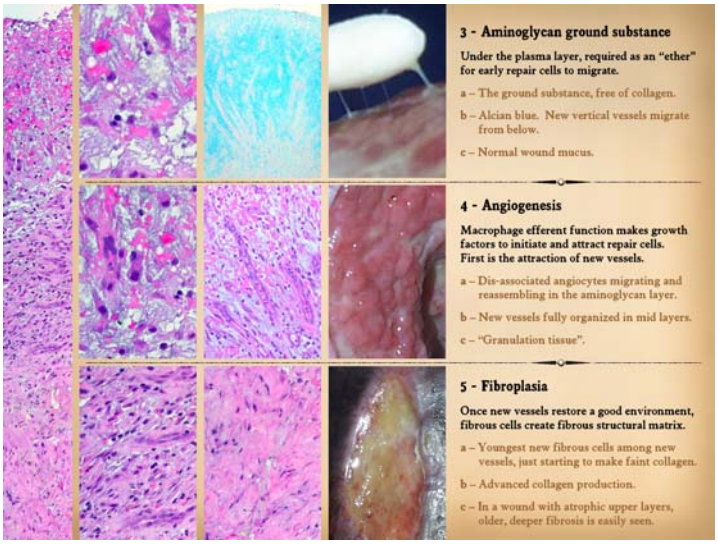
This and the next two slides will show more details about each of the wound events, strata, and physical findings. On the left edge is a vertical wound image. It seems to be split into almost exact thirds, each area with its own distinctive architecture. The "exact thirds" split is just an artifact of how the photograph was cropped, but it does clearly illustrate the progressive development of wound anatomy as the physiological events evolve. The upper third has zones 1, 2, & 3 – inflammation, angio-attraction, and angio-organization – zones made of plasma then aminoglycans without connective matrix, The middle third is the area of young fibroplasia, zones 4 & 5, the histio-attraction and histio-organization layers where fibrous matrix is being made. The bottom third is zone 6, the fibrous collagen layer, the formation of a scar and the conclusion of the mesenchymal wound events. The rest of the illustrations and text on these 3 slides will look at the wound by its timewise physiological events.

0 - Active injury & inflammation. Inflammation is the initial response to injury, to contain damage, clear debris, and prepare for repair. It is also the trigger for repair. Inflammation and repair are integrated sequential processes. **Left:** a normal wound surface. Proteinaceous plasma exudates are the medium, the only environment that exists at this level. What can live and function there are those cells which normally live in plasma – leukocytes. Neutrophils are there in great numbers because they are chemotactically attracted by inflammatory signals. Other leukocytes arrive in the wound more or less in proportion to their concentrations in the blood, monocytes being especially important as the keystone or bridge between afferent (inflammation) and efferent (repair) wound events. **Center:** a close up view of normal wound neutrophils in the upper plasma protein layer of the wound. Their presence indicates active inflammation, disease, or injury of one sort or another. The greater the activity of disease or injury, then the greater the neutrophils at this level and the greater the degree of acute inflammation, and the less likely that the wound can transition into the repair phase. **Right:** an injured leg, inflamed and not healing. This was the result of a superficial laceration in a healthy person. Progressive dermatitis, panniculitis, and ulceration were a consequence of inept care with injurious topical chemicals. Even when sustained injury does not result in progressive ulceration, it will keep repair suppressed.

1 - Inflammation subsides. Because sustained acute inflammation suppresses repair, it must subside for repair to proceed. **Left & center:** healthy wound surfaces well cared for. These show the top stratum, the plasma protein layer. Under the influence of basic hygienic wound care (regular bathing, silver based dressings, edema control), both of these specimens are nearly devoid of neutrophils, stippled basophilia, nor any other evidence of leukocyte activity and acute inflammation. The cells that are present in the upper plasma layer are all large and migratory – monocytes, macrophages, and some arriving angioid cells. The subsidence of inflammation means release from inhibitors that suppress reparative events. Assuming these wounds are otherwise healthy, they can now start healing. **Right:** the same clinical case after 2 weeks of care. Acute injury and inflammation are gone, and the wound is now healing.

2 - Macrophages, eschar separation. Macrophages are monocytes transformed by inflammation. They have multiple functions in the wound. Their afferent function is as phagocytes that clean up the debris and damage of the acute injury and subsequent acute inflammation. **Left:** mononuclear cells are distinctive in the topmost plasma protein inflammatory layer, appearing as typical "compact" (blood borne morphology) monocytes, or in transition as they accumulate cytoplasm and nucleoplasm, or as fully matured macrophages. **Center:** this image shows the cleavage plane between necrotic eschar (above) and viable tissue (below). The cleavage plane represents tissue lysis and processing by neutrophils and macrophages. **Right:** eschar separation seen clinically. This is a pelvic pressure ulcer several weeks after the pressure exposure and necrosis. The separation will continue until complete, all necrosis eliminated, leaving behind healing wound surfaces.



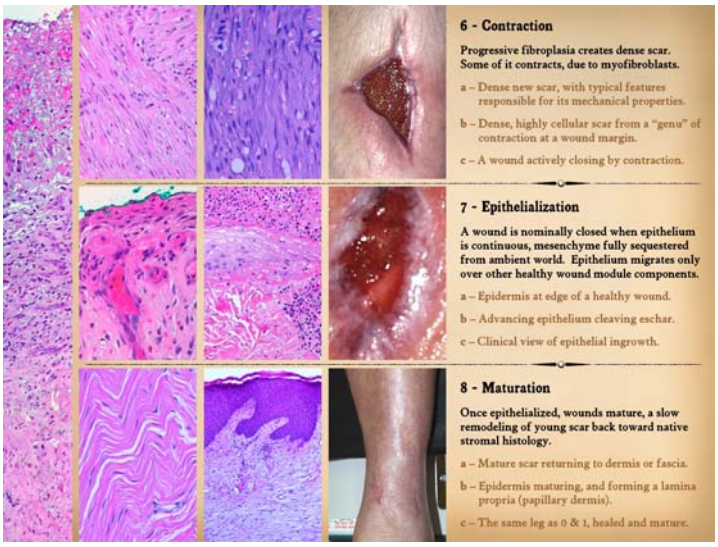
3 - Aminoglycan ground substance. In normal tissues, the glycosaminoglycan (gag) ground substance is the interstitial "gel" that fills the space between cells and connective protein matrix. In normal embryogenesis, it appears as the preliminary medium for histogenetic cells, the "ether" that they require to migrate and organize until connective proteins appear to stabilize their architecture. After injury to fetal tissues, "healing" is simply the production of new gag ground substance, and then the restorative generation of new cells and connective matrix as occurred during primary histogenesis. The post-inflammatory wound healing "program" does not become active until near-term or peri-parturition. (The possibility of suppressing "wound healing" and restarting embryonic histogenesis is one of the "holy grails" of wound healing arts and science). However, the gag's have a crucial role even in normal wound healing. The aminoglycan ground substance is required for the scar or stroma to form, because angiocytes and fibroblasts and the vascular and connective structures they form are just new tissue that needs the gag's as a host medium. Their presence is critically important in the earliest phases of repair, because angiocytes, the first of the repair cells to appear, must have an aminoglycan medium or environment in which to migrate and assemble. **Left:** a view just below the topmost plasma protein layer. Pink plasma "puddles" are present, but most of the "space" here is pale or unstained aminoglycans. Low cell density is typical, with some neutrophils, monocyte-macrophages, and the "advance guard" of arriving angiocytes. There are no connective proteins here, and cell-to-cell organization and assembly which are just beginning are still loose and amorphous. The aminoglycans are made by various cells, but mostly by the arriving angiocytes themselves. **Center:** an alcian blue stain. H&E histology allows the location of the glycosaminoglycans to be inferred, but it does not directly stain the gag's. Alcian blue is the opposite, staining only the tissue gag's (it stains the carboxylated and sulfated aminoglycans of the "ground substance" such as chondroitin, hyaluronan, dermatan, keratan; a red counter stain is used to reveal cells). The plasma protein top layer does not stain, nor do the collagen layers below. In between, the dense blue stain is the aminoglycan zone. It has two strata. The upper half is the angio-attraction layer where, in response to macrophage stimulation, individual angiocytes are streaming and arriving and starting to reassemble into new vascular structures (the scattered lucencies). The lower half is the angio-organization stratum where angiocyte and vascular reassembly is complete, showing the vertical architecture of the angiogenic cords and young vessels. **Right:** normal wound mucus. This is a proper part of any healing wound, and absence of this layer or these chemicals is associated with weak angiogenesis and impaired healing.

The first three events of wound healing – subsidence of inflammation, macrophages, and ground substance – are the afferent wound events, the preparatory or pre-matrix phase, when things are cleaned up and readied for the formation of new stroma. The next four phases – angiogenesis, fibroplasia, contraction, epithelialization – are the efferent wound events, the repair activities. The purpose of wound healing is simply to repair the basic fibrous stroma, consisting of an architectural superstructure (connective protein matrix) and a logistical supply network (blood vessels). This is effected by two mesenchymal cells – angiocytes and fibroblasts. New repair cells are derived from existing local stem-regenerative-pluripotent cells, mainly existing vascular cells in adjacent blood vessels (there may also be a contribution from circulating marrow or other remotely derived stem cells). Keep in mind that the order of appearance and inter-operative dynamics of these cells and their derived structures is different for wound healing as compared to normal embryonic histogenesis. Embryogenesis makes normal stroma. Wound healing makes scar, a dense disordered stroma which must eventually remodel back to a normal stromal histology (maturation).

4 – Angiogenesis. The efferent function of the macrophage is to make cytokines or peptide growth factors which initiate and attract repair cells. In normal wound healing, angiocytes have precedence. They must appear first and make new vessels and re-establish circulation (logistical supply) before fibroblasts can appear and function to make the connective matrix. Because they are operating where there is no structural matrix, they make their own medium, the aminoglycans, where they can migrate and maneuver and reassemble into vascular conduits. (This is different than normal embryogenesis, where local parenchymal cells appear first, and attract new vessels only as required to maintain proper vascular density and circulation in the developing tissue or organ.) New angiocytes are derived primarily from existing nearby blood vessels. Transformative-mitogenic-proliferative angiogenic growth factors from wound macrophages diffuse outward, and where they impinge on surrounding vessels, angiocytes get activated. Cytoplasm and nucleoplasm increase, cells mitose, and they peel off of the parent vessel and start migrating toward the source of the stimulus. As they reach the target zone, they coalesce or reassemble into angiogenic cords which, as they become integrated back into the established vascular network, begin to conduct blood flow. Only once this has occurred can fibroblasts then appear and function to make the connective matrix. **Left:** the upper half of the aminoglycan zone, the angio-attraction stratum, where mononuclear cells (monocyte-macrophage) are signaling angioid cells from vessels below. Dis-associated individual angiocytes (long spindle cells) are streaming toward the source of chemotactic stimulation and reorganizing piecemeal into vascular structures (clusters and cords). These are events which are taking place just below the plasma protein inflammatory layer, roughly 3-5 days after a single-event injury with healthy wound healing. **Center:** the lower half of the aminoglycan zone, the angio-organization stratum, where angiocytes have reorganized into structurally competent vessels connected to the general circulation, with open lumens and conducting blood flow. The vertical or fan shaped arrangement of the new vessels is characteristic. These are still immature vessels, and they will remain young and dynamic for some time yet, because not only do they need time to mature, but they are now themselves the interceptors of macrophage cytokines and the source of new angiocytes for ongoing afferent wound events taking place in the strata above. These events are roughly at 4-7 days in the healthy normal wound. **Right:** typical "granulation tissue", i.e. the clinical appearance of the aminoglycan and angio-organization layers.

5 – Fibroplasia. Once new vessels have restored a good environment, fibroblasts can now proliferate and create the fibrous structural matrix which gives stability and mechanical competence to the regenerating stroma. **Left:** young new fibrous cells and connective matrix (staining pink with h&e) among re-established vessels. This is zone 5, the histio-organization layer, where aminoglycans are no longer the most voluminous substance in the

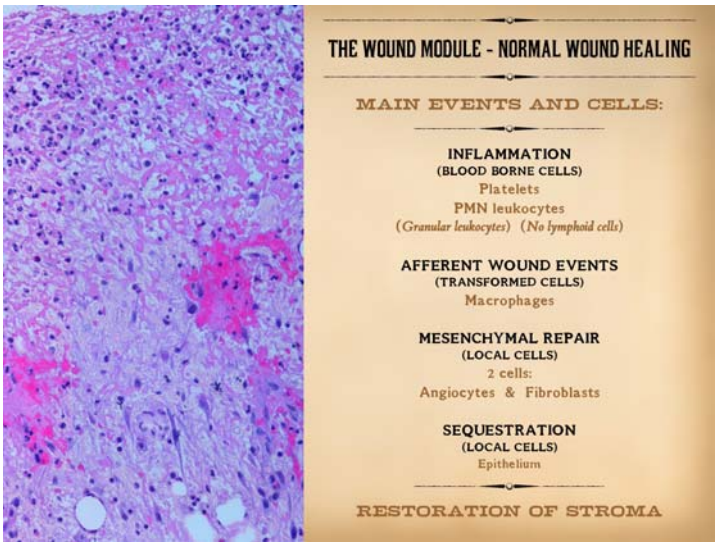
composite material. These events are roughly at 5-10 days in the healthy normal wound. **Center:** advanced fibrous collagen production has occurred, giving the new stroma mechanical stability. This is zone 6, the fibrous collagen layer, the young scar. **Right:** in a healthy wound, fibrosis can be inferred by the mechanical characteristics of the tissues, but it usually is not seen because "granulation tissue" and the upper strata hide what is underneath. However, in a wound with atrophic upper layers (which is pathological and not likely to heal), slowly developing fibrosis is easily seen.



6 – Contraction. As fibroplasia progresses and scar forms, something amazing also happens – scar and wound contraction. Some fibroblasts have actin and myosin and other markers of muscle differentiation. Unlike in true muscle, these contractile proteins do not become highly ordered, periodic, and synched between cells, "crystalline" if you will, nor do they require myoneural action potentials to trigger. However, they are present in certain individual fibroblasts, aka "myofibroblasts", for the same purpose as any muscle – to contract. Their effect is to diminish the surface area of the wound, cranking or ratcheting the surrounding native tissues back together, and thereby also minimizing the proliferative and migratory load on surrounding epithelium which has the final responsibility for "closure". **Left:** new scar, with typical features responsible for its mechanical properties. The stratification, condensed organization, and dense packing of the collagen fibers is obvious. The scar bundles are thick, and different bundles crisscross in different directions, resulting in loss of elasticity and compliance. **Center:** dense, highly cellular scar from a "genu" of contraction subjacent to an infolding wound margin. Myofibroblasts cannot be discriminated from regular fibroblasts by conventional light microscopy color stains (h&e, trichrome, etc.) – rather, e-m and immunos are required. However, gross scar architecture is different in areas of contraction, straighter and more orientated and aligned with the direction of contraction – no surprise since there must be a dominant force vector and loss of isotropy if the scar is to be able to deform in a given direction. This is the "rubber band" of myofibroblast activity and wound and scar contraction. **Right:** a wound actively closing by contraction. This wound is substantially smaller in area compared to its initial size, as evidenced here by the narrow and now-epithelialized vertices, and also by the curling inward and downward of the skin margins toward the wound base. Fibroblasts and myofibroblasts react to tension in predictable ways (see slide 27), and as a self-organizing system (all about this concept in Part 3), the cells will automatically level tensile loads across the surface, meaning a reduction in algebraic order or net geometry of the curvature of the perimeter and surface, i.e. getting progressively smoother and rounder as they get smaller.

7 – Epithelialization. A wound is nominally closed when epithelium is continuous, and the mesenchyme is therefore fully sequestered from ambient world. Complete epithelialization is the nominal endpoint of wound healing for the sake of practical everyday wound management. Epithelium migrates only over other healthy wound module components. **Left:** epidermis at edge of a healthy wound. What were normal basal cells and acanthocytes have become primitive and migratory, streaming outward toward a wound margin that has a suitable wound module underneath, especially well-formed superficial capillaries. Migrating epithelium bears little resemblance to its mature form, but the cells maintain contact with each other as they spread superficially and tangentially in an elongated flattened form. **Center:** advancing epithelium cleaving eschar. Epithelium needs a healthy stroma to migrate on. either a restorative stroma (wound module) or native stroma if the tissues are relatively uninjured. This view is comparable to the eschar cleavage plane seen on slide 8, but here, epithelium is directly finding the boundary itself in an area where acute inflammation and leukocyte-macrophage events have not yet fully developed. **Right:** clinical view of epithelial ingrowth. Active epithelial ingrowth is occurring from all wound margins, covering granulation tissue that has already formed. This process will continue until its growth is inhibited by contact with itself, and the wound is then closed.

8 – Maturation. Once epithelialized, wounds mature. This is a slow involution or remodeling wherein the young scar's dense over-abundant excess of collagen, fibroblasts, and new blood vessels is gradually removed, and the scar progressively returns to the mechanical and histological characteristics of the native stroma. Stroma can of course have various forms – dense fascias, areolar fascias, musculoskeletal fascias-ligaments-tendons, and dermis or various tunicas. Whatever is the correct architecture for the given location, host structure, or intent, the generic young fibro-vascular material, the scar, will respond to local mechanical forces and biological effects to slowly regain the gross and histological morphology of its host structure. **Left:** mature scar returning to dermis or fascia. As scar becomes progressively mature over a period of months to years, fibrocyte and collagen density decrease, and collagen bundles become wavy and springy, with tangential spaces or planes opening between them. Vessel morphology returns to normal, and the number of vessels diminishes back to normal vascular density. This is all apparent in the fully matured scar depicted. The herringbone pattern attests to a final collagen configuration that is once again compliant and mobile. Vessels are sparse, and fibrocyte density is at a normal minimum. While not looking exactly like normal dermis or musculotendinous fascias, it looks very similar. **Center:** epidermis maturing, and forming a lamina propria (the papillary dermis). Regenerated epithelium also matures, slowly developing all of the attributes and functions of its native parenchymal form. For epidermis, this means the restoration of papillae, basement membrane, and the various functional and anatomical strata. It also means the formation of a lamina propria (papillary dermis), a service layer on top of the primary (reticular) dermis to supply the high metabolic requirements and parenchymal functions of the epithelium. **Right:** the same leg as 0 & 1 (slide 8), healed and mature.



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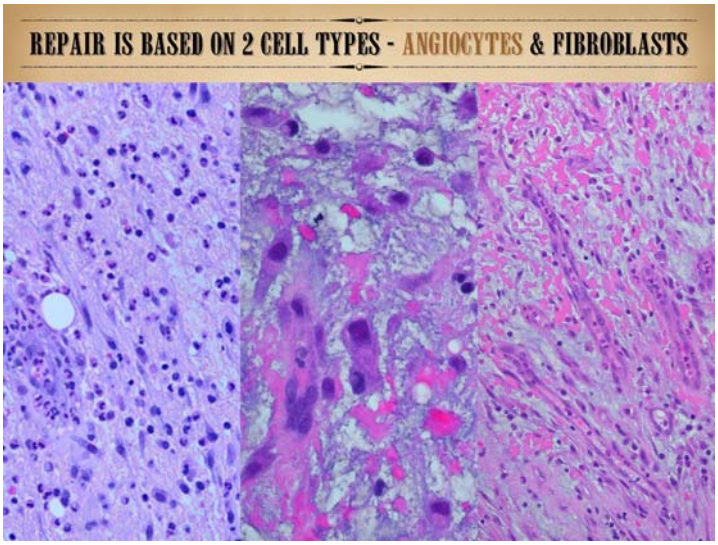
And now, for something entirely redundant. But necessary. As a prelude to everything else that is to follow in these 3 lectures, the details of wound healing must be distilled to a few quintessential concepts. First, the response to injury is an integrated series of linked events. The first event is that something recognizes injury. Thrombosis is usually credited with this accolade, which while inherently true is also an incomplete explanation. Thrombosis does indeed recognize many injuries, but other mechanisms such as allergy and immunity also recognize the primary assault on the body (discussed in detail in Parts 2 & 3). Regardless of how injury is recognized, the response is inflammation. Inflammation is the body's generic protective response, mediated by blood borne leukocytes. As part of their response to injury, they initiate the afferent events of wound healing. These are mediated by macrophages, which are simply blood borne monocytes converted to their tissue phenotype by platelet derived transformative cytokines. Macrophages clean up the injury, paving the way for repair, then they initiate repair by issuing their own set of transformative cytokines which stimulate local repair cells.

The efferent events of wound repair are the restoration of the mesenchymal stroma, then sequestration of the mesenchyme from the ambient world by the restoration of epithelium. Mesenchymal repair is due to two – and only two – cells, angiocytes and fibroblasts. Keep in mind the essential biology of all of this. Multicellular life, with specialization and division of labor among cells (and the complexity and adaptability that they confer), is wholly contingent on just a few crucial elements. The two categorical necessities are (1) an architectural structural framework where differentiated parenchymal cells can be housed, and (2) a logistical distribution network that allows parenchymal cells to deliver and receive items to and from each other. This generic "framing and utilities" is the generalized stroma, and it exists everywhere in the body in one form or another to support epithelia and parenchyma. It is composed of just 2 cells, angiocytes and fibroblasts, and the fibrous and vascular structures that they make. Wound healing is nothing more than this stroma restoring itself, enough to re-establish the structural competence of the injured area and allow parenchymal cells to replenish themselves.

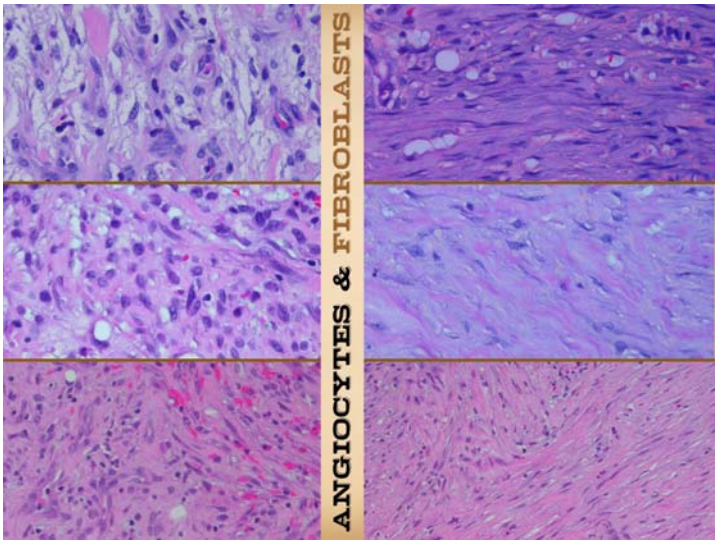
To reiterate, the overall response to injury, inflammation then wound healing, occurs via two general populations of cells – acute inflammation and wound module. Acute inflammation can be subdivided into two groups, the thrombosis or injury-recognition events (plasma, platelets, allergy-immunity, etc.) and the acute inflammation events (leukocytes, especially neutrophils and monocytes). Wound module can also be subdivided into two groups, mesenchymal events (angiocytes and fibroblasts) and epithelial-parenchymal events. Repair per se is contingent on 4 specific cells: (1) blood borne inflammatory monocyte-macrophages are the instigating link between inflammation and repair, (2 & 3) histio-fibroblasts & vascular angiocytes are the mesenchymal cells which restore the generic stroma, and (4) keratinocytes or other epithelia are the parenchymal cells which "hang" on the stroma, sequestering the mesenchyme and restoring functional competence to the injured area.



12

This and the next slide are here to reiterate that the mesenchymal component of repair is based on 2 cell types, angiocytes & fibroblasts, this slide focused on angiocytes and the vascular structure they make.

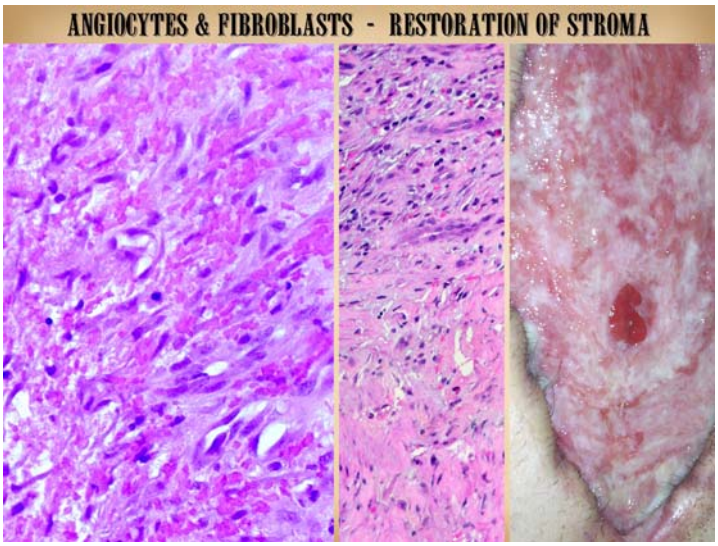
Left: a view of the aminoglycan angio-attraction layer. Spindle shaped angiocytes are streaming from vessels underneath toward macrophages in and above this layer (the other round cells are mainly neutrophils, normal at this level).
Center: a close up view near the top of the wound, at the plasma protein and aminoglycan boundary, having mononuclear (monocyte-macrophage) and angioid cells. The cell cluster is a vessel reassembling from individual angiocytes.
Right: a wide view a bit lower, in the angio-organization layer. Angiocytes have formed long vertical cords and conducting vessels, reaching from mature vessels underneath toward the chemotactic stimuli above. Once these vessels and blood flow are established, the fibrous component of repair can start to develop.



13
This slide emphasizes the vital role of fibroblasts in the repair process. They appear after vessels have established an environment in which other cells can proliferate. As with angiocytes, they are sourced from pluripotent mesenchymal cells in the vascular loci below. Fibroblasts make the connective matrix, and myofibroblasts contract the wound.

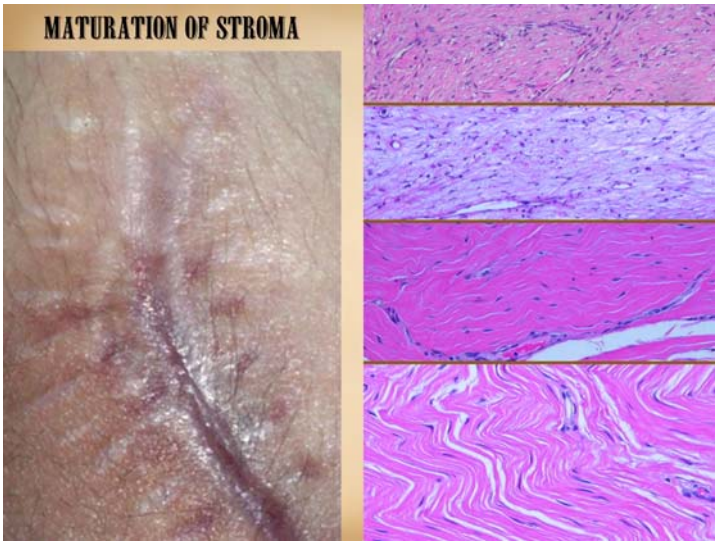
Left upper: early fibroblasts are interspersed among organized vessels in the histio-attraction stratum. They are numerous and small, of non-specific shape. The medium is still largely aminoglycans, but thin strands of eosinophilic young collagen are starting to appear.
Left middle: a view a bit deeper. There are vessels at bottom and upper right, and between them young fibroblasts are larger and denser, consistent with proteogenic activity, and more of the space is occupied by pale pink collagen.
Left lower: a view yet deeper in the histio-organization layer. Young fibroblasts remain dense, and the space is now almost completely filled by young disorganized collagen. The cells are, in general, less round, more spindled, and starting to take on some organization in the form of stratification or lamellations.

Right middle: the next view, going yet deeper into the wound showing young scar. The randomly arranged young fibroblasts are starting to become flatter and layered, trapped and stratified between maturing bundles of wavy pink collagen.
Right lower: at yet a deeper layer, the stratification, organization, and packing of the scar is obvious. The scar bundles are thick, and they criss-cross in different directions.
Right upper: a view from the wound margin subjacent to an infolding skin edge. This is the dense directional scar associated with myofibroblast activity and wound and scar contraction.



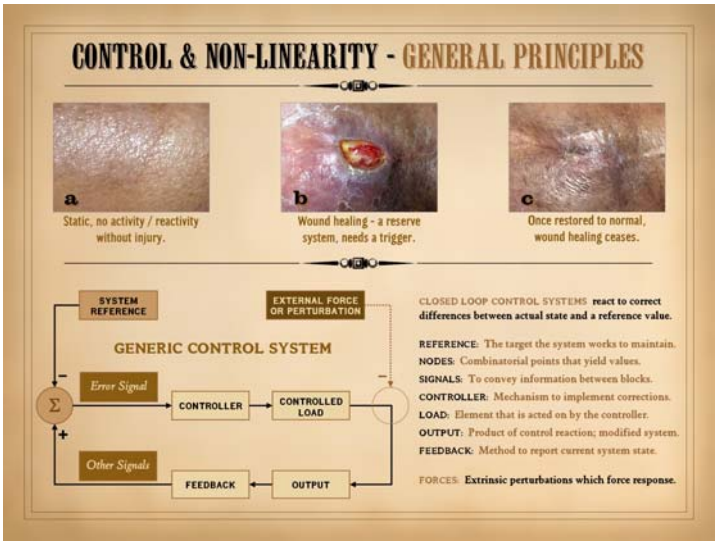
14
This slide reiterates that the reason angiocytes and fibroblasts proliferate is to restore generic stroma, the basic framework of connective matrix and blood vessels that supports parenchymal and epithelial and all other cells.

Left: an example of "granulation tissue" dense with vessels. This is from a wound chamber implanted explicitly to raise a crop of wound activated cells (see slide 27). This specimen has almost exclusively angiocytes and erythrocytes, with no inflammatory cells and only a few young fibroblasts or histioblasts. Fibroplasia will follow, but without the vessels there first, nothing else can grow and be productive.
Center: the middle to lower strata of the wound, where newly organized angiocytes and vessels mix with young fibroblasts and the connective matrix they are making.
Right: the gross appearance of active wound fibroplasia. In this abdominal wound, the aminoglycan-angiogenic strata are atrophic and thin, allowing the deeper layer of fibrosis to be seen.



15
This slide reiterates that the wound and scar is nothing more than the restoration of the stroma, and that although it appears first as a generic "scar", that with time it will mature back to a connective architecture typical of its host tissue.

Left: a set of scars after several operations. Some are young, thick and stiff from excess connective matrix, discolored from excess vascularity and hyperemia. Some are old and mature, being pale and flat, soft and compliant as time has resolved the excesses of the young scar.
Right upper: fibroblasts, collagen, and new blood vessels are seen at the peak of proliferative repair.
Right second: a skin scar weeks after full epithelialization. Vascular and fibroblast density have lessened from their peak in the top image.
Right third: a maturing scar (months), with wavy collagen bundles, spaces opening between them, and decreased cell and vessel density.
Right lower: a matured scar (years) with open compliant collagen bundles and sparse vessels and fibrocytes, much closer to normal dermis or musculotendinous fascias rather than scar.



16

We saw these photos on slide 3. The normal skin is static, without any activity or reactivity absent any injury or provocation. Wound healing is a reserve system, on standby until the proper trigger occurs. Once triggered, it runs its course until the wound is healed. The conventional bioscience aspects of that process were reviewed on the preceding slides. Once the wound-stroma-tissue is restored to normal, wound healing ceases. This brings us back to the initial inquiries of this talk: (1) How does the wound healing process know when to start, how to execute its business, and know when to stop? (2) Why do some wounds fail to heal, even when components of the healing process appear to be active? The answers begin by understanding that the wound is a non-linear control system. The answer to question 1 is in the normal physiological operations of this control system. The answer to question 2 is in the pathological or disordered behaviors of this control system. The basic concepts of non-linearity and control were alluded to on slide 3. Here we will start to explain why normal wound healing is a control system, first looking at some general principles of closed loop control.

As explained on slide 3, biological systems must remain within certain bounds if they are to function properly. The same is true for nearly any system, natural or engineered. Many systems have fairly precise or narrow-range target values. Those target values are the system reference. Closed loop control systems have the "machinery" necessary to sense their own actual state, compare that to the system reference, then react to correct any differences between the actual and the target values. Closed loop control systems generally have the following components.

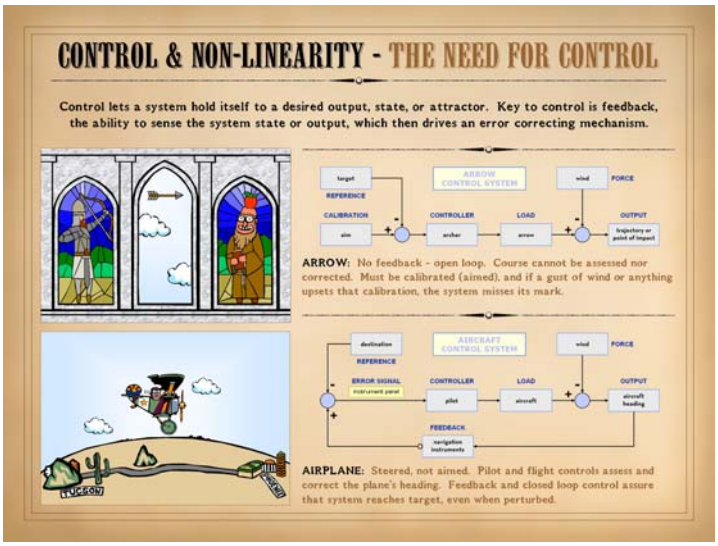
Reference: This is the target or value that the system is meant to be at, that the control loop works to maintain.
Nodes: These are combinatorial points that yield values. The main node, where the system state is compared to the system reference, is usually indicated as a "summation point", a simple comparator where one value is subtracted from (or its inverted value added to) the other.
Error signal: This is the output of the primary summation point, indicating variances of the system away from the reference value,
Controller: This is the component that is directly influenced by the error signal, the first part of the mechanism needed to implement corrections. It is like the executive or general that receives all of the intelligence from the field then must issue orders to the troops to take some action.
Load: This is the element acted on by the controller, the actual productive machinery which will change the system state and try to correct the system error. These are the employees or troops who, commanded by the controller, do the real work of creating the company output.
Output: This is the productive output of the controlled load, that which modifies the state of the system, bringing the system back toward the system reference.
Feedback: Some method is needed to measure the current state of the system and report that back to the comparator node.
Forces: Extrinsic to the control loop itself, these are applied stresses or perturbations which alter the state of the system, forcing a response from the control loop to try to restore the reference state. They can sum into the loop at nodes which, depending on the specific nature of the system, can be located almost anywhere on the loop.

Note that there is no start or stop, no on-off switch. As long as the system as a whole is "on" and active, the control loop is free running, always striving to restore the system to its reference value. If the reference value is reached, then the output of the main summation-comparator node is zero, and there is no force driving the loop. As soon as there is any intrinsic decay or drift in the state of the system, or any perturbation due to extrinsic stresses, then the compared value is no longer zero, and an error signal is generated and the loop is driven.

The feedback and closed loop are by definition "non-linear". In conventional mathematical terms, "linearity" can mean several things depending on context. As applied to dynamics. linearity means a functional relationship between two variables (a function being the classical algebra-calculus-topology definition of a "one-to-one and onto" relationship, embodied in an equation of the form **y = f(x)**, where there is only one allowable value of the dependent variable for any value of the independent variable). For tangible systems in the real world, dependent-versus-independent functional relationships are wildly diverse, They can be as simple as a simple scalar of the form **y = Ax** (straight line "linear" in another sense), or higher order polynomials or angular-trigonometric functions or a zillion other things. The system state (dependent variable) may be a function of time or position or whatever (independent variable), but all have that quintessential definition that the system output value is uniquely determined by the value of the input. In contrast, non-linear systems are those where the system is a "function" of itself. Rather than the value of the system being mapped against time or position or whatever, its output value is determined by its current value. Its value **now** is the input (comparable to the independent variable), and its value **next** is the result (comparable to the dependent variable), but there is only one variable involved, the system parameter that you are observing. In a linear system, you can arbitrarily pick a value for the input variable, and then calculate precisely the value of the output variable. In a non-linear system, you can pick an arbitrary initial value of the system, but then you must sequentially recalculate the next value of **x** versus the current value of **x**. Each time you recalculate, the latest output becomes the next input, each such turnaround known as an **iteration**. If you want to know what the value of **x** is after 500 iterations, you cannot simply plug "500" into time **t** in an equation of the form **x = f(t)**; instead you must run 500 iterations to finally get to the value you seek. We cannot write **x = f(x)**, because that is the notation for a function, and non-linearity is not "functional", so instead we write **x ← f(x)** to denote the self-dependent, recursive, iterative nature of the system.

As will be explained in detail in Part 3, there are many systems in mathematics, engineering, and the natural world that do not have a functional relationship, i.e. many non-linear systems. Compared to the tools of algebra and calculus, the mathematical tools required to work with such systems are of more recent advent, and not so easy to work with, so they get little attention in basic math and science education. Nonetheless, this is the stuff of complex systems, the stuff of the real world. Control systems are all of this form. When a controlled system feeds back, its own state

becomes the next input into the system, Non-linearity is inherent in feedback. Closed loop control means non-linearity. The state of the system does not have a functional linear relationship to time, distance, energy, momentum, temperature, nor anything else. The state of the system in the next iteration (the output) depends only on its current value (the input) as transformed by the operational functions of the system (the **f** in **x ← f(x)**) that govern the physics or chemistry or other dependencies between individual components of the system. For non-linear systems in general, their output or behavior can have several generic forms, as described on slide 23. For closed-loop reference-driven controlled systems, the general idea is for the system to stabilize or converge on a target value.



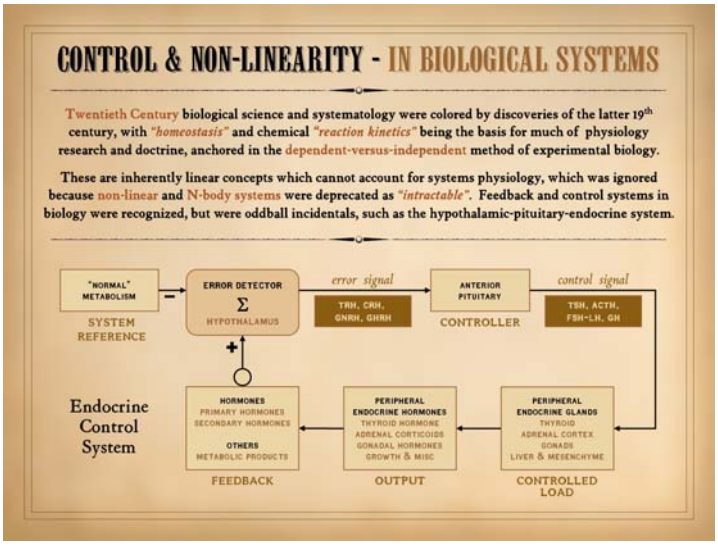
17

We have already established that biological systems must remain within bounds if they are to function properly. That is why there is a need for control. Each system and subsystem needs a mechanism to pull itself back in bounds when it decays, drifts, or is pushed too high or too low out of its effective operating range. Control lets a system hold itself to the intended reference, output, state, or attractor. Key to control is feedback, the ability to sense the system state or output, which then drives an error correcting mechanism.

To understand why control is so important, first consider an arrow. The archer aims, then releases. He hopes his aim was good. However, once the arrow leaves the bow, nothing can be done about it if the aim is off. There is nothing on the arrow itself that can assess nor correct its own trajectory. Even if the aim was perfect, a gust of wind can throw it off. Without feedback, this system is open loop. Without feedback and a response mechanism, there is no control. Open loop systems must be calibrated (aimed), and if anything upsets that calibration, the system misses its mark with no recourse. In this open loop arrow system, we can diagram the "machine". The same terminology applies as for any generic control system: the target is the system reference, the controller is the archer, the arrow is the controlled load, and the output is the trajectory and impact point. But note that there are crucial differences – there is no feedback loop nor error signal. In lieu of feedback (which will continuously update and correct the system), there is instead just a calibration (a one-time up front event).

Many man-made machines are open loop, because they can be made stable, without drift or decay, and they can be isolated so as not to be affected by outside forces. However, biological systems all swim in a sea of stochastic variability, predatory perturbation, and "things that go bump in the night", stresses that by intent or happenstance will strain systems away from their operating ranges. In the kingdom of life, open loop calibrated systems are of marginal value. Biosystems, at small molecular scales and at massive population scales and at every scale in between, all need ways to regulate or correct their state if the system is to function and stay alive.

Contrast the arrow with an airplane. The airplane is steered, not aimed. This is because there is feedback in the system – the pilot and his instruments and flight controls repetitively assess and correct the plane's heading. Just as with the arrow, the destination is the system reference, the pilot is the controller, the aircraft and more specifically its flight control surfaces are the controlled load, and the output is the aircraft's heading. In both systems, external forces such as wind sum into the loop, which for the arrow can mean an irreparable loss of calibration. For the airplane though, perturbation of the system state (the output or heading) can be sensed and fed back via the navigation instruments. If there are variances between heading and destination, then an error signal is generated (the gauges on the instrument panel), and the controller (the pilot) can actuate changes in the load (the flight controls). Feedback and closed loop control assure that system reaches its target, even when perturbed.



18

Are you a conventional bioscientist and finding this discussion about physics and engineering a bit foreign? Does the concept of "system" beyond rudiments like "cardiovascular system" and "musculoskeletal system" seem arcane or irrelevant? Do feedback and control seem to you like concepts on the fringe of biology? If so, you are in good company, because of the quirks of science and science education over the past 150 years. Twentieth century biological science, systematology, and academics are colored by the discoveries of the latter 19th century, when chemistry, organic chemistry, and a scientific pharmaceutical industry turned everyone's attention to biochemistry and the chemical basis of metabolism and disease. Remember when organic chemistry was the 'acid test" of premedical education? Physics, as a rigorous disciplined predicate and also as a sister subject to the study of physiology and pathology has been given little emphasis during this era.

The legacy of this attitude is that everyone assumes that every dynamic in the body is comparable to a chemical reaction, and that everyone is looking for the chemical basis of what goes wrong. A simple example for wounds concerns nutrition. Those who know nothing about wounds all think that all you have to do is stuff the patient with lots of carbs and protein, and that a wound will then heal. As foolish and ignorant as that concept is, it has an historical basis. Twentieth century physiology was informed largely by chemistry, where simple chemical reactions have simple

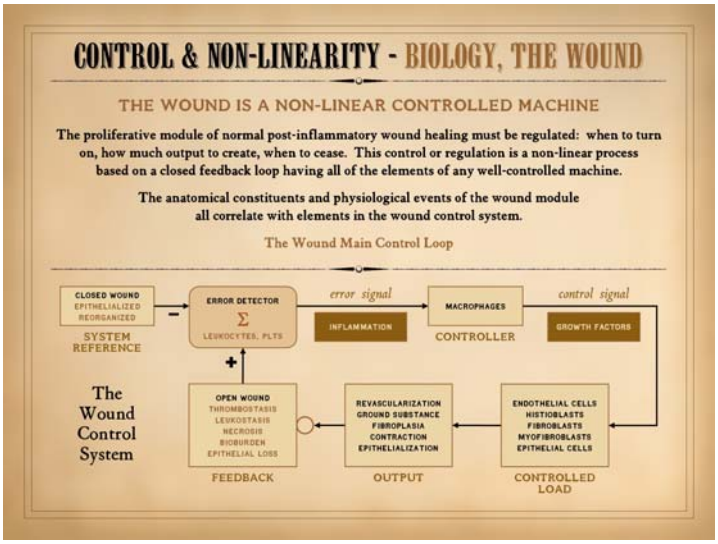
reaction kinetics. Consider a basic chemical equation such as [A] + [B] ⇆ [C]. In a test tube, you can pour more substrate on the left side of the equation, and more product comes out on the right side. Therefore, so the impaired thinking goes, just pour more chicken fried steak and coconut cream pie into your patient, and all things metabolic will speed up, and the wound will churn out collagen faster than you can give it a haircut, and your wounds will then magically disappear.

No, of course not. But that is the legacy of 19th and 20th century science on the mentality of physiology. This approach to physiology was biased towards basic chemical dynamics, biochemical dynamics (e.g. Michaelis-Menten enzyme-substrate conversion), and the linear characterization of how any 2 chemicals react. For example, the Krebs and TCA cycles could be mapped by looking at the basic kinetics of dual-species conversions, such as citrate-aconitate or fumarate-malate. However, understanding the integrated composite behavior of these cycles and their many interconnects could only be reduced to those big highly interconnected wall charts that overwhelmed you in medical school (see Part 3, slides 16 & 17). Trying to understand-model-predict the overall integrated operations or states of highly interconnected complex systems was beyond the analytical or computational means of those times (n-body dynamics; see Part 3). Instead, biological research and doctrine became anchored in the dependent-versus-independent method of experimental biology based on simple linear interactions. This mentality extended from the test tube to the clinic, where RCT's – randomized controlled trials – became the gold standard of exploring the effects of a single agent or intervention on the net behavior of a system. This approach to science has substantial limitations, because it cannot ever elucidate the comprehensive integrated behavior of complex n-element systems. Nonetheless, complex, non-linear, and n-body systems were deprecated as "intractable", and consequently not worthy of study. Within the halls of biomedical academia, there was, and largely still is, no room for understanding nor teaching overall systems biology (but as discussed on slide 5, that is beginning to change).

Much of what we would now consider systems dynamics was simply rolled into the overarching idea of "homeostasis" as defined by Claude Bernard and Walter Bradford Cannon. This concept, that organisms or systems can maintain their "internal milieu" in a stable desirable state, is achieved through various regulatory mechanisms and dynamic equilibria or steady states between components of the system. We recognize easily that this implies "control", that the "desirable states" are the system references, and that the regulatory mechanisms are simply the various control blocks and signals within a control loop. Note though that "equilibrium" was an important part of the way homeostasis was envisioned on the cutting edge of 19th – 20th century biology, rather than recognizing that complex systems are more likely to have stable but non-equilibrated chaotic attractors (see Part 3, slide 20). The confusion is understandable though, because back then chemical equilibrium was an au courant concept of the times, and non-linear dynamics was a fantasy world that few could even envision. The reality though is that non-linearity and control are the core dynamics of all biological systems. However, there is at least one closed loop feedback system that has been a classical part of medical education for a long time - the hypothalamic-pituitary-endocrine system. When it is taught in medical school, it is often portrayed as an interesting oddity that a feedback system even exists . . . not true at all, but this makes it a convenient place to start to illustrate control & non-linearity in biological systems.

The endocrine control system has been abstracted here to a uniform loop that actually regulates several different organs and system references. Whether dealing with thyroid, adrenal, gonads, or growth, the system reference is some manifestation or measure of "normal metabolism". The feedback in the loop is the level of end-organ hormones (thyroid, adrenocorticoid, gonadal, anabolic), either those hormones themselves or some metabolite of them or some further downstream metabolite resulting from their biological effects. The system comparator is the hypothalamus, which through some black-box mechanism monitors the balance between the reference value and the actual concentration of hormone or metabolites. If there is a variance, then the hypothalamus issues an error signal in the form of the releasing hormones (trh, crh, gnrh, ghrh). This signal drives a controller, the pituitary, which amplifies the signal and has the "power" to drive the system load. It drives the load via a control signal in the form of the stimulating hormones (tsh, acth, fsh-lh, gh). The load itself is the peripheral endocrine organs and other tissues – thyroid, adrenal cortex, gonads, and liver & mesenchyme. The system output is the set of peripheral endocrine hormones that regulate whole body metabolism and related functions (thyroid hormone, cortisol, progestins, etc.). They or their effects are then transduced and fed back to the system comparator.

In human engineered systems, such as nuts-and-bolts machines and electronic circuits, systems can be designed around a few well-defined elements. In complex biological systems, there can be dozens or hundreds of relevant elements, so control diagrams such as this are abstractions that roll many elements together into the main control blocks. There will always be a variety of options for how these simplifications are drawn, and how the many elements are represented by the few. For example, this loop could be drawn with the pituitary and even the end-organs just being amplifiers on the hypothalamus output, and some manifestation of general cell and substrate metabolism as driven by the endocrine hormones could be listed as the load and output. Thus in modeling complex physiologies, control loop abstractions, simplifications, and assignments are necessary, meaning there is latitude in deciding exactly where and how to represent each biological element on the circuit. For the wound, which has substantially many contributing elements, abstractions and simplifications are necessary, but the relative roles of the major components of wound healing are clear enough that a standardized wound control loop can easily be defined.

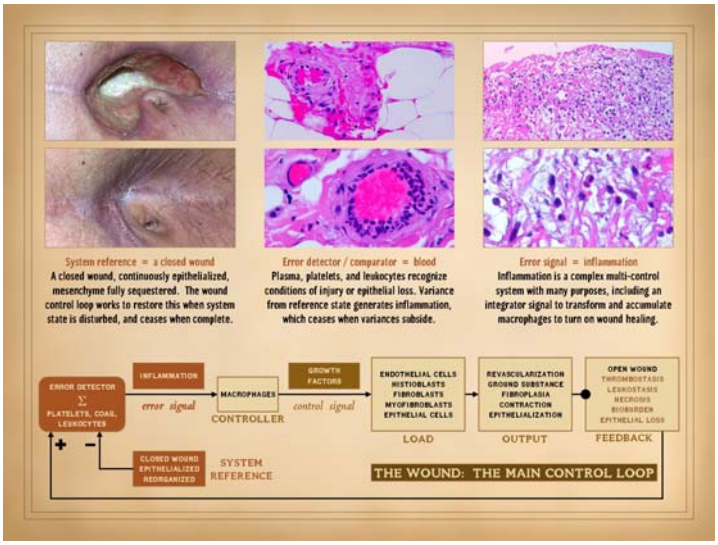


19

Like nearly every other system in physiology, the wound is a regulated controlled "machine". Like everything else, the proliferative module of normal post-inflammatory wound healing must be regulated if it is to remain within its operating range. It must be regulated so it knows when to turn on, how much output to create, and when to cease. This control or regulation is a non-linear process based on a closed feedback loop having all of the elements of any well-controlled machine. The many anatomical constituents and physiological events of the wound module all correlate with basic elements in the wound control system.

Presented here is the Wound Main Control Loop. The next three slides will explain the components in detail. The following is a basic description. The **system reference** is closed (epithelialized) normal tissue free of disease, injury, and inflammation. How the body recognizes this condition, i.e. what physiological or anatomical properties constitute closure are biologically important, but they are not specifically relevant to the basic control dynamics. With regard to the loop and its dynamics, each of these control blocks is the proverbial "black box", made up internally of countless biological parameters which somehow transform an input into an output, but looking to its neighbors as just a single element with a well defined input or output. The system reference is continuously compared to the actual state of the wound, and any variance of the wound away from "closed" generates an error signal. The system comparator and **error detector** are the recognizers-sensors-transducers of injury, mainly leukocytes, platelets, and related chemicals. These are the initiators and mediators of acute inflammation, and what they do, their activities and consequences – their output – is inflammation itself. Inflammation, its various chemical and cellular effects, is the **error signal**, the way that the system comparator tells the system controller what to do. The **system controller** is the macrophage. The error signal boosts macrophages and turns them on to do their afferent and efferent wound functions. Their efferent function is to issue pro-proliferative growth factors, the **control signal** which will actuate and drive the system load. The system **controlled load** is the set of cells which does the actual work of rebuilding tissue and trying to correct the open wound conditions which generated an error signal. These cells are the angiocytes and fibroblasts which restore the mesenchymal stroma, and the epithelial cells which close and sequester the mesenchyme. These cells are the load. What they create is the **system output**, mainly ground substance, vessels, connective matrix, contraction, and epithelialization, those things that are explicitly the new tissue which reduces "open wound" and corrects the system error. Finally, the new state of the system must be reported back to the system comparator. The **feedback** is the residual open wound, with various "black box" attributes of "openness" being transduced by the error detectors. Note the circle leading into the feedback block. The system output has the effect of diminishing the state of "open", so that effect on the "open wound" block is a negation, an inhibitory or inverting input, represented by the circle.

As described on slide 16 concerning general control systems, note that there is no start or stop, no on-off switch. As long as there is an open wound, the control loop is running, always striving to restore the system to its reference value of closed. If the reference value is reached, then the output of the main summation-comparator node is zero, and there is no force driving the loop. On slide 16 we also stated that "as soon as there is any intrinsic decay or drift in the state of the system, or any perturbation due to extrinsic stresses, then the compared value is no longer zero, and an error signal is generated and the loop is driven." For the endocrine control loop, where the reference value of hormone levels or metabolism is some positive value, then intrinsic decay and drift are the variances which drive the system, and the system will be continuously active and continuously driven from birth until death. For the wound, extrinsic perturbation is what drives the system, in the form of injury. When the wound settles back to "closed", there is no error, and the loop remains quiet. Injury instantaneously creates a condition of "open wound" which enters the loop at the feedback block, immediately generating an inflammation error signal, and the loop then runs autonomously until the wound is closed. Knowing when to start and stop is not a matter of knowledge or intelligence within the system, it is just a matter of a well-tuned control loop operating as designed.



20

This slide focuses on the front end of the Wound Main Control Loop, the system reference, the error detector and comparator, and the inflammation error signal that they create.

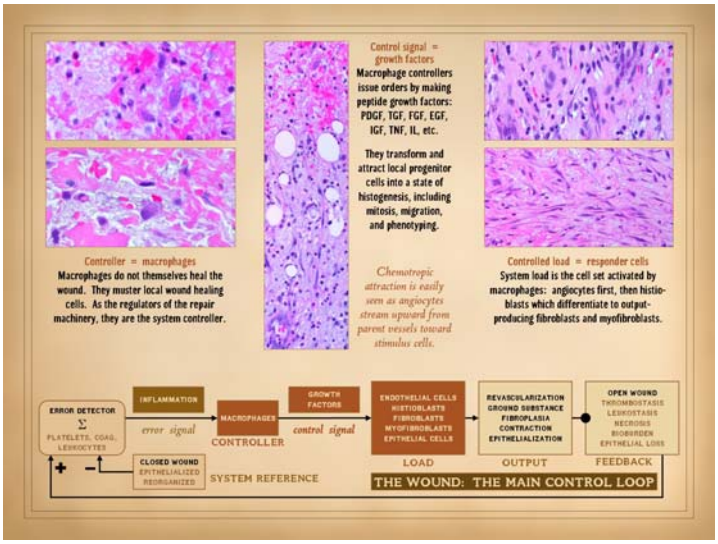
System reference is a closed healthy tissue free of injury and inflammation. This condition means that the tissue is continuously epithelialized, the mesenchyme therefore fully sequestered from the ambient world, and also that no acute injury or defensive activities are present – just normal healthy tissue carrying on normal metabolic or vegetative functions. The wound control loop works to restore these conditions whenever the system state is disturbed, and the loop ceases when the restoration is complete. Remember that the state of "closed" is a complex set of biological parameters that must be transduced and measured by the system comparator. The specific biological and biochemical details of how that basal state is measured is a matter of conventional biology, and knowing and understanding these "nuts and bolts" is a vital part of understanding the whole picture of wound healing and wound pathology. However, from the dynamics and control

point of view, these various parameters are hidden inside the "black box". (The same is true for every black box block on the loop.) Actually, while these comments are true as a matter of general principles, the basal state of "closed" is not transduced at all. Rather, the basal closed state (normal tissue) is passive, and the error detector simply ignores it and stays quiet. The normal state is ignored because there are no active positive tangible markers of openness. As explained in the paragraph on the feedback element (slide 22), the condition of "open" is what is actively transduced and sensed. The system reference therefore serves as a ground or zero or bias on the comparator. This comparator "knows" when the wound is open when positive markers of openness are presented by the feedback block. The comparator "knows" when the wound is closed when the markers of "open" vanish, making the now-healed wound equal to normal tissue which likewise has no markers of "open". (The **photos** illustrate this fundamental restoration, from open to closed.)

Error detector & system comparator is based on blood borne elements. "Blood borne" includes solid and cellular agents (leukocytes and platelets), soluble agents (plasma protein coagulation system and acute phase paraproteinemias), and miscellaneous blood and vascular parameters such as vascular stasis, rheological effects, micro-angio-alterations, and any other acute state of the blood that triggers some sort of protective reaction. These elements recognize and react to conditions of injury or epithelial loss, thereby sensing the positive markers of "openness" or "wound". How it is that plasma, platelets, and leukocytes are activated by injury is basic bioscience knowledge and need not be reviewed in detail here. What is important from a dynamics and control point of view is that the state of injury-wound-openness is recognized and transduced by elements within the comparator block which then generates an output signal based on those inputs. The internal workings of the black box are not relevant as long as the "transfer function" or mapping from input to output represents a consistent physiology or set of rules. The error detector and system comparator is the keystone element in the control loop. It has two inputs, the state of the system (wound or openness) and the system reference (normal tissue and closed). An error signal (inflammation) is generated when there is a variance between the inputs. As mentioned in the last paragraph, the state of "normal" is a passive input, i.e. normal tissues do not excite or activate the error detectors (plasma-platelets-leukocytes). The state of "wound" is an active input due to tangible chemistry that is not present in the "normal" state. Note the "plus +" and "minus -" signs on the comparator inputs. The minus indicates that this is an inverting or negating input, and the plus indicates a positive or summating input. "Negative" is subtracted from the "positive" to get the output value of the node. When the absolute value of "open wound" exceeds the absolute value of "system reference", then the output is positive and the loop is driven. See slide 22 for specifics of how the system recognizes the state of openness and therefore the difference between closed and open. (The **photos** are a simple view of blood stasis in vessels near a wound, illustrating indirectly the plasma-platelet-leukocyte events that are triggered by injury and which become the basis for detecting the condition of "wound".)

Error signal is acute inflammation. Acute inflammation is that complex set of acute phase cellular and biochemical events that is meant to defend the body in conditions of injury and disease, then clean up the mess and prepare for repair, then initiate the repair system. As for every element on the control loop, this is another example of countless items being rolled up into one black box. The individual effects of kinins or interleukins or prostanoids are irrelevant to the control loop. As discussed on slides 24, 25, 27, 28, the Wound Main Control Loop is an open model that allows any and all individual elements of the entire system to be specifically added or exposed in order to simulate their effects. However, the core process, the Main Loop, is a collection of quintessential black box control elements, and the aggregate condition of acute inflammation is the error signal, with one origin or input (plasma-platelets-leukocytes) and one effect or output (macrophages). One effect or output? Yes, it is true that inflammation as a whole has many effects on many events after injury (acute defense, immunity, cleanup, etc.). These operate as other controlled loops, either within the black box, or else on parallel planes with inflammation being a point of intersection between several regulated systems. However, for the sake of driving the wound healing control loop, there is just a single output, the effect of accumulating (integrator) and initiating (transforming) macrophages from monocytes. The **photos** illustrate the upper strata of the wound, the topmost plasma protein inflammatory layer and its junction with the gag-angio-attraction layer. Acute inflammatory cells are present in the plasma, and so are some large mononuclear cells (monocyte-macrophages) and some migratory spindle cells (chemotropic angiocytes). The release of platelet cytokines and related chemicals is how monocytes are transformed to macrophages, establishing the link between the error detector and the system controller.

Note that the set of plasma-platelet-leukocytes is diagrammed as the error detector whereas acute inflammation is diagrammed as the error signal. Isn't this self-contradictory, redundant, or artifice? Isn't inflammation just the operations and consequences of plasma-platelets-leukocytes? The answer is that they are a pair. There are actually three such pairs on the Wound Main Control Loop. These are all splits between anatomy and physiology, between actuator and effect. First, there is a tangible element that does something, then second there is the something that it does. The three pairs are: **(1) error detector & error signal** = detector (plasma-platelets-leukocytes, the mediators and controllers of inflammation) and signal (the state of inflammation that they cause); **(2) controller and control signal** = controller (macrophages) and signal (the growth factors they produce); **(3) load and output** = load (repair cells) and output (the new stroma and epithelium that they produce).



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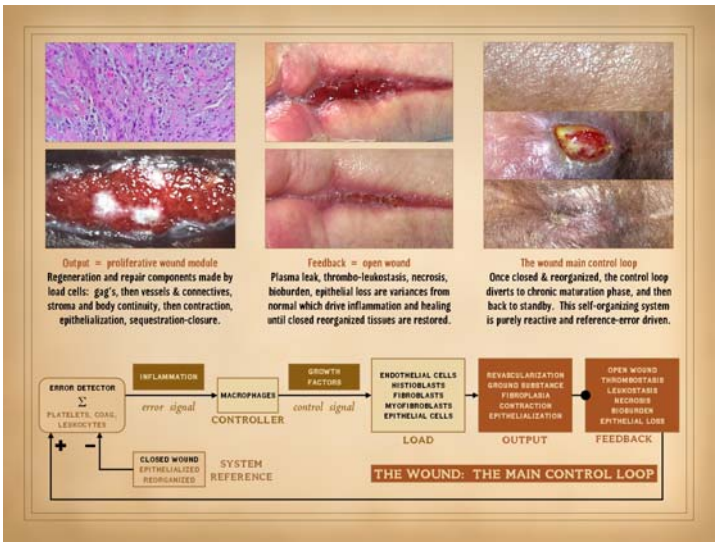
This slide focuses on the mid cycle of the Wound Main Control Loop, the system controller, the control signal, and the system load that is actuated and controlled by the controller.

Controller is macrophages. Macrophages do not themselves heal the wound. As the system controller, they regulate the repair machinery. Like any system controller, macrophages are executives who are informed about the status of the system and then issue orders for the workers to take some action. Macrophages start off in a standby form, the blood borne monocyte. They are informed about the status of the system by the error signal, inflammation, which has the effect of transforming the monocyte. The error signal is issued by the system error detector, and with respect to monocyte-macrophage transformation, it comes in the form of platelet-released peptides such as PDGF. These transformative cytokines cause a committed phenotypic change from the circulating planktonic monocyte to a local amoeboid cell. As mobile phagocytes, the macrophages have an afferent function to cleanup the injured area. As proliferation controllers, the cytokines that they issue muster the local stromal cells to begin repairing the wound. From a biological point of view, it makes sense that these two functions – (1) cleanup the mess and then (2) let us know when you are done (initiate repair) – are integrated in one place, the macrophage. With regard to the wound control loop, the phagocytic cleanup functions of the macrophage are an embedded or parallel loop within or intersected with the black box of the controller block. It is the proliferative growth factors that they make which feed forward as the control signal to drive the next element of the loop. (The **photos** show the macrophages, large migratory cells in the upper strata of the wound, the plasma protein inflammatory layer, and the aminoglycan angio-attraction layer. Being in the inflammatory zone, they share the scene with numerous neutrophils. The macrophages themselves, as the system controllers which regulate and attract repair cells, are the cause and target of angio-attraction.)

Control signal is growth factors. The macrophage controllers must issue their orders to the system load, and they communicate by making peptide cytokines and growth factors: IL's, TNF, PDGF, VEGF, TGF, FGF, EGF, IGF, etc. These factors have a multitude of regulatory effects on local events after injury, helping regulate the latter phases of inflammation, and serving as the commands or initiators of repair. Along with their phagocytic functions, their cytokines that influence late phase efferent inflammation can be rolled into black box functions within or parallel to the control block. The control signal which drives the repair loop is the set of potent pro-proliferative growth factors such as pdgf, vegf, fgf, tgf-b, igf, egf which signal local repair stem cells or progenitor cells to come to life. These pro-proliferative cytokines and growth factors all have the usual functions of such peptides – transformation and phenotyping, mitosis, and migration. Transformation induces a state of activity or phenotypic maturation in progenitor cells, allowing them to become recognizable angiocytes or fibroblasts with specific metabolic or structural functions. Mitosis allows the activated progenitor cells to replicate and increase their numbers to meet the needs of repair and stromal rebuilding. Migration is due to the chemotactic effects of these growth factors and the chemotropic nature of the responder cells, serving as a guidance system to bring the new activated repair cells to precisely where they are needed. The net effect of the control signal is to induce a state of histogenesis, in which local responder cells will turn on to rebuild stroma and close the wound. The **photo** shows the effect of macrophages to produce a chemotactic-chemotropic vector that draws activated angioid cells into the wound. The plasma protein inflammation layer is at the top, and below that is the full length of the angio-attraction layer. There is a background of neutrophils as expected. In the plasma layer there are large amorphous mononuclear cells – the macrophages. At the bottom an organized vessel can be seen. In between are vertically oriented spindle shaped cells. These are migratory angiocytes. They arise from vessels below, activated by the mitogenic and chemotactic effects of vegf and other angiogenic cytokines. They migrate through the intervening aminoglycan layer toward the source of the angiogenic stimulation, the macrophages above.

Controlled load is the set of responder cells which, commanded by the controller (macrophages), will do the actual work of trying to restore the system and correct the system error. These are the local mesenchymal cells – angiocytes first, then histioblasts, and lastly epithelium – which will restore the damaged stroma and close the wound. These cells exist normally in a standby form, either as passive stem cells awaiting the call, or else as mature functioning components of existing stroma that are still capable of proliferation or pluripotent re-differentiation. There seem to be 3 sources of the regenerative new mesenchyme. The stem cell reservoir of regenerative angiocytes and fibroblasts has long been ascribed to angio-pericytes, the perivascular cells. In recent years, the honor has also gone to circulating presumably marrow-derived pluripotent stem cells. There certainly is evidence that both play a role in repopulating the healing wound. However, one need only to look at any H&E or trichrome stain of any random wound biopsy to realize that a preponderance of regenerative cells come from existing angiocytes in nearby normal blood vessels. In the normal tissues subjacent to the wound, angioid cells all become hypertrophic with cytoplasm and nucleoplasm, an effect of pro-proliferative pro-mitogenic pro-migratory angiogenic factors. Angiocyte mitoses are easy to find in or near vascular walls, and the peeling or breaking away of angiocytes from existing vessels as a prelude to their spindle-shaped migration is abundant. The origin of fibroblasts between coalescing vessels in the mid strata of the wound is not so obvious. Remember that there is extraordinarily little biological difference between angiocytes and fibroblasts and myofibroblasts, and they can share or trade phenotypes. For example, in conditions of tissue growth and vascular adaptation, new vessels begin as minute capillaries of just one or a few circumferential cells. As they grow, mature, and develop a larger angiosome, they must get a progressively larger diameter and thus they must also get thicker (Wolf's and Laplace's Laws), eventually getting a muscular media and elastic lamina, etc. It is all the same cell doing these different tasks, taking on these different avatars based on local signals, promoters and inhibitors, based on the principles of parsimonious self-organization (see Part 3). Perhaps new angiocytes in the healing wound derive largely from existing angiocytes, whereas fibroblasts are more likely to come from pericytes and circulating stem cells. Perhaps they all contribute equally. This is where conventional bioscience is necessary to answer unresolved questions. What is important from a control point of view is that the system controller issues a control signal, and any and all potential responder cells that happen to be there (or are chemotactically attracted there) can then respond. The **photos** show the assembly of new stroma as a consequence of the system load responding to the controller. The upper photo shows mid-level

wound events in the histio-attraction layer, where young fibroblasts are making young collagen and matrix interspersed between organized but still immature vessels. The lower photo shows events deeper in the histio-organization layer, denser flattened fibroblasts packing the space with fibrous collagen.



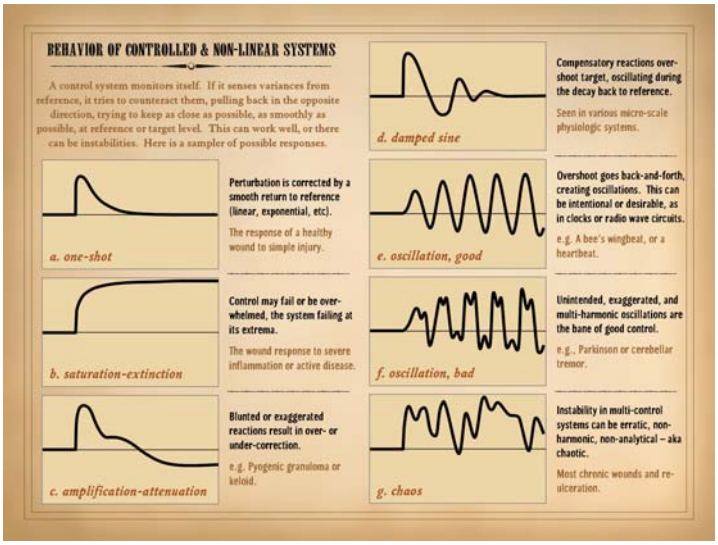
22
This slide focuses on the back end of the Wound Main Control Loop, the system output created by the controlled load, and the way that this feeds back into the system comparator.

Output is the proliferative part of the wound module, the tangible substance and structures that result from the activities of the system load. Remember, from a control point of view, it is the responder cells, the active agents of production, that are the load that must be controlled. The system output is the stuff that they make – fibro-vascular matrix and wound closure. Specifically, the load cells produce (1) restoration of mesenchymal stroma via aminoglycans and ground substance, then vessels, then fibrous matrix, and (2) wound closure and mesenchymal sequestration via contraction and epithelialization. (The **photo** shows a histological view of the wound at the level of the histio-attraction layer, demonstrating all of the mesenchymal components of system output and tissue building. The other photo is a gross view of the new stroma – "granulation tissue" – plus the signs of closure – contraction and epithelialization.) The generation of output products directly modifies the state of the system, i.e. more tissue and less wound. This has the dynamical effect of bringing the system back toward the system reference. That of course is the whole purpose of any control loop, to drive a perturbed system back toward reference. As the load cells create output which corrects the variances in the system, the discrepancy between actual and ideal state, as measured by the error detector, will diminish. As the error diminishes, the error signal and the driving force in the loop starts to diminish, eventually ceasing when the discrepancy is zero. In this explanation of the Wound Main Control Loop, all that remains to complete the loop is feedback, some way to report the current state of the system back to the system comparator.

Feedback is the open wound, i.e. some detectable and transducible set of properties of the state of "openness". Recall from the discussions on slide 20 the differences between the ways that the system comparator senses the feedback and the reference. System reference (normal closed tissue) is a passive condition. The error detectors (plasma-platelets-leukocytes) simply "ignore it", meaning that they are not activated by any of the properties of normal tissue – which is of course the way that they are tuned – plasma, platelets, and leukocytes do not activate when the are contained in healthy uninjured blood vessels and unstressed tissues. What the comparator elements are actively sensing are the byproducts of injury and inflammation and openness – these are the conditions which trigger or activate plasma, platelets, and leukocytes. Specific activating stressors or triggers include hemorrhage, plasma leak, stasis of blood-plasma-platelets-leukocytes, necrosis, bioburden, fluid and hydration changes, vascular reflexes, etc. How it is that these conditions initiate and auto-amplify thrombosis and inflammation is the stuff of conventional bioscience and need not be reviewed in depth. What is important from a control point of view is that any open wound will have some degree of any or all of these attributes, and these are the conditions which activate or perpetuate plasma, platelets, and leukocytes. Thus "open wound" is a positive input on the system error detector, whereas healthy and healed is a neutral input. For the system comparator and error detector, an open wound and its attributes are always a detectable discrepancy from normal, and the discrepancy elicits a response, the error signal of active inflammation. As long as there is "open wound", there is a positive error signal, and the loop keeps driving until the error is gone, meaning no more "open wound", i.e. healed. (The **photos** show a healthy wound actively healing. The progressive closure is a consequence of output from the load cells, but until it is fully closed, there will be conditions which feed back to continue driving the process.)

The **Wound Main Control Loop** is an abstraction of the real process of wound healing. It is a means of distilling an extraordinarily complex physiological process with innumerable players into a valid conceptualization that clearly, simply, and precisely explains the core dynamics of the system. As will be shown on subsequent slides, it is also an open model that maintains a place for all of the biological realities of the system. It is crucial to remember that for complex systems, conventional biosciences are required to discover and characterize individual components of the system, but dynamics – physics – is required to understand how the many elements inter-operate. The loop is autonomous. It starts and stops correctly depending on the circumstances of normal tissue versus open wound. Once an injury or wound is closed and reorganized, the control loop ceases to run. However, the inherent machinery and dynamics are still there, in an unseen quiet or standby state ready to reactivate if new injury or wound occurs. Note that when the wound is epithelialized and nominally healed, that related biological activity is not yet fully finished. The restored tissue is not fully reorganized until it is completely matured, a process which will take months or years. Maturation is also a regulated process, but it operates sequentially after the main wound healing process and control loop are settled. Thus, maturation can be seen as a parallel or sequential process, important biologically, and intersected but otherwise separate from the Main Loop. The **photos** are the same as those on slide 3, reminding that normal skin does not spontaneously start healing, and that a healthy wound will go through the process properly until healed, and then the process ceases.

Finally, notice some of the key dynamical attributes of the Wound Main Control Loop. This system is self-organizing – as the loop runs, tissue reassembly is automatic and correct. The loop and wound healing are purely reactive – they do not start and stop of their own initiative. Rather, they start and stop only in response to explicit trigger conditions. The loop and wound healing are reference or error driven – they operate only as required to correct a variance from normal. Thus, assuming that the process is healthy, it will accurately restore the injured tissue to normality. Reactive self-organizing error-driven dynamics are of vital importance to the health of the system and host. This is true for all systems with these attributes, especially for life-and-death biological systems, as these properties confer robustness and fault-tolerance against the noise and perturbations of normal variability and extrinsic stresses.



There are many reasons for modeling or abstracting a complex system. Among them are being able to observe and study the behavior or output of the system, and being able to understand what is wrong when the system varies from normal. For the moment, we will leave wounds behind and return to control systems in general. There are certain types of generic responses or behaviors that all controlled and non-linear systems will potentially exhibit, and we will look at them here.

Remember the basic purpose of control. Systems need to be at a certain value or within a certain operating range in order to function as intended, or the system needs to maintain a certain state or value for the sake of its output. Once a system is at a desired value, it might not stay there. There may be decay or drift of intrinsic parameters, or it may be perturbed by extrinsic forces. A control system monitors itself. If it senses variances from reference, it tries to correct them and restore the system to the reference value. The system is trying to counteract changes, pulling back in the opposite direction of the unwanted change. If the system drifts low, the control loop pulls up. If the system is pushed high, the control loop pulls down. The control loop is always trying to reverse the direction of unwanted variances, trying to keep the system as close as possible to the reference or target level. Ideally, these corrections are made as quickly and as smoothly as possible. However, there can be errors or instabilities in the correction itself, leading to over correction, under correction, or a variety of dynamical behaviors. Whether or not the system behaves as needed depends on the quality and tuning of the circuit. In engineered systems, man made machines can deliberately be designed and tuned to have specific types of responses. In natural and biological systems, the quality and tuning of the responses represent evolutionary adaptations based on principles of thermodynamics and non-linear dynamics (see Part 3). Here is a sampler of possible generic responses in controlled systems.

One-shot. An incidental perturbation or trigger suddenly knocks the system to a new value. The change from baseline is corrected by a smooth return to reference or baseline - **convergence**. The dynamics or mathematical nature of the return can vary based on the nature of the system. The decay could be at a constant rate (zero-order, linear), or at a rate proportional to its current value (first order, exponential), or with some other dynamic. First order exponential decay is probably the most common dynamic in natural systems (chemical, electrical, mechanical, physiological, etc). This will appear as the typical curved asymptotic graph illustrated. This is the ideal response to perturbation, the way engineers would want to make most machines behave. Common examples from biology include the serum concentration of a drug after a one-shot injection, the relaxation of tension in a stretched scar, and the response of a healthy wound to simple injury. Normal wound healing, its aggregate closure as well as many individual components, and especially the overall dynamics of the Wound Main Control Loop, will exhibit this response to the initial injury, assuming that the system is healthy and that the injury was a one-time instantaneous event.

Saturation-extinction. Under certain circumstances, control may fail or be overwhelmed. (1) The intensity of the perturbation can drive the healthy system out of bounds of its operating range (if you will, the extrinsic disturbing force is greater than the strength of the system to respond). (2) If the system is intrinsically inadequate or has become faulty, then it cannot respond as designed to forces which would otherwise be within the response range. The effect is that the system fails at its extrema, reaching its maximum physical capacity to respond, or exceeding its operating range and design limits - **divergence**. It could go into saturation, failing at the upper or loaded end of the range, or it could go into extinction, becoming zero. Either way the system was meant to be operating somewhere in between, and it has now been forced up or down out of the operating range. Since the system is no longer in its operating range, it can no longer function and respond to the altered state, and thus it cannot correct itself. The system will then remain in its saturated or extinguished state until the perturbation is relieved (or the system machinery is repaired). As a biological example, consider a patient with congestive heart failure, cardiomyopathy, and coronary artery disease who is currently compensated and free of acute symptoms. Suddenly the patient goes into florid failure due to a hypertensive crisis or the onset of pneumonia or peritonitis. Sudden changes in afterload or cardiac work and preload will change many parameters such as myocyte fiber length, ventricular diameter, ventricular dp/dt, etc., i.e. those physiological parameters that must be properly "tuned" if the myocardium is to remain in its proper operating range and be an effective pump. Even oxygen delivery and energy utilization are important for the myocardium to function effectively, but those parameters are impaired because of the coronary artery disease. See what is happening? The acute extrinsic stressors result in decompensation of fiber length and ventricular diameter which push the pump out of its operating range. The coronary artery disease is loss of an intrinsic component, loss of a "degree of freedom" in the system's innate ability to react and compensate. As the failure begins, conditions which provoke failure get even worse (a non-linear amplification), and the decompensation progresses until there is no chance of the system ever pulling itself back into the proper operating range. For any specific parameter you are looking at, they are all out of bounds, either high or low, saturated or extinguished. If the pump is physically damaged e.g. by an infarct, then corrections may be impossible. However, if the machine stays inherently undamaged, then interventions to restore the proper operating range will allow the system to come down from its saturated state and start once again to actively control and properly regulate. Treating hypertension or volume overload or systemic metabolic load or correcting coronary artery occlusion are the types of interventions which will be needed to pull the system back into operating bounds. As a wound example, saturation is the response to severe inflammation or active disease. Under conditions of active disease and ulceration, the repair process cannot prevail over the destructive events. The repair process and control loop become saturated – on full time "overdrive". What is happening is that the error detector sees a widening discrepancy between normal and actual state, so the system gets driven to maximum capacity. However, the control loop is "blocked" or "open circuit" at the system output stage, because output is being suppressed or destroyed by the active injury and inflammation. No output means no correction of the system state. Therefore "open wound" feedback does not diminish (in fact it gets worse due to disease and inflammation). The system error persists and the front part of the control loop remains maximally driven and operating at its peak physical capacity, i.e. saturated. So, the system is saturated, but nothing productive happens. Alleviating the active disease and injury will allow the control loop to

reenter its operating range.

Amplification-attenuation. The response of the system to perturbation might be qualitatively correct but quantitatively incorrect – too much or too little. If the response is amplified or exaggerated, then there will be an over-correction. If the response is blunted or attenuated, then there will be an under-correction. If the loop continues operating, there will be subsequent opportunities for the system to correct itself, but if the imbalance is system wide or consistent, then it will repetitively miss the mark. Biological wound examples that seem to fit this dynamic are pyogenic granulomas (see slide 28) and keloids, in which the wound healing process is amplified, exaggerated, and overshoots the intended target, making too much wound healing and too much new tissue.

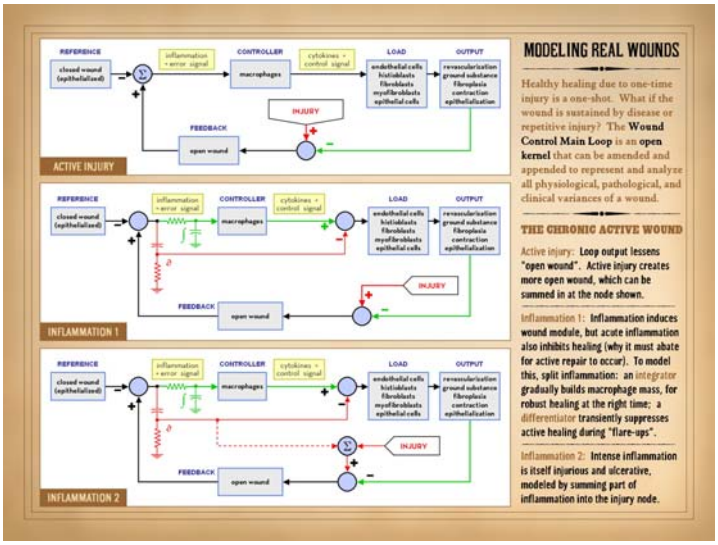
Damped sine. A controlled system needs time to sense errors and actuate corrections, and the corrections need time to propagate and feed back. The consequence of such time delays is the possibility of over- or undershooting the target, or getting oscillations. When the controller initiates a correction (implemented by the load), that correction will have a certain velocity (speed and direction), a rate at which the system state changes in the desired direction. If the rate is perfectly tuned, the system will settle on the correct value. If the correction is a bit too fast or strong, it risks overshooting the target. If it does overshoot, this generates an error condition of the opposite sign, and the control system then tries to bring the system back the other way. If it could all be sensed and actuated instantaneously, the return to baseline would always be smooth. However, because of time delays, the controller is unaware of the overshoot until it has occurred, and any corrections it then makes will need time to kick in, allowing the system to continue moving the wrong way before it can start moving back the other way. Imprecision and delays will be consistent, so the same thing happens going in the other direction. The net effect is that the system "rings", a transient oscillation as it decays back to reference, The peak-to-peak envelope (or else the root-mean-square values) will be the expected exponential decay. This oscillation shaped by the decay is a damped sine wave (or a damped whatever if the oscillatory waveform is something else). This is the brief waveform you might see on the screen when you turn the power off to a conventional CRT display (television, monitor, oscilloscope, etc). It is also the transient wiggling in a spring that is stretched and released, and the slowing of a pendulum due to friction. Damped sine waves are seen in biological systems in somewhat arcane laboratory circumstances, usually related to neurological, sensory, and certain cellular and chemical events, i.e. micro-scale physiological systems. If you work with spine injured patients and have a chance to observe the reflex spasms that occur briefly in response to stimulation, that is a perfect clinical example of a damped sine wave as the spasm triggers then decays.

Oscillation (good). Not all oscillations are bad, and not all are transient or damped. Oscillation is in fact the goal and healthy productive state of many systems. The principles of feedback, reactive correction, overshoot, and timing and delay can all be engineered and tuned in such a way that the back-and-forth over-correction under-correction becomes a sustained oscillation. Of course, all systems have energy losses which will damp an oscillation, so for it to be maintained, new energy must be repetitively restored to the system, and when it is, oscillation can be sustained indefinitely. These principles allow us to build clocks and radio wave circuits and engines and all kinds of things. Biological examples of autonomous periodic, i.e. oscillatory systems include a bee's wingbeat and a heartbeat. While overt fixed rate oscillations are not evident in wounds, these principles do apply to issues of thrombosis and inflammation and the sustentation of necrosis and ulceration, as explained in Part 2 of this series, "Auto-Immunopathy and the Intrinsic Disease of Wound Healing".

Oscillation (bad). In mathematics, all periodic or harmonic (oscillatory) functions can be represented by a polynomial of sines and cosines (a Fourier series). Engineers use this principle to combine different frequencies to create any arbitrary waveform. The same principle works in reverse to get information out of combined waveforms, such as decoding a particular channel from all of the information that comes through a single television cable. So, multi-harmonic oscillation can be a good thing, but when it shows up unintentionally, or it is exaggerated, or it is the result of unintended subsidiary circuits and feedback loops in complex systems, then it is unwelcome and can be the bane of good control. All clinicians are familiar with the concept from looking at ECGs that are blurred by 60 Hz crosstalk from nearby electrical devices and wall power. As an impairment of control in biological systems, Parkinson's disease and cerebellar and other tremors are examples of inadequate or delayed control resulting in problematic single or multi harmonic oscillation.

Chaos. When systems and circuits and machines seem erratic and unstable, the problem could be one of unwanted oscillation, or it could be one of noise and random variability. However, variability in multi-control systems is usually due to something else – chaos. In fact, if you measure any complex natural multicontrol system carefully enough, especially biological systems, you will find that even repetitive functions like breathing are not strictly fixed rate sinusoidal oscillations. The behavior of complex systems can at times seem very erratic, non-harmonic, and non-analytical (not reducible to conventional functions of algebra and calculus). Yet at the same time, these systems are following precise physical rules and principles. This is chaos, the behavior of complex non-linear systems. "Chaos" is an unfortunate term because in physics and math it does not mean the same thing that in means in the vernacular vocabulary. What it means is that systems can behave in seemingly complex ways that cannot be described by simple harmonics or other equations, nor are they random and noisy. Chaotic systems are highly deterministic and rule-driven, and highly organized and structured. The trick is that you need to know how to look for that structure, and how to describe that structure if the face value data stream is to make any sense. Most chronic wounds are chaotic – i.e. they have chaotic dynamics, how they behave over time. The origins of chaos in the wound are introduced on slide 32, and chaos is explained in detail in Part 3, "Chronicity and the Physics of Wound Failure".

If you are an electrical or a mechanical or an aeronautical or a control engineer, you are apt to deal with any and all of the types of responses and instabilities described here. Examples have been given of how all of these behaviors can be seen in controlled biological systems (and remember, virtually all biological systems are controlled). However, any system has its own set of physical and dynamical realities, and consequently its own characteristic ways of failing or misbehaving. For wounds, not all of these responses apply. Oscillatory or harmonic behaviors are not relevant. We often see amplification and attenuation in response to various therapies that are applied, but this rarely changes the intrinsic dynamics of a given wound. The core dynamical behaviors and misbehaviors that are a part of everyday wound physiology, pathology, and clinical management are three: one-shot convergence, saturation-extinction-divergence, and chaos. As described in explicit detail on subsequent slides and in Part 3, the **healthy wound** has a normal one-shot response to the injury, converging on the desired state of "healed". The **sick wound**, subject to active disease and injury, is diverging, its controller saturated or extinguished as it becomes progressively inflamed, necrotic, and ulcerated. The **impaired wound** which is not actively sick from acute injury, but which nonetheless will not heal, is chaotic. Part 2 of this series will explain the biological basis for that chaos, and Part 3 will explain why, once that state has occurred, it can be difficult or impossible to get the wound to behave otherwise.



Healthy healing due to one-time injury follows simple one-shot dynamics. Both the displaced values of system parameters and the overall dynamics of the loop will settle smoothly to baseline or pre-morbid values if the system and its components are healthy and there is no perturbation by outside forces. What happens though if the wound is sustained by disease or repetitive injury? How will the control loop behave, and what will a graph of the system state or dynamics look like over time? As for any properly abstracted model, the Wound Control Main Loop is an open kernel that can be amended and appended, opened up and plugged into. For the sake of modeling real wounds, any parameter, sub-parameter, physiological reality, pathological challenge, and clinical variance can be represented and analyzed on the loop. We will now look at how the chronic active wound, sustained by ongoing disease and repetitive injury, can be represented by the loop, and how the dynamics of the loop will change due to sustained stressors.

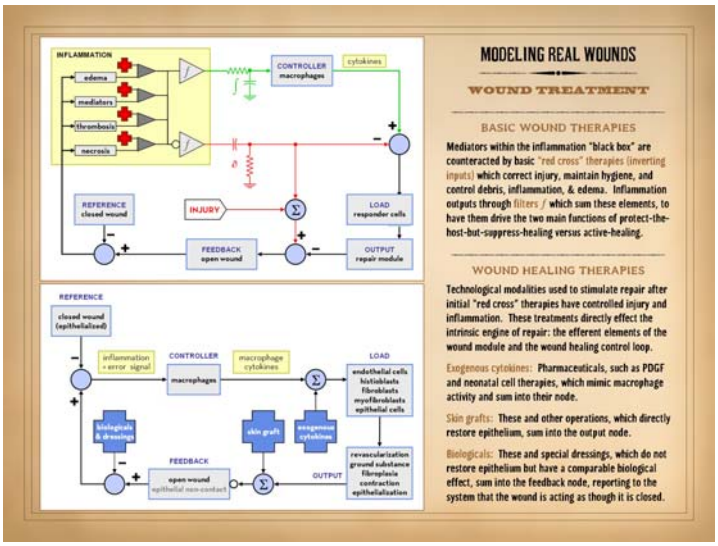
Active injury: The top panel shows the most trivial amendment to the Wound Main Control Loop, how to represent sustained exogenous injury. The effect of the loop output block is to lessen "open wound". Active injury does the opposite, it creates more open wound. These two effects obviously counteract each other, adding or subtracting from the state of the wound. To represent this, injury is summed with the system output, introduced at the node shown. The composite effect of wound healing (less wound, negating effect [-] on "open wound") and new injury (more wound, additive effect [+] on "open wound") determine the net state of "wound" which is then the feedback into the error detector which then decides how hard to drive the loop.

Inflammation 1: Acute inflammation is the proverbial "double-edged sword". Inflammation induces wound healing, but inflammation also inhibits healing. How, why, what, huh? First, recall that acute inflammation induces the wound module – this is the normal physiological sequence of events and the trigger that turns wound healing on after injury. However, inflammation is just a big "black box" event with many players, roles, internal operations, and intersections with control loops other than wound healing. It is not "inflammation" per se, its aggregate or many activities which turn on wound healing. Rather, it is just one component of inflammation that has that task, the macrophage. So, yes, it is true that inflammation induces wound healing, but . . . Acute inflammation is inherently destructive and inherently inhibitory. It is meant to destroy non-autogenous foreign biotes and chemicals. It has a variety of mechanisms via enzymes, ionic radicals, and cell killing activities that will chew up your own ground substance, connective matrix, and cells as readily as it chews up the invaders. In fact, normal healing cannot begin, the stroma reconstructed, until the injured area is first deconstructed in preparation for repair. This is all just the normal set of afferent wound events. One of the effects of acute inflammation is also to directly and explicitly inhibit the repair process. Many of the acute phase chemicals and mediators of inflammation have a direct inhibitory effect on many of the reparative events, such as angiocyte and fibroblast proliferation, that are yet to come. Teleologically, this is a beautiful mechanism that keeps the body from wasting its repair resources until the time is right. Dynamically, it allows the integrated system of injury recognition-response-repair to function correctly with proper short term dynamics and without long term adverse sequelae. These issues are discussed on slide 32, and then at length in Parts 2 & 3. Simply put, the inflammatory phase of a response to injury is itself a one-shot ramp-up then decay of defensive activities that keeps the repair phase suppressed until the right time. As acute inflammation wanes (assuming a single one-shot injury event), inhibitors of the next repair phase diminish, allowing repair to turn on. However, repair also depends on an active promoter, and the period of inflammation has allowed the numbers and effects of macrophages to accumulate. Pro-repair regulators build slowly but steadily in the acute wound, whereas initial or recurrent acute phase inflammation has a more immediate effect to suppress repair (which is why it must abate for active repair to occur). From a mathematics and dynamics point of view, these events sound like typical integrator and differentiator functions.

To model this duality of acute inflammation (middle panel) we can introduce a split, a bifurcation in the wound circuit at the inflammation error signal. An integrator gradually builds macrophage mass, for robust healing at the right time, the bridge between the inflammation one-shot and the wound healing one-shot (see slide 32). The parallel differentiator transiently suppresses active healing during "flare-ups" of acute injury and inflammation, keeping the body from wasting resources (and minimizing the chances of exposing endocellular materials to possible auto-immunization – see parts 2 & 3). If you are unfamiliar with basic principles of electricity, the circuit elements shown are two basic types of R-C resistance capacitance circuits. The green circuit feeds the error signal through a resistor, and a capacitor bridges the output to ground. Resistors impede – slow down if you will – the flow of electricity. The function of the capacitor is to accumulate charge, preventing it from flowing through the rest of the circuit until the capacitor is full. The net effect is that if a signal or voltage appears at the head of the resistor, it will take time for that voltage to build up on the tail end of the resistor. The build up of voltage is a logarithmic rise or fall which mathematically is the timewise integral of the current. Thus this circuit – the voltage on that capacitor – is an analog integrator. Also, capacitors have their own impedance which lessens as frequency gets greater. Thus, for currents flowing through this circuit, high frequency information will get shunted to ground before getting to the next block, whereas low frequencies will have time to develop their voltage on the capacitor, delivering that information to the next block. Thus this circuit is an integrator but also a low-pass filter – low frequency information gets through to the next stage, and high frequency information vanishes. The red circuit is the inverse, feeding currents through a capacitor which is shunted to ground through a resistor. Voltages applied to the head of the circuit will be immediately transmitted across the capacitor, but as charge builds up, the capacitor gets saturated and current stops flowing. Thus the waveform will be one of a voltage spike that then decays logarithmically as charge on the other side of the capacitor flows out through the resistor. This is the mathematical as well as the physical inverse of the green circuit, making its output voltage the first derivative of the current. Thus, this circuit – the voltage on that resistor – is an analog differentiator. A corresponding effect is that high frequency information will get across the capacitor, but lower frequencies are stopped as the capacitor has time to saturate. Therefore this circuit is differentiator but also a high-pass filter.

What does all of this have to do with the wound, inflammation, and the wound control loop? Everything. Remember, characterizing the "nuts-and-bolts" biology of these systems is the stuff of the conventional biosciences. Understanding the dynamical operation of the wound (or any complex) system is physics. There are of course no electrical components in the wound loop, but electrical components are frequently used as ways of demonstrating the nuts-and-bolts structure of various physical and dynamical elements when modeling systems. We could of course just replace the electronic diagrams with simple blocks containing an $\int$ or ∂ to denote integrator and differentiator. What is crucial to remember is that however we choose to represent these elements and events, it is their interoperations and dynamics that are important. To reiterate, acute inflammation causes the buildup of macrophages, a low frequency integrator function that in the healthy wound ensures the one-shot coupling of inflammation to the reparative wound module. However, sustained or repetitive or recurrent acute inflammation will have an immediate inhibitory effect on the wound by negatively affecting the controlled load, the actual repair cells. This suppression is a high frequency differentiator function that ensures that repair resources are not wasted in the early inherently destructive phase of response to the injury.

Inflammation 2: The defensive and destructive aspects of acute inflammation do more than just inhibit or delay repair. By its very nature of being destructive, it is injurious to the host matrix or tissue. With sustained injury and pathological wound conditions, acute inflammation is the proximate mediator of active ulceration. Clinically, this is especially evident in suppurative and in autoimmune ulceration where the inflammatory-lytic pattern of active ulceration is overt and aggressive (see Part 2). The ulcerative effects of acute inflammation can be modeled by summing the immediate defensive "differentiator" limb of acute inflammation into the injury node.



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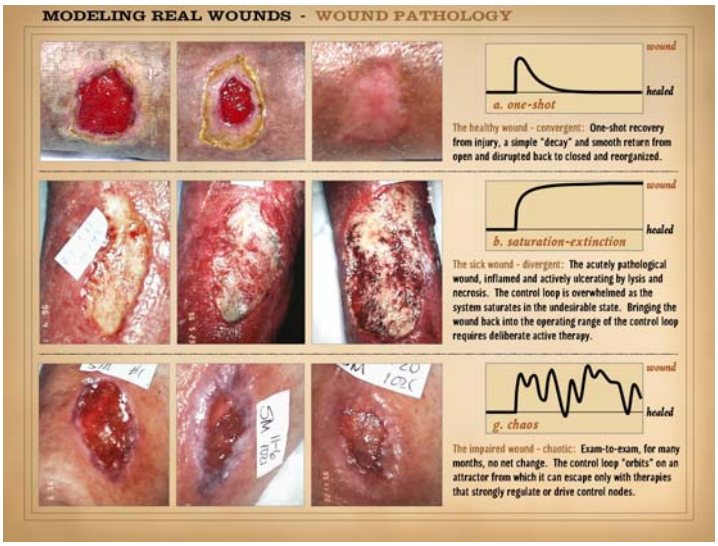
As a further example of how the Main Wound Control Loop is a comprehensive open model that can accommodate all aspects of the wound, we will now look at the role of wound therapies and clinical interventions in affecting the operations and dynamics of the loop.

Basic wound therapies: The first panel demonstrates the effects of basic wound therapies treatments. These are the general, diagnosis non-specific, common treatments that are the first and mandatory set of therapies that control a wound, arresting its progress, alleviating symptoms, and generally ensuring the safety of the patient. These include basic wound hygiene and debridement, topical care, and control of inflammation and edema. Each of these interventions counteracts some detrimental aspect of the wound, host, or injury that can be represented as a component of inflammation. To illustrate these ideas, we start with the "Inflammation 2" panel from the preceding slide, and then we open up the inflammation "black box". Within the box, four aspects of inflammation have been selected to illustrate these dynamics: edema, chemical mediators, thrombosis, necrosis. (These four are important aspects of inflammation, but they were selected arbitrarily and limited to just 4, so as to keep this illustration simple.) Each of these inflammation mediators is shown feeding into a two-input amplifier (grey triangle, direct and inverting inputs), paired with a "red cross" therapy which corrects injury, maintains hygiene, controls debris, inflammation, & edema, etc. The therapies counteract the pathologies, and depending on which has the greater strength, the output of each amplifier will flip positive or negative. All of these individual elements with their counter-therapies then sum through output filters f which determine the net value of the state of inflammation. The two output stages are inverses of each other (the circle input is a negation or inversion). Depending on the net state of acute active destructive inflammation versus settled treated conditions, one or the other limb will be driven, either the integrator function which builds the system controller (the treated state, leading to active healing), versus the differentiator function which inhibits healing (the acute inflammation state of protect-the-host-but-suppress-repair).

Wound healing therapies: Wound healing therapies are the technological products and modalities used to stimulate repair after initial "red cross" therapies have controlled injury and inflammation. These treatments directly effect the intrinsic engine of repair – the efferent elements of the wound module (controller and load cells) and the dynamics wound healing control loop. Three examples are shown. **(1) Exogenous cytokines** include pharmaceuticals such as PDGF, and neonatal living cell therapies. These either mimic or go beyond natural macrophage activity to make pro-proliferative growth factors. As such, they are acting as off-the-shelf wound controllers or control signals. They are represented by summing into a node that combines with the native macrophage growth factors. **(2) Skin grafts** and other wound repair operations have the effect of directly restoring epithelium and immediately closing the wound. They have the same effect as "system output", the proliferative tangible "stuff" which restores tissue and diminishes "open wound". As such, they are represented by summing into a node that combines with the output. **(3) Biologicals** are non-living biologically derived materials that are meant to be used as skin substitutes or temporary wound closure. They also include non-biological materials that are engineered to serve the functions of skin and biological covers. These materials do not restore actual living epithelium, but as skin substitutes they have a comparable biological effect (even if only of limited duration). They do not create genuine durable living "system output", but they do have the immediate effect of diminishing the state of "open wound". As such, they are represented by summing into a node that combines with the system feedback, thereby reporting to the system that the wound is acting as though it is closed.



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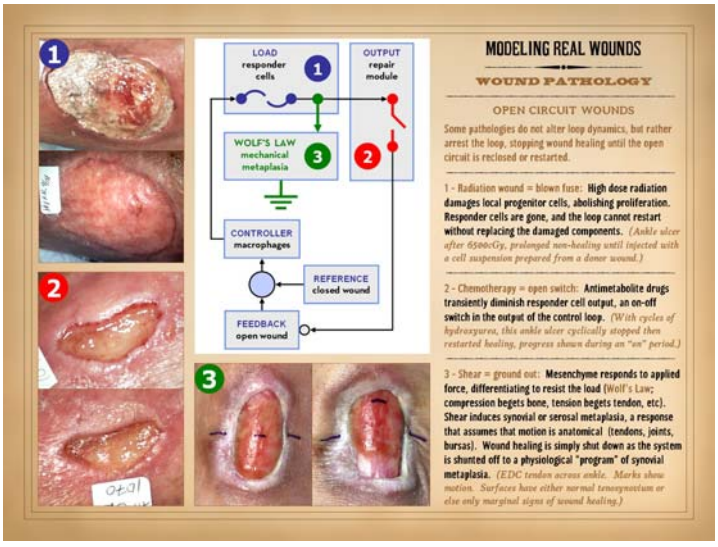
On the last few slides we saw how we can diagram the wound control loop to account for perturbations and altered states and other variances from normal healing, including how therapeutic interventions can fit into the system. A diagram is one thing, but the goal of having it is to understand how the system actually operates and responds, what the output of that diagram will look like, and how to use it to understand the behavior of the system. Here we start to look at what happens when the wound is stressed or pathological, i.e. how the core loop (or the core with circumstance specific amendments) reacts to normal and abnormal conditions. Keep in mind that even when the wound seems to be in whacky and crazy or frustrating or exasperating states of misbehavior and non-healing, that does not necessarily imply that the wound healing machinery is broken, The main loop and its physical-biological components are meant to respond to inputs and perturbations, and if the inputs get crazy, then the responses can seem crazy too. As will be seen in detail in Part 3, the wound control loop and its core elements rarely get inherently sick. Rather, their responses to complicated sustained adverse inputs can become disorganized. On slide 23, we looked at generic responses of controlled systems to perturbation. The kingdom of biology has examples of all of those types of response, such as harmonics and oscillations, but for wounds and most other biological systems, controlled behavior tends to fall into three general responses or attractors.

Convergence: This is the healthy wound. The dynamics are a one-shot. Injury or disease suddenly "jumps" the wound up to an elevated state. From there, the control system brings it back down to normal (healed) along a simple trajectory of "decay", ideally a smooth return from open and disrupted back to closed and reorganized.

Divergence: This is the sick wound. Primary disease and injury are at work, causing active necrosis and ulceration by thrombo-infarctive and inflammatory-lytic mechanisms. The control loop is either overwhelmed by the strength of the perturbation, or it is suppressed by one means or another, and the wound rises into the undesirable dynamical state of saturation, a "full blown" actively pathological wound. Bringing the wound back into the operating range of the control loop, where individual components of the control loop are not overwhelmed or suppressed, where the wound healing machinery can then operate to heal the wound, requires deliberate active treatments to abate the disease and injury. No magic wound potions and no legitimate wound healing therapies will succeed when the wound is acutely sick and inflamed. There are plenty of tangible chemical, cellular, biological reasons for this as well, but as a dynamical system, it is also easy to see how a saturated system has to be brought back within the operating range of the repair system before it can work. Clinically, that means first things first: control disease, injury, and inflammation, and only then when they are controlled can you move on to step 2, discretionary therapies to make the wound heal, or make it heal faster.

Chaos: This is the impaired wound. These are the wounds from slide 2, the premise of this talk. Exam-to-exam, for many months, there is no net change in the wound. It does not necessarily get any worse when low levels of underlying disease are alive, and it certainly does not get any better in spite of a wide range of diligent treatments. The control loop "orbits" on an attractor, going back and forth a bit, but never getting too far, and from which it can escape only with therapies that strongly regulate or drive control nodes.



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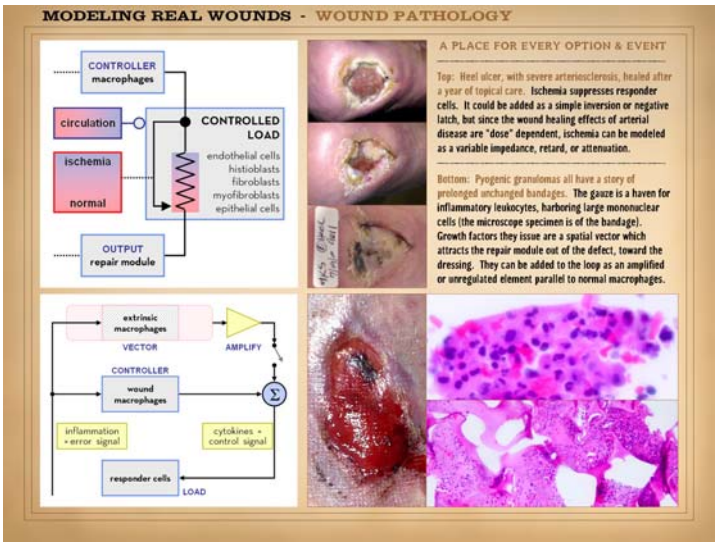
The last slide looked at wound pathology from a dynamical sense, looking at the attractors or generic dynamics that can occur in healthy and sick wounds. Here, we look at how the control loop can be adjusted to account for specific diseases or clinical disorders. Here are three examples of "open circuit" wounds in which the control loop is interrupted in one way or another, thereby arresting wound healing until the open circuit is reclosed or restarted.

1 - Radiation wound = blown fuse: High dose radiation damages cells in ways that keeps them from dividing. That is its therapeutic intent when used for cancers, keloids, heterotopic bone, etc. However, the same effect on stromal tissues does latent damage to local progenitor cells, inhibiting their ability to have a proliferative response to injury. Mesenchymal responder cells, normally in wound healing standby mode, are rendered incompetent to do their job if eventually needed. With responder cells gone, the controlled load in the system is missing, and the loop is thereby opened at that position. The effect is modeled as a blown fuse rather than an open switch. The difference is that switches can be flipped on and off, whereas a blown fuse is an irreversible fault in the circuit which can be corrected and the loop restarted only by replacing the damaged component. The example shown is an ankle ulcer after 6500cGy for mycosis fungoides (cutaneous lymphoma). Prolonged non-healing was corrected by transplanting wound competent cells back into it from a donor wound. This was done by implanting a perforated plastic chamber under the skin in a healthy part of the body (abdomen in this case). Two weeks later, the "granulation tissue" wound module within the chamber was harvested, then minced-trypsinized-suspended, then injected by syringe and needle throughout the wound. The top photo is the appearance at two weeks, with granulation tissue starting to appear. A second

harvest and transplant was done at that time, after which 100% of the wound proliferated, and then a skin graft was placed. The lower photo is the healed result a few months after the process was started.

2 - Chemotherapy = open switch: This is another ankle ulcer in a patient with a myeloproliferative disorder being treated with hydroxyurea. The ulcer would start and stop in synch with cycles of the drug. Unlike radiation which damages the proliferative cells, thereby damaging the load element, antimetabolite drugs have only a transient effect to diminish the metabolic output of the responder cells. Thus it is acting as an on-off switch. Although its immediate biological effect is on the load cells, its dynamical effect is on-off for the output element of the control loop. The two photos show the progress made during one cycle of drug = off and healing = on.)

3 - Shear = ground out: One of the most important principles of mesenchymal biology is its ability to respond to mechanical force – compression, tension, shear. Under load, mesenchyme responds or differentiates in such a way as to resist the load, thereby minimizing stresses or strains in the material. This is a crucial part of normal musculoskeletal and cardiovascular embryology, histogenesis, and organogenesis. It is also the principle that governs much of the pathology, sequelae, and care of countless problems in clinical medicine. In its earliest enunciation and best known form, it is Wolf's Law – compression begets bone, tension begets tendon, etc. With shear, the need to minimize stress within tissues is corrected by having the stroma (fibroblasts and angiocytes and the structures that they make) undergo synovial or serosal metaplasia. This is a response that assumes that motion is anatomical (tendons, joints, bursas, coelom, etc). The healing wound is nothing more than active biologically plastic mesenchyme. When shear is present, normal wound healing is simply shut down as the system is shunted off to a physiological "program" of synovial metaplasia. Of course, that is the teleological way of seeing it. The reality is that there is no difference in this situation between "healing" as we envision it and the serosal metaplasia. It is just mesenchyme – fibroblasts, etc. – responding to the applied inputs and cycles of the control loop. When shear is present, the young mesenchyme rearranges itself into mature flat fibrous lamellae and then goes to rest so that no adhesions form between surfaces that were meant to move. (It is also the shearing of blood flow against the angiocytes in forming blood vessels that matures the angiocytes and "solidifies" their aggregation into formal vessels.) In embryology, this process is essential. It also works to your advantage when you are trying to rehabilitate a tendon or joint, but it works against you when you are trying to make flaps heal on their tangential surfaces or when you are trying to get skin to heal over a moving tendon. Shown is an EDC tendon across ankle (extensor digitorum communis). The ink marks show the range of tendon gliding with active ankle motion. The wound surfaces have either normal tenosynovium or else only marginal signs of wound healing. Observe on the left that the fully exposed surface has slight amounts of angiogenesis, etc., i.e. granulation tissue. On the right, with the tendon transposed upward, the areas that are shearing directly against another surface have white, flat, non-angiogenic, non-healing normal serosal-synovial surfaces. The wound margins are obviously contracting, but they are doing what they should, following the topological surface, meaning that instead of the defect constricting, edges are curling under themselves. This is completely 100% normal appropriate behavior of a healthy wound, following the innate genetic and dynamical program that ensures that embryological structures form and that most wounds heal properly. The problem is that it can be very frustrating clinically to have to bear with this until the correct treatment is done. In plastic surgery, this is a straightforward issue of "essential coverage", a situation that mandates some sort of flap (or flap substitute) or else it will not "heal". Dynamically, the situation is comparable to grounding the circuit. The load, the mesenchymal responder cells are simply exiting the loop, shifting away from the stromal repair model of proliferation to the synovial metaplasia model of maturation. As will be explained in greater detail in Part 3, this is all part of the process of automatic self-organization. These are the same cells, following the same code, but they are now in a different state space, a different "subroutine" than the wound healing space.



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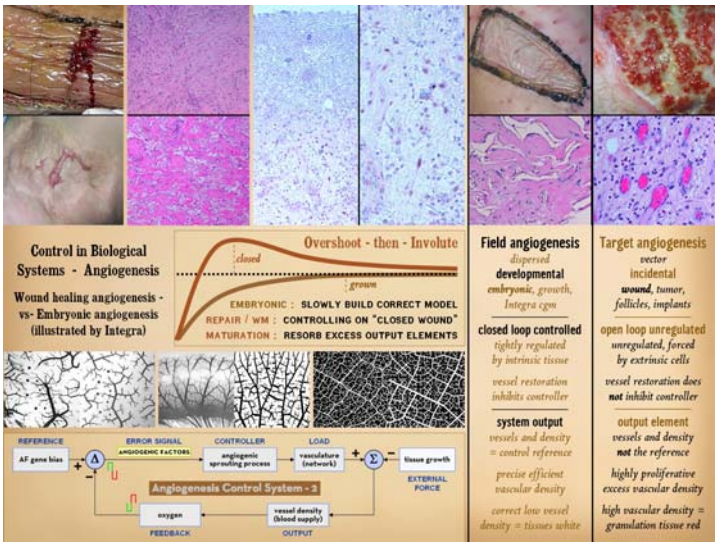
All numerical and engineering models are abstractions of the real world. That is the purpose of modeling, to reduce complex systems to quintessential elements that are computationally manageable yet still accurately portray the system for the sake of experimentation or solution. That means that no model can be all-inclusive without reverting to the complexity that made modeling necessary in the first place. However, if the model is well constructed, then it is "open", allowing components to be added or linked to account for special circumstances. The main wound control loop, as the "backbone" of the whole process, does indeed allow any circumstance of disease, risk factors, and injury to be accounted for. Here are two examples.

Top: This is a heel ulcer with underlying unreconstructable arteriosclerosis. As for most heel ulcers, it healed with topical care alone, but with retarded dynamics, taking one year to close. Ischemia suppresses or delays responder cells which are the load in the loop. The block labeled "circulation" is one way to show this effect. It represents arterial disease (not blood supply). It acts as an inverting control on the load, suppressing the load when disease is high (since the vascular occlusion does not vary, this block could also be a switch or latch as opposed to a continuous attenuator). Alternatively, to more clearly portray the "dose" dependency of arterial disease, ischemia can also be modeled as a variable impedance to act as a retard or attenuator. Then, when the system is normal, the impedance is bypassed and the loop operates properly, but when ischemic, loop dynamics slow due to lower "currents" and downstream "voltages". Note that the arterial disease block is an additional factor extrinsic to the core elements of the control loop.

Bottom: Pyogenic granulomas are just excessive wound module. They form from otherwise normal wounds. Their name is a misnomer, since they are not really suppurative, and they are not granulomas at all in the histo-pathological sense. They are just beads of granulation tissue, and they almost all have one feature in common: they occur after a period of inept care, usually poor wound hygiene covered by bandages which are unchanged and left in place for prolonged periods, a week or more at a time – "Band-Aid disease" if you will (with apologies to the Band-Aid company). The problem is that normal inflammatory cells get into and function within the gauze. The microscope picture is a specimen of an

unchanged "sheer strip" bandage, a haven for healthy active inflammatory leukocytes, including large mononuclear cells. This has the effect of elevating the inflammatory layer of the wound off of the surface. As in any wound, the monocyte-macrophages issue growth factors which create a chemotaxis-chemotropism vector that draws the wound module upward. In the case of a pyogenic granuloma, that attraction of the wound module does not cease at the level of the surrounding skin, but instead keeps reaching for the source of the stimulus, the dressing. This effect can be added to the loop as an amplified or unregulated element parallel to normal macrophages. As diagrammed, there is an on-of switch showing that this effect can be started and stopped instantaneously but putting on or taking off an overrun dressing.



Addendum to Slide 28
This slide is an addendum to the original presentation, added to further illustrate the multi-faceted significance of control in biological systems and wound healing. It looks at angiogenesis, the formation of blood vessels, and the difference between the closed loop feedback regulation of normal embryonic angiogenesis versus the dynamics of angiogenesis in wound healing.

Normal embryonic angiogenesis, meaning the formation and morphogenesis of a life-and-death critical vascular distribution network, is itself a non-linear closed-loop controlled process. As such, its "laboratory" visualization depends on modeling and computational simulation. The controlled dynamics of embryological angiogenesis is explained by the VT (Vascular neT) model of angiogenesis, which will be explained briefly here. Then, in order to directly demonstrate the consequences of normal regulated embryonic angiogenesis, photos are shown of histogenesis in a regenerative bio-matrix (Integra® collagen-aminoglycan artificial skin). (See the Arimedica website for extensive information about Integra collagen-gag matrix and artificial skin. See especially http://www.arimedica.com/content/arimedica_integra histogenesis_gottlieb-me_v2003.pdf and http://www.arimedica.com/content/arimedica_integra_in situ tissue engineering_2005.pdf for information about the histology and dynamics of its regeneration. For information about the VT model and angiogenesis, see "Developmental Angiogenesis and the Biophysics of Vascular Network Formation", May, 2006. http://www.arimedica.com/content/arimedica_vt_(slides)_2006-0516.pdf)

To begin, we will look at normal vasculogenesis and the dynamical controls that regulate formation of blood vessel networks. The VT (Vascular neT) model of angiogenesis is a numerical model of the non-linear physiology of embryonic vasculogenesis. The model has just four inputs, a "growth model" that governs how the host tissue grows, and three intrinsic angiogenic parameters: "ischemia threshold" which is how far away from the vascular network a cell in the host tissue must be to "feel" the ischemia and trigger angiogenesis by cytokine stimulation; "reach" which governs how far from its origin on the network to the stimulating cell a new vessel grows; "anastomosis" factor (multiple sprout multiplier) which governs the number of new vessel sprouts which respond to angiogenic stimulation. The iteration of this model begins by "growing" the host tissue, meaning that the whole domain is enlarged, then it is subdivided or "tiled" into individual cells of uniform base size. Next, the distance-to-network is calculated for each cell in the tissue. For any cell that is too far from the network, it is ischemic, forcing it to generate angiogenic factors which diffuse back and stimulate the network to sprout a new vessel. The new vessel grows toward the stimulus, the ischemic cell, thereby relieving the conditions which triggered the response. Obviously, this process is iterative (recursive), non-linear (its next state depends on its current state), and regulated by feedback (oxygen or distance to network) and closed loop control (comparison to a reference measure of ischemia, i.e. the numerical threshold or the bias on the vegf gene). This is how the normal biological process works, and the numerical model simply simulates the reality with a strictly deterministic set of rules based on the physics of diffusion and the physiology of angiogenesis and angiogenic factors. Not surprisingly, model outputs closely mimic real anatomy, not just vascular morphology, but also the mathematical and engineering parameters of the network. The model also recreates many other branched networks in a diverse array of biological systems.

Lower: This is a control diagram of normal vasculogenesis. The ***feedback*** is oxygen levels at each cell (in the VT model, it is the distance-to-network). The ***system comparator*** is the host or parenchymal cell which checks oxygen feedback against the ***system reference*** (real = bias or stimulation threshold on the vegf gene; model = ischemia threshold). Note that feedback is inverted at the system comparator, so that when oxygen is low, comparator output is high and there is a "green light" go on driving the whole process. The biological ***error signal*** is the expression of angiogenic factors. The ***system controller*** is the new vessel sprouting mechanism (numerically and biologically). The ***load*** is the entire vascular network. The ***system output*** is vascular density or the r-net. In topology, an r-net is a system in which no point or element in the space is greater than distance r from the network. Note that the new vessels are not the system output, as you might have thought. That would be the case if the system was trying to build a vascular network using a vascular "blueprint" as the reference, but it is not. The new vessels change (control) the network (the load) leading to a subsequent change in the output of the network. The output of the network is the unfettered availability of oxygen, which anatomically means correct vascular density or r-values. This is a crucial point to understand. The blood vessels do not exist for their own sake. They are a supply network that serves the host cells. The host, which includes the system comparator and controller, is not explicitly trying to build a vascular network nor does it care what the network looks like or how it is constructed. The host only cares that it is getting enough oxygen. Each host cell will actively control and drive the system until its metabolic demands are met. For these demands to be met, every host cell must be within a certain distance of a capillary. Each capillary can service several cells (higher or lower depending on the metabolic rate of the given tissue), and ultimately what results is that there is a given density of vessels in the tissue, or a given value of r (equivalent to the ischemia threshold). Growth of the host tissue is the ***external force*** which perturbs the system and diminishes vascular density and thereby drives the formation of new vessels.

Note that growth of the embedded vascular network is strictly reactive and space driven, rather than being autonomous and network driven.

New vessels grow only in response to needs dictated by the host cells, and this space-regulated reactive growth leads to a controlled vascular density. It is true that nature produces a wide variety of morphologies in vascular networks, different patterns from one tissue to the next, one species to the next. However, these variances form a set or family of patterns, and vascular networks throughout nature all fall within this family. Morphological differences are determined not by any variance in the nature of the angiogenic control loop, but solely by the growth characteristics and metabolic demands of the host tissue. More important though is that dynamically, vascular networks are all equivalent in that just one single process creates them all, described by three parameters representing the diffusion and reactions of oxygen, angiogenic cytokines, host cells, and angiocytes. Also, they are all mathematically identical – topological fractal r-nets. Fractals are a form of geometry in which similar morphologies repeat at different size scales due to the repetitive application of a recursive dynamical process. They can look complex, but since they typically form from simple recursions with feedback and control, those complex looks can be deceiving. For vascular networks, the driving imperative is that no cell be greater than some distance r from the embedded network. If you were to take a vascular network (VT or natural), and then draw on it a simple geometric lattice (cartesian or euclidean) using the same r-value (the way an engineer or architect might design a distribution network), and the lengths of the two networks are compared, the vascular network is typically no more than 10% longer than the idealized network, sometimes less. And since biological systems build the network only as needed, rather than preemptively, there is even greater economy over the long run of complete embryogenesis then adult maturation. This economy, known in biological and other complex systems as "parsimonious self organization" means that, for blood vessels, the body never makes any more blood vessels than just what is needed to supply the logistical needs of the host tissue – no more, no less – just right.

Mid lower: Here are three images of natural vessel networks juxtaposed against outputs of the VT model. On the **left** are meningeal vessels (the little dots demonstrate "locality", a crucial topological feature of vascular networks). In the **middle** are mesenteric and small intestinal vessels in a segment of ileum. On the **right** are veins in a plant leaf. Note first the patterns or geometry of the meningeal and leaf veins. The vessel patterns are isotropic, meaning that vessels are present equally in all directions or angles. There is no net "directionality" to the overall field. You can rotate an image 90° and it still looks essentially the same. This pattern results from "multiplicative" growth of the host, when the growing host tissue is expanding uniformly throughout its entire substance. The center image intestinal vessels have a somewhat different morphology, anisotropic and directional. They have long vertical branches with acute angles which all appear to be "reaching" toward the top. Between them are the non-directional isotropic vessels that characterize the other images. The directional anisotropic reaching pattern is what happens when the forming tissue has "additive" growth along a margin, new tissue forming as a fringe at the boundary of the established tissue. This will be relevant below when talking about wound and target angiogenesis versus embryonic and field angiogenesis.

Center: This is a conceptual graph showing the state of the wound during and after healing. It essentially shows the state of the tissue or stroma, and the net accumulation of output from the wound control loop (the replacement substance that restores the tissue). The horizontal dotted line represents the target level of "stuff" that makes the tissue. First look at the **brown curve**. This is not wound, but rather normal embryonic growth and development. The tissue starts on the left with a small amount of substance. Tissue will be made, growing up to the intended size, reaching the target dotted line at full growth. The asymptotic rise reflects that the formation of the stroma, both the fibrous matrix and the embedded vascular network, will grow with orderly regulated reference-controlled dynamics. The growing tissue makes only what it needs when it needs it. Embryonic growth is slow, but it is never excessive, and when complete, vascular density will be precisely correct for the needs of the host cells, as expected of normal embryonic vasculogenesis and as demonstrated by the VT model. (Collagen and matrix production have their own controls which likewise lead to proper healthy embryonic histo-anatomy.) Now, look at the **red curve**, the wound curve. On the left, the curve starts in negative territory, a deficiency of tissue due to injury. Unlike for normal growth and development, wound healing is very rapid, and the production of stromal elements during normal wound healing overshoots the normality line. There is too much stuff, too much matrix and vessels by the time that the wound is actually closed. In the interests of then going from the hyperplastic scar back to normal anatomical stroma (or dermis or fascia), the body must now start to dismantle and remodel the excesses. This process of "overshoot-then-involute" is what the maturation phase is all about, managing the involution. Dynamically, this is equivalent to one cycle of a damped sine, in which the system has "overcorrected" and then has to pull back the other way. Or is it? . . .

Isn't the purpose of control to keep everything on track without significant variances, deficits, or excesses? Yes. Then isn't this overshoot contrary to everything we have already said about wound regulation and control? No. The explanation is in keeping all of the details and the actors and their roles straight. Wound healing, the Wound Main Control Loop, regulates on the state of being closed. It makes fibrous matrix and vessels as part of system output in order to reach the condition of closed. As the wound closes, the loop is less driven, output diminishes, and matrix and vessel production will eventually cease. The key thing is that the loop is neither sensing nor comparing and controlling on the collagen and vessels. The loop is controlling on the state of closed, and when that endpoint of epithelialization and closure is reached, matrix and vessels will be whatever they are – high, low, or correct. And as it turns out, they are almost always excessive. Once the endpoint of closure is reached, then the hyperplastic new stroma, the scar, begins remodeling, i.e. the maturation phase.

Top left: Two clinical and two histological photos illustrate important aspects of regeneration in a regenerative biomatrix. The material shown is Integra® collagen-aminoglycan artificial skin. When placed on a wound, platelet and inflammatory events are arrested, and defensive responses and normal wound healing are completely turned off. Over a period of several weeks, new tissue forms in the spongy matrix. Histogenesis starts when mesenchymal stem cells wander into the sponge, then bind onto the matrix. Once bound, they begin proteogenesis and mitosis, forming little clusters of collagen producing cells, cells which share all of the features of the embryonic dermatoblast. These clusters compete for available gases and substrate diffusing from the wound below, and when demand outpaces supply, the clusters make vegf to attract vessels. New vessels arrive from the nearest vessels in the tissues below, and as they arrive at the clusters, vegf turns off. The new blood supply now allows the dermatoblasts to go through another round of proliferation, creating the classical fibroblasts and connective matrix that fill the voids in the sponge and make tissue. When the process is complete, the temporary silicone barrier layer is removed, and real epithelium is restored with skin grafts. The final regenerated material is histological and mechanically quite similar to normal dermis and fascias, and quite distinct from post-inflammatory wound healing and scar. The dynamics and histology of the process is completely analogous to normal embryonic mesenchymal or stromal histogenesis, and entirely unlike normal wound healing and scar formation. Likewise, the dynamics, geometry, and topology of the blood vessels that regenerate in the matrix are completely analogous to embryonic vasculogenesis, both the real process and the process abstracted in the VT model. (See the links cited above for more information on the very close analogy between normal dermatogenesis

and vasculogenesis and their counterparts in the regenerative matrix.)

In the **upper clinical** picture, a bead of "granulation tissue" – normal wound healing – pokes through a seam between pieces of the collagen gag-matrix. Note the red capillary-dense appearance of the normal wound versus the pale pink appearance of the regenerated matrix. In the **lower clinical** picture we see a similar situation (different patient) after the skin grafts have been placed and healed. The healthy regenerated matrix looks largely like normal skin, whereas the granulation tissue has become a bead of hyperplastic scar. The **upper histology** image is normal young scar in a healing wound. It is excessively dense with fibroblasts and young collagen, without any porosity, reflecting its clinical characteristics of thickness and hypertrophy, stiffness and non-compliance. This young scar represents the hump on the red curve in the center panel, the peak of "overshoot", now awaiting the prolonged "involute" of the maturation phase which will very slowly remodel back to a normal dermal appearance. The **lower histology** image shows recently regenerated collagen-gag matrix. Each pore or domain of the sponge is filled with fibroblasts and collagen that have the characteristics of normal dermis rather than scar. In between each domain there is considerable porosity, maintaining the mechanical properties of normal skin rather than scar. Just like in normal embryogenesis, the regeneration of this tissue follows a convergent asymptotic trajectory similar to the brown curve in the center panel. This trajectory is slower than wound healing, but it never overshoots, resulting in correct histogenesis in which stromal elements are of proper density. The correct tissue model results because this regulated process controls on vessel and matrix density, not on "closed" (the collagen-gag matrix is closed to begin with because a silicone outer layer serves as a temporary epithelial substitute). To summarize, normal wound healing, which is referenced to the state of "closed", rapidly proliferates scar and overshoots correct vascular and connective matrix density, and in so doing obliterates any semblance of organized substructure within the scar (time frame = days). The scar must then mature, remodeling back to the histology and mechanical characteristics of normal dermis and fascias (time frame = months). In comparison, the regenerative process in the collagen-gag matrix reflects normal embryonic histogenesis. By being referenced to vascular and collagen density, a correct model of normal tissue, with normal dermal characteristics, is achieved by steady regulated building of the tissue (time frame = weeks).

Top middle: These two photos are of a normal wound, wide and zoomed in, immunostained to show vegf. In the boundary between the plasma and aminoglycan layers there are large mononuclear cells which stain heavily for vegf. These are the system controller monocyte-macrophages, the source of the chemotactic attraction for the angiocytes and vessels which are streaming up from below. Review the wound histology images of slides 6-12. The initial new vessels are long and vertical, just like the intestinal vessels on this slide. This is the effect of having a perimeter or leading edge of attraction – a target – that is extrinsic or on the boundary of the existing network. This is quite different than the proper-density isotropic distribution of vessels that exists in normal tissues, fully remodeled scar, and Integra® collagen-gag histogenesis.

Top right: Two clinical and two histological photos illustrate the difference in vascular density in normal wound healing versus embryonic histogenesis (as illustrated by histogenesis in the Integra® collagen-gag matrix). The **left clinical** image shows collagen-gag matrix fully regenerated several weeks after placement. It is seen through the silicone overlayer, awaiting final skin grafts. Note that the color of the regenerated material is nearly identical to the surrounding normal skin, more white than red, more collagenous than vascular. This is because both have the same – and the proper – vascular density. Keep in mind that both the native skin and the regenerated matrix are alive. This might seem like an obvious trivial statement, but it points out that both have the correct amount of circulation, just the right number of vessels to meet the metabolic and respiratory demands of the cells in that tissue. In both, vasculogenesis and vascular density were determined by the process of normal developmental angiogenesis, a controlled process that ensures proper vascular density. The **left histology** image shows the regenerated collagen-gag matrix. Blood vessels are few, with a density comparable to normal dermis and fascias, and a density which is precisely correct for the needs of the tissue. Vessel caliber is small and uniform, typical of normal capillaries and pre- and post-capillaries. In comparison, the **right clinical** image shows "granulation tissue" in a normal wound. Usually taken as a sign of a healthy wound and wound healing competency, this red pebbled appearance represents the vessels that have formed in the mid strata of the wound. Seen in comparison to the paleness of normal tissues, the saturated red color is indicative of significant vascular hyperplasia and increased vascular density. The **right histology** image shows granulation tissue from a wound similar to the clinical photo. It shows organized vessels in the histio-attraction layer at the transition between the aminoglycan and fibrous zones. It is shown at roughly the same scale as the left histology image of regenerated matrix. The angiogenesis of normal post-inflammatory wound healing is seen as dense, closely distributed large vessels carrying large volumes of blood. This high blood density is why granulation tissue is seen grossly as an exuberant saturated red color.

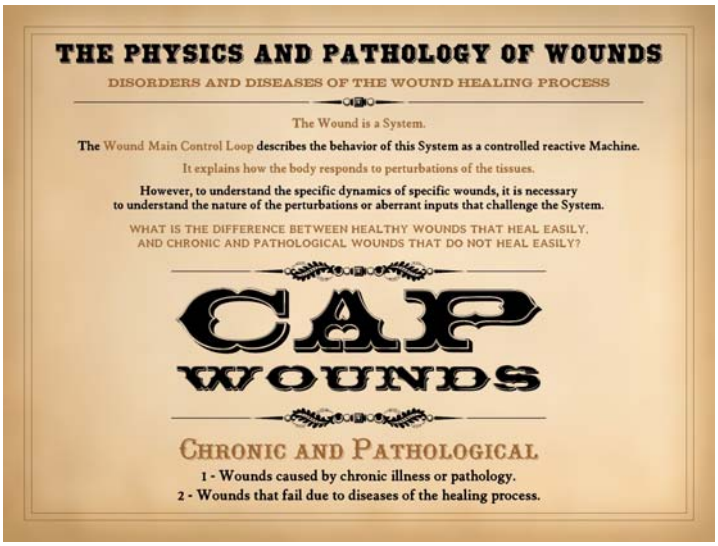
The collagen-gag matrix undergoes a histogenetic process that is comparable to normal embryogenesis. When looking at normal tissues (dermis, fascia, regenerated matrix) they appear pale. They also have normal vascular density, the correct vascular density to meet their metabolic needs. This is because the non-linear regulatory mechanism that controls angiogenic embryology controls on vascular density, striving to make precisely the correct number of vessels needed to supply the host cells. Thus, these tissues appear pale because normal vascular density is what it is, and that is the way the tissues look at proper density. This is the color of the correct circulation to meet the metabolic needs of the host. In comparison, the bright red "granulation tissue" in a healthy wound has excessive vascular density. However, the metabolic requirements of the wound are not extraordinarily different than those of normal skin and fascia. The vascular density of the granulation tissue is thus excessive, exceeding the metabolic requirements of the host tissue. If embryonic angiogenesis is a reference-regulated control process, and if wound healing is likewise a reference-regulated control process, then why is there such a difference in vascular density?

We have just looked at a potpourri of different items on this one slide. What is the common thread? Simple. We can now explain why the vascular (and collagen) density in normal embryonic tissues and in regenerative matrices is low (normal) versus why it is so high in post-inflammatory wound healing. It all comes down to basic elements of control – what are the system reference, feedback, and comparator, and what are the controller, load, and output? What are they (which cellular and chemical elements of the system), and also where are they, in which strata or locales? (Let us first reiterate that normal embryonic histogenesis and regeneration in the collagen-gag matrix are essentially the same thing. They have the same basic dynamics and anatomical organization. The explicit details of their similarities are beyond the scope of this presentation, but can be reviewed at the links given above. For the sake of this slide, the collagen-gag matrix was used as a simple and readily available way to observe these dynamics, in lieu of actual embryological specimens. Below, when embryology or matrigenesis are mentioned individually, generally each implies the other as a type of "normal histogenesis".)

In **normal histogenesis**, the system reference and regulatory controls are all about the matrix itself, the density of vessels and collagen. Angiogenesis per se is controlled. (This is easy to demonstrate and understand via the VT model of vasculogenesis.) The feedback is a function of the vessels, their density, and the oxygen they deliver. The system reference is the amount of oxygen expected by the vegf gene. The system comparator or error detector are the developing local parenchymal or histiogenic cells which are trying to form the tissue. Angiogenesis is not triggered until "native" cell mass and metabolic load in scattered independent locales exceed the existing blood supply. When need does exceed supply, when new host cells need their own vessels, then they summon the formation of new vessels via the error signal of angiogenic factors. This stimulates the controller, the vessel sprouting mechanism, and new vessels are sent directly to the locales where beckoned. The load, the actual vascular network, is thereby modified, and the output as perceived by the host is the density of the network which governs the oxygen feedback parameters. As new vessels arrive and the blood supply needs of the host cells are satisfied, then the angiogenic cytokine error signal turns off, and there is no further stimulus on the vessels. The feedback and regulation, i.e. the closed loop control, are thus between the growing embryonic host cells and the blood vessels they attract. It is the vessels themselves that are regulated, leading to a defined vascular density. Turning angiogenic stimulation on only when vessels are needed, and then turning off, means that only just the necessary number of new vessels appears, and the host tissue has precisely the right density of blood vessels needed for normal development and function. One clinical consequence of this is that normal tissues appear "pale", desaturated of red color because the required vessel density and blood volume in normal tissues is only a slight fraction of net tissue mass.

In **post-inflammatory wound healing**, matrigenesis is the system output, but regulatory control is not about the matrix itself. Regulatory control is between platelet-plasma-leukocyte error detectors and the state of open-versus-closed, and the state of closed does not exist until epithelium fully sequesters mesenchyme. It is also true that epithelium cannot grow and close unless the underlying matrix is properly proliferated. The output of collagen-vascular stromal matrix is thus make-or-break essential to the system, but nonetheless it is not the controlled parameter. If we focus on the interaction between stimulatory macrophages (the system controller) and the responder angiocytes (the controlled load), the process is one way only, a strictly open loop direct relationship between these two events. Macrophages trigger the creation of new vessels, but revascularization does not suppress the macrophages. When new vessels arrive at the controlling macrophages, the macrophages just keep on pumping out angiogenic cytokines and triggering more vessels. This is because the macrophages are being transformed and stimulated by inflammation, not ischemia. Inflammation and the error detectors neither know nor care what vessel and matrix density are. The feedback that works its way clockwise through the control loop and eventually back to the macrophages must do so through several other intermediary control blocks, creating time delays or phase lags. By the time the wound starts closing and macrophages are down-regulated, the output of vessels and matrix will be whatever they are, and they are dense, far in excess of what is normally needed by healthy tissues. The clinical and histological appearance is of excessive numbers of unnecessarily large blood vessels, a substantial percentage of wound volume (in the relevant strata), forming bright red "granulation tissue".

With regard to angiogenesis and vascular density, here are the important contrasts between normal and wound healing histogenesis. **(1)** In normal histogenesis, host cells (comparator, the system master) sense inadequate vessels then attract new ones. Restored vessels then inhibit the attraction – a straightforward system of simple closed loop control. *In wound healing, the macrophages are the controller (only a system agent) which attract vessels, and being regulated by inflammation they are completely indifferent to the state of the vessels. The arrival and restoration of vessels does not inhibit the attraction – an open loop unregulated system.* **(2)** In normal histogenesis, the host cells which attract vessels are an intrinsic part of the developing tissue. The host cells and the vessels they attract will have a lifelong fixed relationship to each other, so it is crucial that they have a mutually regulated interaction to build proper tissue architecture. *In wound healing, the cells which force and attract vessels, the macrophages, are transient players extrinsic to the regenerating stroma. They have no other relationship to the new vessels, and they clear the scene after they have served their purpose.* **(3)** In normal histogenesis, vessels and density are the measure of the system that is compared to the reference. Vessel growth and density are thus explicitly and precisely controlled, accurate and efficient, neither more nor less than what is required by the host. *In wound healing, vessels-density-oxygen are not the measure of the system. Vessel growth and density are unknowingly overdriven, overshooting normal values by the time the wound is closed, resulting in the excessive vascular density seen as "granulation tissue".* **(4)** In normal development, the developing host cells are dispersed or distributed evenly throughout the histogenetic field. This results in "field angiogenesis", and the resulting vascular patterns are isotropic with no overall directionality, exhibiting the orderly topological feature of "locality". *In wound healing, the macrophage and angio-attraction zone is a layer near the top of the wound. This results in "target angiogenesis", with all new vessels attracted along the same spatial vector, from below to above, creating an anisotropic directionality to the new vessels.* **(5)** Developmental angiogenesis, in which vessels and correct vascular density are explicitly controlled, include conditions of embryonic and maturational growth and development, and also embryonic type neo-histogenesis such as in Integra® collagen-gag matrix and in acellular cadaveric dermal matrices. *Non-regulated excess density angiogenesis occurs from incidental conditions of trauma, pathology, and targeted stimulus, such as wounds, tumors, placenta, and certain implants.*



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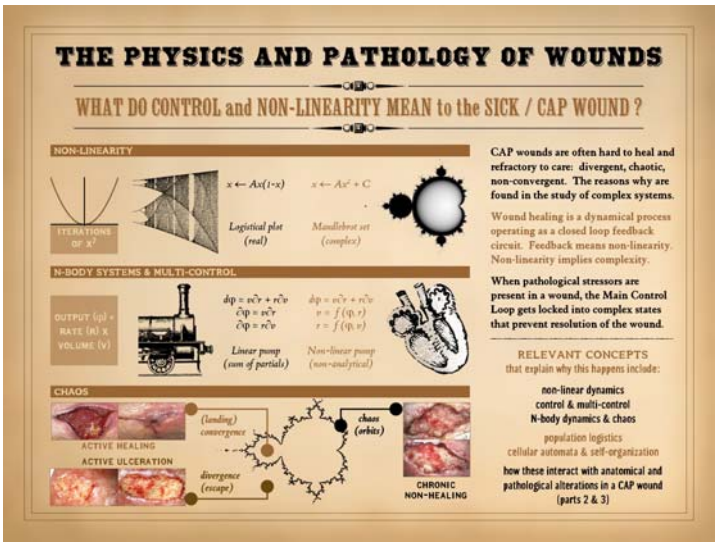
Most cursory or perfunctory discussions of wounds focus on a few major categories of causative or associated diagnosis – arterial, diabetes, pressure, etc. What is ignored is that there are also diseases of the wound healing system. The whole premise of this series of lectures is to elucidate what are the core intrinsic diseases of wound healing. However, unlike the way we think about most diseases, the intrinsic wound diseases are not due to a single gene mutation, not due to an excess or deficiency of a specific metabolite, not due to dysplasia or degeneration of any specific cell or tissue. Simple biochemistry, proteomics, and genomics cannot explain wound failure and wound healing incompetence. Understanding the intrinsic pathologies of wound healing depends on understanding the wound as a physical system. The relevant science is physics.

Understanding wound physics begins with understanding that the wound, like most complex biological processes and structures, is a non-linear controlled system. We have just described the main control loop of the wound as the core of this controlled reactive system. The loop explains how the body responds to perturbations of the wound and the wound healing process. When wound disorders or pathologies occur, they can be of two types with reference to the intrinsic loop. They might be (1) aberrant behavior of the control loop due to diseases or derangements that sicken or impair its elements, or (2) aberrant outputs or loop dynamics due to proper reaction of the loop to aberrant or extreme inputs (extrinsic stresses) which perturb the system. As will be discussed in detail in Part 3 of these lectures, there are not many diseases of the loop elements, i.e. conventional biological diseases of macrophages or angiocytes and the like. More often, wound failure and incompetence is due to the types of stresses, perturbations, forces, inputs that challenge the system. The main question to ask is, what is the difference between healthy wounds that heal easily, and chronic and pathological wounds that do not heal easily? The answers to this question are the purpose of these lectures, and they are forthcoming.

A definition is needed here: the CAP wound – Chronic and Pathological. Chronic and pathological CAP wounds are those that are either (1) caused by a chronic illness or pathology, or (2) fail due to disease or incompetence of the healing process. Some cap wounds are due to identifiable extrinsic diseases, such as arterial problems or repetitive trauma. They are the ones that are usually easier to heal, as long as the primary disease is resolved. Cap wounds that are due to inherent wound healing problems – i.e. wound healing is "broken" – are the ones that are difficult to heal.



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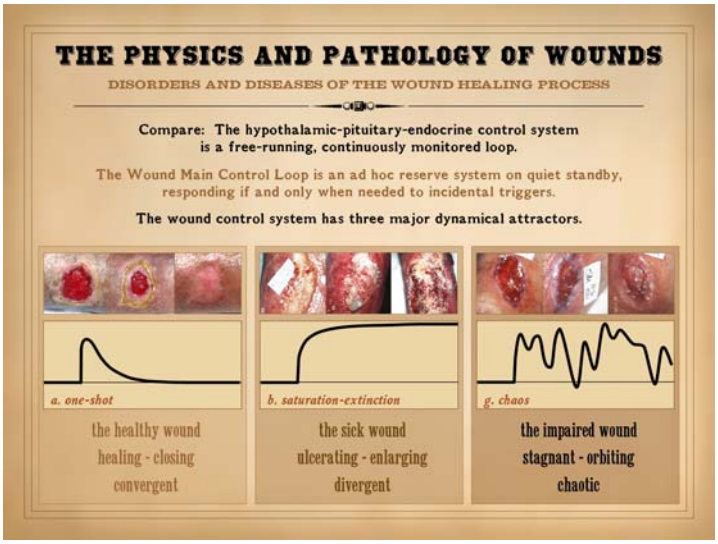
So far we have explored the issues of closed loop feedback, non-linearity, and control, and how those principles underlie the dynamical timewise physiology of the normal wound, and even the stressed or impaired wound. What does all of this have to do with the sick wound and the CAP wound, chronic and pathological?

CAP wounds are often hard to heal and refractory to care. Dynamically, the control loop and the behavior of the wound are non-convergent, i.e. they are divergent or chaotic. The reasons why are found in the study of non-linearity, complexity, and complex systems. These reasons will be explored in detail in Part 3. As a prelude, consider that these faulty dynamics mostly occur when pathological stressors are present in a wound, forcing the Main Control Loop to get locked into complex states that prevent resolution of the wound. The relevant concepts that explain why this happens, subtopics of non-linear dynamics, include: control & multi-control, N-body dynamics & chaos, population logistics, and cellular automata & self-organization.

The graphics are mathematical items from non-linear dynamics that illustrate these concepts. They are: (top) logistical maps and mandelbrot sets illustrate non-linearity and population dynamics; (middle) pumps illustrate N-body dynamics and multi-control; (bottom) mandelbrot sets are matched to corresponding wound dynamics. In Part 3, it will be shown in detail how the dynamical properties of complex systems, as illustrated by these objects, interact with anatomical and pathological alterations in a wound to create chronicity. The following 2 slides are a preliminary introduction to one crucial component of these dynamics – the concept and origins of chaos in the wound.



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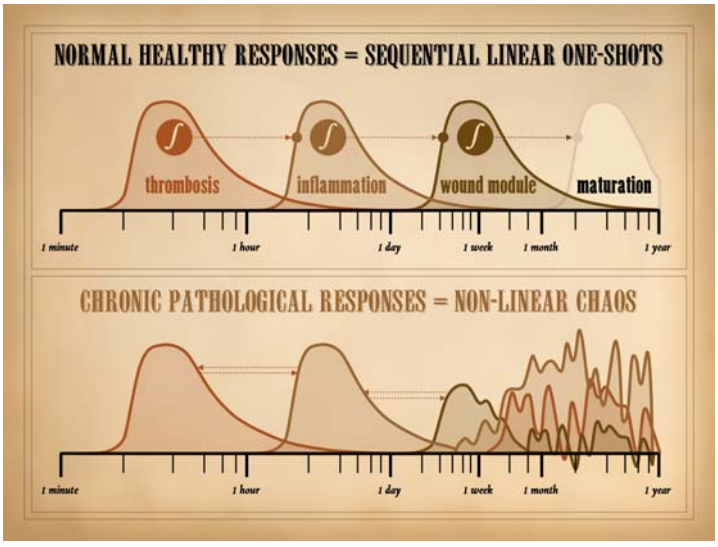
Control is control, and the principles and physics do not vary. Yet different systems can appear to behave very differently due to the nature of their elements, their reference, and the nature of the perturbations or stresses on the system. Go back to the endocrine and wound comparison of slides 18 & 19. The hypothalamic-pituitary-endocrine control system is a free-running, continuously monitored loop. There is a constant level of thyroid hormone output. If it varies, the system will respond to restore the required level. There are diurnal periodicities and incidental reactions to various stresses, but at all times there is a basal system output that must be monitored and regulated. The sustained basal rate of hormone production might be due to an autonomous pacer or load controller in one of the glands, versus the basal level just reflecting control around the system reference, whatever that might be. Either way, control dynamics are what they are for all systems, the same generic principles reacting to the different physical realities of each particular machine.

In comparison to the endocrine system, the Wound Main Control Loop is an ad hoc reserve system on quiet standby, asleep if you will, reacting if and only if and only when it is needed in response to incidental triggers. On slide 23 we showed that there are some standard ways in which controlled systems might respond to perturbation. These responses depend on the intrinsic quality and tuning of the machine, and then on the nature – strength, profile, duration – of the perturbations. As we first saw on slide 26, that for the wound and for many other biological systems, three responses are likely to occur: convergence, divergence, chaos. For the endocrine control system, convergence is on the basal level of hormone production, which is a continuous positive outflow of hormone. For the wound system, convergence is on a healed wound and repaired stroma, a zero in the system, after which the system goes quiet, in hibernation until it hears the next knock on the door of injury. Divergence is an undesirable morbid state in which control is overwhelmed by strong inputs versus illness within the loop which keeps it from reacting properly. Chaos reflects complexity within the system, either its natural state, or its state under the influence of stressors.

The three likely dynamical states – attractors – of the wound control system each reflect certain clinical and pathophysiological states of the wound. (1) The **healthy wound** is healing properly and closing, dynamically convergent. (2) The **sick wound** is actively pathological, ulcerating and enlarging, dynamically divergent. (3) The **impaired wound** is stagnant, "orbiting", getting neither better nor worse, dynamically "chaotic". The disorders and diseases of the wound healing process all obviously have a tangible physical reality based on chemistry and biology, but how those physical elements inter-operate to get the job done, or fail, is a matter of physics. Looking for the reasons why wounds do not heal will not be resolved by discovering the combative chromosome or the evil enzyme or the lazy leukocyte. Every biochemical and biological discovery made adds another vital piece to the whole puzzle, but the rules of how to assemble and solve the puzzle are dynamical and operational – physics. As will be detailed in Part 3, the chaotic non-healing impaired wound actually has nothing really wrong with any of its constituent parts. It is only their integrated operations that have gone awry, because of the reactions of the wound control system to the circumstances of the moment.



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The dynamics of the impaired wound will be discussed in great detail in Part 3. As an introduction though, we will look here at some of the dynamical events that shift the wound control loop from one of convergence and proper healing to one of disordered dynamics and failure to close. This starts by understanding that dynamically, normal unimpaired wound healing is a convergent one-shot. The aggregate response is a one-shot, and the response of each of its constituent subsystems is a linear series of one-shots. The dynamic of the impaired wound – chaos – begins when these orderly convergent one-shots are disturbed, either by extrinsic stresses on the system or by illness within the system.

In the **upper panel**, time is represented by a logarithmic scale. The curves shown are idealized abstractions of the wound and its dynamics. This is the conventional linear-sequential concept of wound healing, and it is essentially equivalent and mappable to the closed loop control model for a particular subset of wound dynamics, the healthy-healing-convergent wound. For a moment, just focus on the "wound module" curve. This is a non-rigorous conceptual curve. As the curve goes high and then low, it represents the load and output of the wound repair cells, their kinetics as driven by the wound control loop, ramping up as they become activated, then decaying back to standby as they wrap up business as the wound closes. Next, note that this conceptual kinetic curve or curve-of-state is repeated for different main events: thrombosis, inflammation, wound module, and maturation. All of these events are related to each other, but each is also its own control system. Recall from the Main Wound Control Loop that inflammation, the error signal, is a black box element, but within that black box is a highly complex system that "knows" how to turn on and turn off and do its business in response to injury, disease, and the blood-based error detector. Consider the roles and dynamics of each of these major events in the response to injury:

Injury: Consider what happens when a one-time incidental self-limited injury occurs – a cut finger, a surgical incision, a ruptured tendon, whatever.

It occurs in the time frame of seconds-to-minutes, and then it is over. It too will have the same general shape as the other curves shown, and it could / should be added to the diagram as another hump on the left side, the "injury" cusp. The effect of the injury is to trigger thrombosis. The injury itself was brief, and by the time that thrombosis is really getting underway, the injury itself is over.

Thrombosis: The term "thrombosis" is used here in a loose sense to refer to the aggregate injury recognition events that are based on a variety of blood borne elements – mainly plasma proteins, platelets, and leukocytes. In the historically conventional sequential-linear view of wound healing, thrombosis is the on-off switch which recognizes injury and gets the rest of the process going. In the non-linear control view of the wound, thrombosis is the error detector which is comparing current status to normal tissues and issuing an error signal in the form of inflammation. (These two views are essentially the same thing when injury and response are simple healthy one-shots, but they diverge when wound dynamics become chaotic, which is why a proper non-linear model is best suited for non-linear real events.) In the wound control loop, "thrombosis" or the error detector is a black box, but unto itself within that black box is another complex control system. When "thrombosis" is triggered, there are two factors which cause thrombosis to "rise" or ramp up: (1) the more potent and the more sustained the injury or trigger is (the integral of the trigger with respect to time), then the more potent is the net response; (2) thrombosis is also an auto-amplifying event which raises its own kinetics. Thrombosis will eventually reach a peak, and then start to decay. If injury was a one-time limited event, a one-shot, then there is nothing further to trigger thrombosis beyond the initial event. Thus, thrombosis gets its one-shot trigger, then it runs its course, and then it decays and extinguishes. (The term "one-shot" comes from electronics, jargon for a "monostable vibrator", a circuit which can switch state (on-off, positive-negative) when triggered, and then it returns to baseline by discharge through an R-C circuit. The basis for many timing circuits, the output waveform and the time-dependent nature of the electronic event is completely analogous to the phases of injury-response as shown here.) Aside from its immediate effect to staunch bleeding, thrombosis has a crucial dynamical effect – it feeds forward into the control loop by triggering inflammation. Thrombosis events build then decay in the time frame of minutes-to-hours. By the time that inflammation is building, acute thrombosis events have largely subsided (there will still be old fibrin thrombi and platelet plugs in the field, but no new platelet aggregation, fibrin catalysis, nor clot formation is occurring).

Inflammation: In the historically conventional sequential-linear view of wound healing, inflammation is the next downstream event after thrombosis and the trigger for subsequent wound repair. In the non-linear control view of the wound, inflammation is the error signal which activates the system controller. (Again, these two views coincide for single event "healthy' injury and one-shot responses, but they diverge when wound dynamics become chaotic.) Inflammation is turned on by an integrator effect of thrombosis. Thrombosis events build or accumulate the stimuli needed to trigger inflammation, and once inflammation begins, it can be self-sustaining, even as active thrombosis wanes. In the wound control loop, inflammation is abstracted to a single element, the control signal, but within its own black box it is its own complex multicontrol system. It too is self-amplifying, so between the thrombosis trigger then its own dynamics, it builds to a peak effect. Assuming that injury and thrombosis were one-time one-shot events, then there is no further provocation for inflammation, and inflammation events will soon enough start to decay and wane. Aside from its important short term effects to defend the host and clean up the injury, inflammation also has the crucial dynamical effect of feeding forward into the control loop by spawning and regulating the system controller, the macrophage. In the same way that thrombosis triggers inflammation, inflammation is itself an integrator function that gradually builds the controller in such a way that the controller can be self-sustaining as inflammation subsides. Inflammation events build then decay in the time frame of hours-to-days. By the time that the macrophage population is built and load control and system output begin, acute inflammation events have largely subsided.

Repair: Note the dynamical similarities between the injury-thrombosis-inflammation cusps, and the analogous transitions from one phase to another. That trend now continues in the jump from inflammation to the repair phase, the proliferative wound module. Once triggered, repair events will build to some peak of productive activity. As system output increases and thus the wound diminishes, the control loop is less driven, and wound module kinetics start to wind down. Wound module ramps up then decays in the same way that the preceding phases did, but in a time frame of days-to-weeks. Wound module is also an integrator function that triggers another downstream one-shot event – maturation. Maturation begins only once the wound is epithelialized, meaning that maturation waxes only as wound module wanes.

Maturation: The integrated output of the wound control loop, i.e. the net production of the output control block is the reconstituted stroma that negates the condition of "open wound". However, it is not normal stroma, it is scar, a form of stroma that is excessively dense with vessels and connective matrix. This undergoes a process of remodeling or maturation that eventually restores it to proper architecture and density of the stromal structures. The way wound healing and the wound control loop have been defined here, the wound is healed and the loop settles when epithelialization and closure are complete. Maturation is therefore a tangential or derivative event, and like everything else it is its own control system within its own black box domain. The scar is the integrated output of the wound module, but also the feed forward input into the maturation block and curve. As for the other major events, maturation rises to a peak dynamic then fades, with its time frame measured in weeks-to-months.

In the dynamics of the normal wound, in the progression of injury → thrombosis → inflammation → repair → maturation, there is a crucial additional relationship implicit in the diagram – the anatomical stratification and timewise separation of these events. Yes, these events are contingent, each downstream event being triggered by the preceding cusp. However, aside from a handshake and the flip of a switch to get things going, these events and phases, and their constituent cells and structures, actually have only limited contact with each other. Recall from the review of basic wound anatomy that the wound is not just an amorphous bowl of pudding. It is highly structured, even within the few vertical microns or millimeters of its existence. The vertical anatomy of the wound is a timewise historical view of what has happened after injury, with the top happening now, and the strata underneath being progressively older. Thrombosis events are in the topmost layer. Inflammation is there and in the aminoglycan layer underneath, repair events are below that, and maturation occurs later (more on this in Part 2, see especially slide 52). These events overlap to some extent, vertically and in time, but the overlap is comparable to the overlap of cusps in the sequential phase diagram shown here, with preceding events waning as latter phases appear. As long as these phase relationships are maintained, and as long as there is no new injury, then each event runs its course with one-shot dynamics, and the net dynamics of the wound runs a smooth course from injury to maturation.

In the **lower panel**, we can start to see where chaotic dynamics come from. This diagram likewise is missing the acute injury cusp at the left. However, imagine it is there, and consider this scenario. A single injury occurs, putting the other events into action, each following the other, each having one-shot dynamics, and each conforming to the general shape of the cusps shown. Keeping an eye on the logarithmic timescale at the

bottom, imagine that at 12 - 24 hours another injury event occurs. Within a few hours, the downslope of the inflammation cusp will suddenly have another rise. This will result in new uprises in the other downstream events. Each event curve will then seem a bit more complex, although obviously the new blips represent the superposition of the new curves on the original ones. If more injury events were to occur, then more event curves or phase cusps would have to be added. Assuming that each injury event leads to an exact repeatable profile of downstream events, then the resulting composite activity curves would be the superposition of x-number of original events, and the curves might start to seem very erratic or complex. Understand the significance of the phrase "exact repeatable profile of events". This means that the system response adheres to a precise set of rules – i.e. it is strictly deterministic. Although the composite activity profiles might seem complex, they just represent the superposition of multiple copies of the same "waveform". If you knew the precise shape or dynamics of a single response curve, then the entire data stream could be "deconvoluted" (a mathematical method) to see all of the individual injury events and the time that they initiated. The point is that with repetitive injury or events, the composite data stream might seem very erratic or "non-functional" or "non-analytical" (unlike a simple equation or standard function), yet there is strict rhyme-and-reason to the data.

The real situation in real wounds actually gets even more complex, but the principles of deterministic rules and the superposition of waveforms remain in force. In the lower panel, note the double arrows between the thrombosis, inflammation, and wound module cusps. In the normal healthy unstressed response to injury (the normal wound control loop), all of the dependencies between one control block and another feed forward, and the control loop runs clockwise. The net effect is the smooth one-shot convergent dynamics of normal healing. These double arrows, back and forth, imply a retrograde stimulus or feedback. How is that possible? It is possible, it does happen, and it is abnormal. "Abnormal" in the words of medicine is "pathology". These retrograde, events are the consequence of sustained injury or diseased and disordered states of the wound. Consider first the inter-relationship between thrombosis and inflammation. This is discussed at length in Parts 2 & 3 (e.g. slides 2-46 & 3-07). First, thrombosis triggers inflammation. From the point of view of conventional biology, thrombosis is explicitly how injury is recognized which then turns on inflammation. Dynamically, this is the normal feed-forward relationship of injury-recognition (system comparator) to injury-response (error signal), with one-shot thrombosis waning as one-shot inflammation is rising. However, inflammation can also cause thrombosis. Inflammation creates a variety of chemical mediators, mechanical-geometric-flow changes in vessels, changes in blood rheology, activation of leukocytes, and even further stasis and activation of platelets, all of which are potent thrombogens. The retrograde trigger of thrombosis by inflammation occurs under special circumstances, circumstances in which acute inflammation is sustained by some condition of continued injury or disease. When inflammation is sustained beyond its ideal one-shot profile, then it begins to act as an integrator or accumulator of those factors which risk triggering thrombosis.

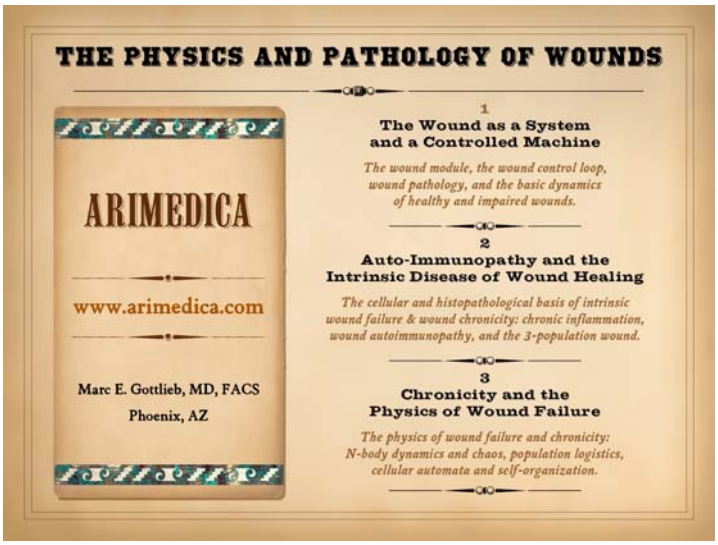
Inflammation can also cause another "retrograde" dynamic – creating more injury. This was explained on slide 24. This is an effect of the destructive histiolytic components of inflammation. These lytic effects are normal, meant to destroy exogenous pathogens if present and especially to dissolve damaged stroma so that phagocytic and repair cells can perform their tasks. Normally, these lytic effects act on the original injury and damaged tissue. By the time that wound module and repair are ramping up, inflammation is waning, and inflammation will not have destructive effects on the re-forming stroma. However, if some condition of repetitive injury, thrombosis, or other triggers of inflammation re-activate or even perpetuate acute inflammation, then inflammation will become fully active concurrent with active repair. Under those conditions, the destructive effects of inflammation not only suppress repair, but risk creating damage and destruction of restored or surrounding tissues, thereby causing progressive ulceration. Consider too that thrombosis can have a retrograde effect to cause injury. Normally, thrombosis is a response to injury and damaged tissues. By the time that normal first-round inflammation and then repair are active, thrombosis is long gone, and it has no effect on subsequent new tissues. However, if some act of recurrent injury or sustained inflammation is present, enough to cause thrombosis when it should be gone, when things are trying to repair, then what happens? Thrombosis obstructs blood flow and causes infarcts. In chronic wounds, wound infarcts can and do occur. Small and multifocal, the aggregate effect can be quite damaging to the wound as a whole, undoing what has already been built, or causing progressive ulceration. Infarcted granulation tissue is a common observation in chronic wounds, such as coagulopathic and immunopathic wounds when there is a flareup of active disease. Next, note the wound module cusp on the lower panel. It never rises to full activity because of suppressive effects of sustained inflammation. These abnormal inter-phase dependencies can feed forward and be inhibitory as well as feed backward and be stimulatory. Finally, the lower panel also shows a backward arrow from repair to inflammation, another abnormal retrograde event that is not a normal property of healthy clockwise wound healing. This abnormal dependency is explained in detail in Parts 2 & 3.

The effect of new injury events, new thrombosis events, and all of these counterclockwise retrograde events is that if one occurs, then a preceding phase is bumped up to a new wave of response and reaction. This new bump then feeds forward, clockwise, sustaining overall wound dynamics. What should have been an up-and-down one-shot event then becomes disordered as the "waveforms" of each of these new rounds of activity are superimposed. The immediate response and dependency of one phase on another remains strictly deterministic, strictly driven by the physical, chemical, and biological rules of the system. However, by the time that enough of these aberrations have occurred, the waveform or data stream of sustained unsettled loop activity can seem anything but orderly. Instead of each phase or event rising and falling in sequence, eventually all events are simultaneously sustained and active. This is seen on the right side of the lower panel, where each event has a continued but erratic profile that continues without termination as long as the chronic wound persists.

If you were to record some parameter of all of this – wound size, loop dynamics, an inflammatory chemical concentration, density of a certain cell, whatever - what would you find? We have just seen that things might seem erratic, but we also know that the responses are strictly rule based and deterministic. If the responses or transfer functions between phases or events are standard, understandable, and analytical, and if all of the superimposed states or values of the system can be convoluted or deconvoluted, then shouldn't we be able to parse or decode the data stream into its constituent events? If the cusps have a periodic or standard time-delayed relationship to each other, then shouldn't we be able to find some evidence of periodicity or harmonics in the data? Or is the erratic data so disordered that there is no order, that instead it is just noise, i.e. random? No, no, and no. No, you will not be able to decode or deconvolute the data stream to a set of original simple or primary waveforms (unless you know with infinite precision every detail of the system, aka LaPlace's Demon). No, neither a fast-inverse fourier transform nor other relevant method is likely to have a decipherable power spectrum, i.e. no frequencies or periodicities in the data. And surprisingly, the data is not likely to have a uniform or gaussian distribution to imply strict randomness. If you know how to map or graph the data properly, there will be structure in the data (explained in Part 3). What is that structure? Chaos. The nature of deterministic chaos was explained on slide 23, and now on the right side of the lower panel on this slide you can see what it looks like. Remember, "chaos" in math-physics-engineering isn't the same as vernacular chaos. It is

strictly rule based and non-random. It can be highly complex and at first seemingly undecipherable, but it is not arbitrary. Everything you see in the data stream has a mappable cause-and-effect relationship to something else or even to its own antecedent or resulting state. It looks wild and crazy and unpredictable, but it is the result of highly deterministic analytical functions which become entangled or superimposed or have abnormal feedbacks which retrigger or amplify the system in complex patterns.

We have been discussing that wound and wound loop dynamics will tend to fit three profiles of response: one-shot convergence, saturation-divergence, or chaos. You can see here what chaos really means to the system, and why it happens. In the wound, it happens when the orderly progression of wound phases and the orderly sequencing of control block dynamics as a set of single-cusp one-shots gets disordered. The disorder results from repetitive primary injury extrinsic to the loop, and from abnormal intrinsic feedbacks and retriggers. These abnormal or unanticipated events and dependencies have an effect to sustain the wound and loop dynamics. This sustentation can eventually reach a point where the repetitive or persistent interplay and recurrence of injury-thrombosis-inflammation can become a self-sustaining state that can no longer find a path to convergence. Since healthy wounds heal properly, what types of disease can induce a wound to get locked into a chaotic "attractor" of this sort? Keeping in mind the circular dependencies of thrombosis and inflammation, and the effects of both to create more injury, the diseases that induce chaotic wound dynamics are sustained conditions of primary injury, primary thrombosis, and primary inflammation. These include repetitive trauma, autoimmune states, atopy-allergy disorders, chronic infections, and hypercoagulable and micro-occlusive disorders of blood or blood vessels. Parts 2 & 3 of this series will explain this in detail.



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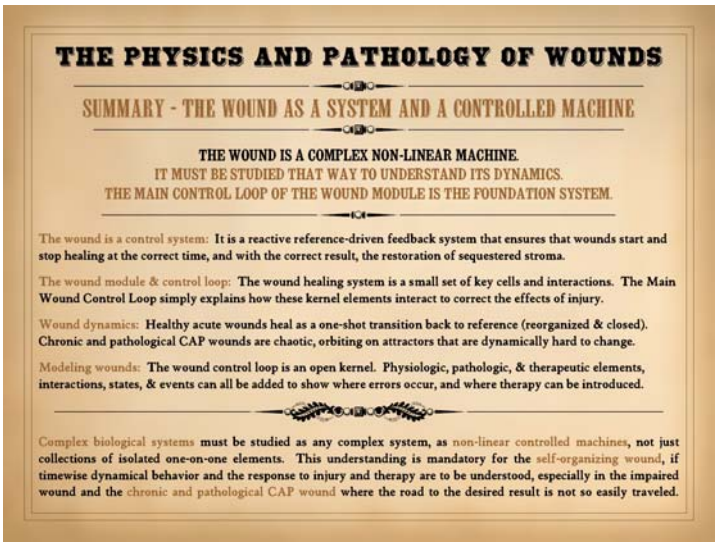
This slide is a reminder that this is a three part presentation that looks at wound pathology from the point of view of its applicable physics, elucidating the intrinsic dysfunctions of the wound as a result of dysdynamia, especially when stromal auto-immunization has occurred due to prolonged population admixture in a repetitively injured wound.

Part 1 – The Wound as a System and a Controlled Machine
The wound module, the wound control loop, wound pathology, and the basic dynamics of healthy and impaired wounds.

Part 2 – Auto-Immunopathy and the Intrinsic Disease of Wound Healing
The cellular & histopathological basis of intrinsic wound failure & wound chronicity: chronic inflammation, wound autoimmunopathy, & the 3-population wound.

Part 3 – Chronicity and the Physics of Wound Failure
The physics of wound failure and chronicity: N-body dynamics and chaos, population logistics, cellular automata and self-organization.

These presentations and supplementary materials are all available at **www.arimedica.com** .



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We have now concluded the first part of this series on The Physics and Pathology of Wounds, looking at wound healing as a system and a controlled machine. As for any complex system, biological or non-biological, it is the presence of non-linear feedback and control which governs the basic dynamics and timewise behavior of normal wound healing. As a complex non-linear controlled machine, the self-organizing wound must be studied as such (and not just as a collection of isolated one-on-one elements) in order to fully appreciate both its normal healthy dynamics and also its response to perturbation and pathology. The complex non-linear "machine" that is wound healing can be diagrammed as a foundation closed-loop control system, the Main Wound Control Loop. Understanding these dynamics will be essential in Part 3 of this series where we will look at how and why wound dynamics misbehave and wounds fail to heal. The following comments summarize the key points.

The wound is a control system. It is a reactive reference-driven feedback system that ensures the correct result – the restoration of the mesenchymal stroma and its sequestration by a regenerated epithelium. Control also makes sure that the wound healing system stays asleep until needed, then comes to life, builds a morphologically correct structure with just a few rules and cell types, then winds down and reenters standby mode once its job is complete. As a complex controlled system, no amount of conventional bioscience experimentation will elucidate the operational physiology of the impaired wound. Required are the relevant tools of physics and engineering – non-linear dynamics.

The wound module is a control loop. The wound healing system is a small set of key cells and interactions, each with a designated time, place, and function within the anatomical wound. They also have their place on a basic engineering control loop that has feedback and a way to correct variances from a reference state. The Main Wound Control Loop simply explains how the kernel elements of repair interact to correct the effects

of injury. The healthy wound and all states of pathology can be modeled on this loop.

The wound is dynamical, it has timewise behaviors: Healthy acute wounds heal as a ***one-shot convergence*** back to reference (reorganized & closed). Actively pathological and ulcerating wounds are sick, in a state of ***saturated divergence***, getting progressively worse and with a loss of control. Chronic and pathological CAP wounds are the impaired wounds, ***chaotic***, orbiting on attractors that are dynamically hard to change.

Modeling wounds: The wound control loop is an open kernel. Dynamical behaviors, healthy and altered, can all be studied or understood from this perspective. Physiologic, pathologic, and therapeutic elements, and also their interactions, states, and events can all be modeled into the loop to show where errors occur and where therapy can be introduced.

In human engineered and technological systems, machines are designed to have a particular stability or output. Good control is based on understanding how a signal or state must be transformed through a control block (transfer function), and it is important to design a system with correct feedback and responses to avoid oscillations or instabilities. In support of these goals, there are robust math and engineering methods, such as second order differentials, convolutions, and LaPlace transforms, which permit the exact design and precise numerical specification of a planned machine and its operations and output. In principle, precise disciplined engineering could apply as well to the wound . . . in principle. However, we have too little knowledge of wound physics to have precise engineering specs and numerical characterizations of the wound machine. However, until complexity and the science of systems is acknowledged for wounds, and the relevant 21st century tools and means of research are put into practice, there is a risk that we will never elucidate thoroughly dependable therapies. We risk never getting out of our current method of haphazard, hit-or-miss, try-this-try-that management for the hard-to-heal impaired chaotic wound. For the chronic and pathological CAP wound, where the road to the desired result is not so easily traveled, and for which conventional 20th century bioscience has yet to even reveal a failsafe road, understanding systems level behavior and failure is long overdue.

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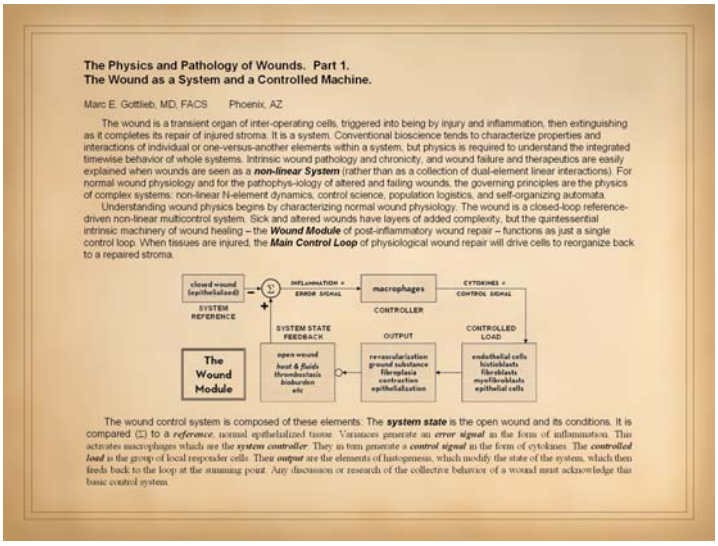
End


The Physics and Pathology of Wounds. Part 1.
The Wound as a System and a Controlled Machine.

Marc E. Gottlieb, MD, FACS Phoenix, AZ

The wound is a transient organ of inter-operating cells, triggered into being by injury and inflammation, then extinguishing as it completes its repair of injured stroma. It is a system. Conventional bioscience tends to characterize properties and interactions of individual or one-versus-another elements within a system, but physics is required to understand the integrated timewise behavior of whole systems. Intrinsic wound pathology and chronicity, and wound failure and therapeutics are easily explained when wounds are seen as a ***non-linear System*** (rather than as a collection of dual-element linear interactions). For normal wound physiology and for the pathophys-iology of altered and failing wounds, the governing principles are the physics of complex systems: non-linear N-element dynamics, control science, population logistics, and self-organizing automata.

Understanding wound physics begins by characterizing normal wound physiology. The wound is a closed-loop reference-driven non-linear multicontrol system. Sick and altered wounds have layers of added complexity, but the quintessential intrinsic machinery of wound healing – the ***Wound Module*** of post-inflammatory wound repair – functions as just a single control loop. When tissues are injured, the ***Main Control Loop*** of physiological wound repair will drive cells to reorganize back to a repaired stroma.

The wound control system is composed of these elements: The ***system state*** is the open wound and its conditions. It is compared (Σ) to a ***reference***, normal epithelialized tissue. Variances generate an ***error signal*** in the form of inflammation. This activates macrophages which are the ***system controller***. They in turn generate a ***control signal*** in the form of cytokines. The ***controlled load*** is the group of local responder cells. Their ***output*** are the elements of histogenesis, which modify the state of the system, which then feeds back to the loop at the summing point. Any discussion or research of the collective behavior of a wound must acknowledge this basic control system.

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Abstract (as submitted in advance of the meeting)

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The Wound as a System and a Controlled Machine.

Marc E. Gottlieb, MD, FACS Phoenix, AZ

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THE PHYSICS AND PATHOLOGY OF WOUNDS. PART 1.
THE WOUND AS A SYSTEM AND A CONTROLLED MACHINE.

Original presentation February 22 - 26, 2010, Maui, at the
The John A. Boswick, M.D. Burn and Wound Care Symposium 2010

The presentation and related materials are accessible at:
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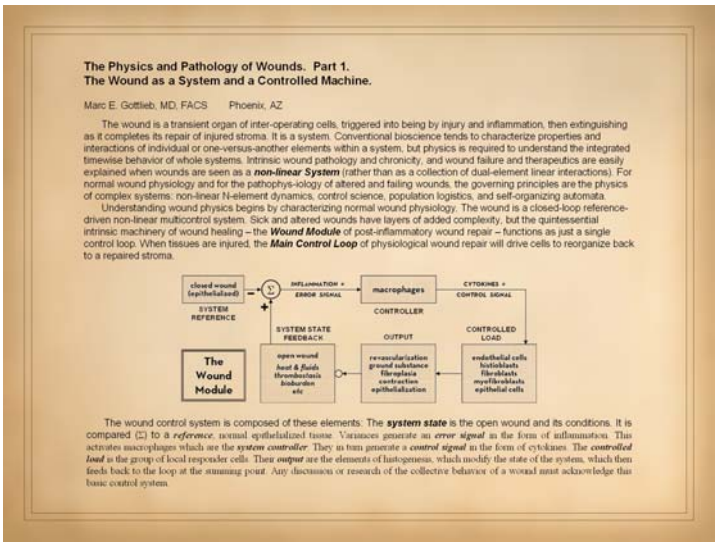
Contact information is on the slide.

Revision 01a, February 22, 2010



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Marc E. Gottlieb, MD, FACS Phoenix, AZ

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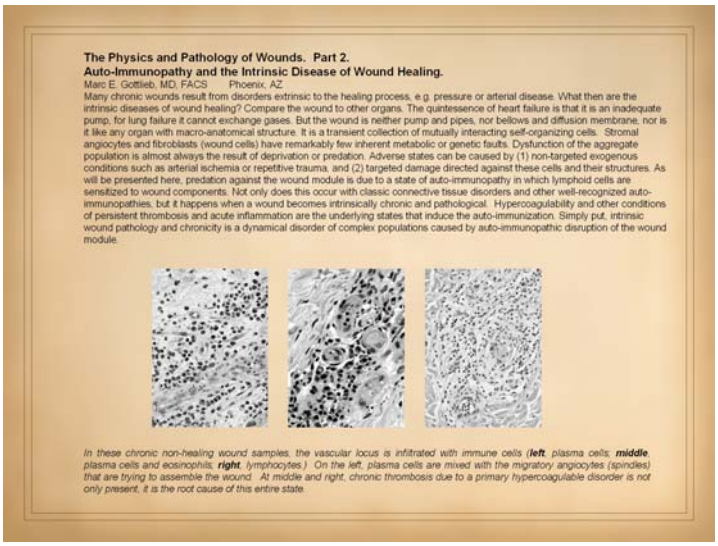

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The Physics and Pathology of Wounds. Part 2.
Auto-Immunopathy and the Intrinsic Disease of Wound Healing.

Marc E. Gottlieb, MD, FACS Phoenix, AZ

Many chronic wounds result from disorders extrinsic to the healing process, e.g. pressure or arterial disease. What then are the intrinsic diseases of wound healing? Compare the wound to other organs. The quintessence of heart failure is that it is an inadequate pump, for lung failure it cannot exchange gases. But the wound is neither pump and pipes, nor bellows and diffusion membrane, nor is it like any organ with macro-anatomical structure. It is a transient collection of mutually interacting self-organizing cells. Stromal angiocytes and fibroblasts (wound cells) have remarkably few inherent metabolic or genetic faults. Dysfunction of the aggregate population is almost always the result of deprivation or predation. Adverse states can be caused by (1) non-targeted exogenous conditions such as arterial ischemia or repetitive trauma, and (2) targeted damage directed against these cells and their structures. As will be presented here, predation against the wound module is due to a state of auto-immunopathy in which lymphoid cells are sensitized to wound components. Not only does this occur with classic connective tissue disorders and other well-recognized auto-immunopathies, but it happens when a wound becomes intrinsically chronic and pathological. Hypercoagulability and other conditions of persistent thrombosis and acute inflammation are the underlying states that induce the auto-immunization. Simply put, intrinsic wound pathology and chronicity is a dynamical disorder of complex populations caused by auto-immunopathic disruption of the wound module.

In these chronic non-healing wound samples, the vascular locus is infiltrated with immune cells (left, plasma cells; middle, plasma cells and eosinophils; right, lymphocytes.) On the left, plasma cells are mixed with the migratory angiocytes (spindles) that are trying to assemble the wound. At middle and right, chronic thrombosis due to a primary hypercoagulable disorder is not only present, it is the root cause of this entire state.

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The Physics and Pathology of Wounds. Part 3.
Chronicity and the Physics of Wound Failure.

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The wound module is a transient set of interacting cells which collectively restore in-jured tissue to normality, a fibrous stroma of angiocytes and fibroblasts. Its healthy aggregate behavior is a well behaved machine, governed by the physics of control systems. A sick system can result from various extrinsic perturbations, but the core mechanism of self-sustaining persistent dysfunction, the true intrinsic disease of wound healing is chronicity itself, the paramount cause being wound module autoimmunization. This state is disruptive but not fully toxic or lethal, thus immunopathic wounds have complex behaviors, at times better-worse-stable-variable, often looking healthy, but always frustrating as they refuse to cross the finish line. How does one explain such variable behavior and the differences between normal and chronic-and-pathological (cap) wounds?

Simply stated, intrinsic wound pathology and chronicity is a dynamical disorder of complex populations. The physics governing complex behaviors in complex systems is non-linear dynamics (nld). In addition to control, three aspects of NLD are especially important to wound pathology. (1) Population logistics. Healthy healing is a sequence of one-shot self-completing linear events: primary injury & thrombosis –then– acute inflammation –then– wound module. Pathology creates abnormal population dependencies (nutrition, starvation, predation, cultivation) and a new population, chronic inflammation. Non-linear perpetual complexity arises in the logistics of injury & thrombosis –vs– acute inflammation –vs– wound module –vs– chronic inflammation –vs– injury & thrombosis. (2) Cellular automata & self-organization. The "cellular" agents of the wound module (real biological cells in this case) have a small set of deterministic rules of interaction with each other. When allowed to function properly, stromal rebuilding is automatic and correct. Under pathological conditions, self-organization, i.e. wound healing is disrupted. (3) Chaos & N-body dynamics. The net effect is that the wound, a set of several interacting cell populations, has 3 attractors (basins, dynamically stable states or behaviors): convergence (healing), divergence (ulcerating), and self-sustained chaotic orbits (chronicity).

Basic methods to demonstrate non-linear dynamics: left, the logistical map of competing populations; middle, diffusion-limited-aggregation, an

example of self-organizing automata; right, attractors and chaos in the Mandelbrot set of complex-plane iteration. While seemingly abstract, these structures are directly correlated with wound events.