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Reverse Engineering the Inflammatory “Clock”: From Computational Modeling to Rational Resetting

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Abstract

Properly-regulated inflammation is central to homeostasis. Traumatic injury, hemorrhagic shock, septic shock, and other injury-related processes such as wound healing are associated with dysregulated inflammation. Like many biological processes, inflammation is a dynamic, complex system whose function, like that of an analog clock, cannot be discerned simply from a laundry list of its parts (data). The advent of multiplexed platforms for gathering biological data, while providing an unprecedented level of detailed information about the inflammatory response, has paradoxically also proven to be overwhelming. This problem is especially acute when the datasets involve time courses, since typical statistical analyses and data-driven modeling are geared towards single time points. Various groups have addressed this problem using dynamic approaches to data-driven and mechanistic computational modeling. These modeling tools can be thought of as the “gears” and “hands” of the “clock,” and have led to insights regarding principal drivers, dynamic networks, feedbacks, and regulatory switches that characterize and perhaps regulate the inflammatory response. In parallel, mechanistic computational models have given an abstracted sense of how the inflammatory “clock” works, leading to *in silico* models of critically ill individuals and populations. Integrating data-driven and mechanistic modeling may point the way to a rational “resetting” of inflammation via model-driven precision medicine.

Keywords

Inflammation; mathematical model; computational biology; systems biology; trauma

Introduction

The current manuscript is focused on the role of computational modeling in deciphering novel aspects, some with clinically relevant insights, of the acute inflammatory response. Due to space limitations, this article is focused predominantly on our group's work in the

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area, which spans over a decade. Readers who wish to delve into this topic in greater detail are directed to recent publications [1,2]. The primary goal of this article is to help define inflammation as a complex system, especially in the context of critical illness stemming from severe injury or infection.

Acute Inflammation: A Dynamic, Complex System that is more than its Parts

Biology is by definition complex, where complexity is defined not as “complicated” but as being non-linear, controlled by positive and negative feedbacks, and highly dependent on initial conditions and reaction rates [3,4]. It is by now a cliché, but in a complex system, the whole cannot be discerned from the sum of its parts. The typical reductionist approach to understanding complex biological systems is analogous to the scene depicted in Fig. 1, which shows a conglomeration of gears and other parts (data; Fig. 1A), with the underlying assumption that they must somehow be interrelated. The difficulty lies in determining if the parts are actually interrelated, in defining what larger structure those parts actually form (for the sake of argument, let us assume that they form an analog clock; Fig. 1B), and in describing how that device works.

The process of inflammation, in which the body informs itself that a stress-inducing change has occurred or is occurring, either quickly or slowly, is a prototypical complex system [2]. If we continue the clock analogy, knowledge of the state of the system (clock) at a single moment in time (the hour hand is on “2” and the minute hand is on the “12” in Fig. 1B) does not actually tell us how the clock is organized or how it works. We do not know if the clock functions “clockwise” or “counterclockwise” (or some combination of both), and so we cannot be certain of what time it will show the next time we look. Furthermore, a laundry list of the parts of the clock (Fig. 1A), while useful and necessary, is insufficient defining the mechanical interrelationships among those parts. Similarly, knowledge of a given inflammation biomarker at a single time point does not tell us what that level (or the associated health parameter) will be at the next time point. Also, a laundry list of the various inflammatory cells, mediators, etc. does not facilitate the task of predicting the course of inflammation over time, nor does it tell us how to modulate inflammation to improve outcomes [1]. The implications of these concepts of structure and dynamics will be revisited below.

A clock can be used to tell time, to wake us up for important appointments, and for many other useful purposes. However, a clock can also be a source of stress, reminding us of the fleeting nature of our existence (or depriving us of much-needed sleep). Similarly, inflammation can be both beneficial and harmful. It is necessary for proper healing from injury or infection, and also for the subsequent process of tissue regeneration. Inflammatory processes are initiated by damage or dysfunction to cells, tissues, and organs, and communicate this stress via myriad pathways involving both inflammatory mediators and other, highly interrelated processes (neural, cardiovascular) in order to drive a response (pathogen killing, tissue repair) [1,2].

A dysregulated inflammatory response, on the other hand, is thought to underlie myriad inflammatory diseases, key among them a constellation of syndromes induced by severe injury or infection termed “critical illness.” The term dysregulated includes both hyper- and hypo-inflammation [5], much like a clock that is dysfunctional because it runs either too fast or too slow. Importantly, critical illness can both result in and result from dysregulated acute inflammation. Multiple attempts, based on relatively simple reductionist paradigms, to antagonize individual inflammatory mediators have generally failed to ameliorate critical illness. While it is possible that these negative results have occurred because multiple organ dysfunction is not driven directly by acute inflammation [6], another possible interpretation is that the interactions among injury, hemorrhage, infection, inflammation, and multiple organ dysfunction or nonlinear and highly dependent on initial conditions and individual rates of reaction – that is to say, that critical illness is a prototypical complex system [1,2]. This therapeutic failure might be akin to attempting to reset the time on an analog clock by removing or interfering with random parts of a clock without an understanding of the intricate way in which these parts are arranged.

A Data Deluge in Injury, Infection, and Critical Illness

Starting approximately 15 years ago, the vexing challenge of critical illness spurred intense interest utilizing then-emerging “omics” technologies in order to define the critical illness disease state space as fully as possible [7], largely encompassed within the U.S. National Institutes of Health-funded Inflammation and the Host Response to Injury Glue Grant [8]. This and other programs have leveraged multiple bioinformatics-centric approaches to analyze “omics” data and led to important insights, e.g. the concept of the “genomic storm” following traumatic injury [9]. It is important to note that these bioinformatics tools – clustering methods, network discovery algorithms, and data reduction tools such as principal component analysis (PCA) – are all based on statistical methods [10]. Thus, they are still subject to issues that affect the underlying data, though variants of these methods, under the heading of computational intelligence techniques [11], may allow for “fuzzy” associations that otherwise might not be obtained using standard statistical or bioinformatics methods. It is also important that the “omics” work evolved from a focus on single time points [12] to a more dynamic view [9,13].

Inferring the Dynamically Interconnected “Gears” of the Inflammatory “Clock”

Our group has focused almost exclusively on dynamics, namely inferring how dynamic networks of inflammatory mediators (assessed at the protein levels) characterize the various etiologies or outcomes associated with traumatic injury in both pre-clinical and clinical contexts. These data-driven modeling approaches and their application to trauma and sepsis have been reviewed extensively elsewhere [1,5,14], but key aspects are summarized in Table 1.

Let us continue for a moment with the clock analogy. If we imagine that the parts depicted in Fig. 1A come from an analog clock (Fig. 1B), one could conceive of the various data-driven modeling approaches to discovery of biological causality (or, more accurately, quasi-

causality) as being akin to defining how the parts function in such a clock. In the setting of acute inflammation, this would entail defining how inflammatory mediators interact with each to drive the ultimate disease phenotype and clinical outcome of critical illness. In the clock analogy, the hour hand could be thought of as the primary disease phenotype, i.e. the main feature of interest to clinicians or researchers (since, when we ask “what time is it?” we are concerned primarily with the hour, and only secondarily with the minutes).

In the clock analogy, the minute hand is actually more like a disease mediator/biomarker: something whose changes over time presage the onset of the principal disease characteristic of interest, and whose levels are of secondary importance but still useful in predicting the onset of a clinical change). However, targeting such biomarkers would be akin to wanting to spin the minute hand manually to change the hour on the clock. Clearly, it is more logical to turn a dial or a knob, which in turn affects numerous, intertwined gears (Fig. 2).

We hypothesize that the biological equivalent of the clock's gears would be dynamic networks (Fig. 2). In the experimental setting, analyses of dynamic networks of systemic inflammation suggested that, despite a large degree of overlap in inflammatory mediators induced by experimental injury alone vs. injury combined with hemorrhagic shock, the latter group exhibited very different dynamic networks (including a phenotype that can be termed “elevated but not connected”, i.e. an elevation of key inflammatory mediators without concomitant connection of these mediators to other mediators) [15]. In the clinical setting, a comparison of dynamic networks of systemic inflammation in blunt trauma patients that also suffered spinal cord injury vs. similarly injured patients that did not suffer spinal cord injury pointed to a potentially novel role of the chemokine IP-10/CXCL10 as a regulator of the key anti-inflammatory cytokine IL-10, associated with a profound immunosuppression observed in the trauma/spinal cord injury cohort [16]. Dynamic network patterns could also distinguish blunt trauma patients that went on to experience nosocomial infections from those that did not [17], and suggested that pediatric acute liver failure patients who were transplanted had networks similar to those who survived spontaneously but different from those patients who died [18].

Perhaps the most striking of these network-based discoveries concerned the differences in dynamic inflammation networks in blunt trauma patients that succumbed to their critical illness vs. highly matched patients that survived, despite nearly identical injury and treatment characteristics [19]. That study derived multiple novel insights either directly or indirectly from computational modeling of dynamic networks of systemic inflammation: 1) that trauma-induced mortality which occurs in the intensive care unit is not simply a function of elevated injury severity, but rather appears to be a feature specific to a minority of trauma patients; 2) that trauma survivors and non-survivors can be distinguished by their inflammatory networks very early following injury; 3) that survivors exhibit inflammation that in many way resembles chronic inflammation whose hallmark is the production of lymphoid-derived mediators, rather than the classic concept of acute inflammation characterized primarily by innate immune mediators; and 4) the presence of self-sustaining inflammation network characterized by early activation of the Th17 axis in non-survivors, with dynamics that mirror those of multiple organ dysfunction [19]. In the clock analogy,

this would imply the potential discovery of a key gear that might affect how the clock functions.

From Minutes to Seconds: Insights into Principal Drivers of Acute Inflammation in Critical Illness

To complete the clock analogy, we could conceive of the second hand (which must turn multiple times in order to allow for a small change in the minute hand and a barely perceptible change in the hour hand) as the something like the principal component analysis: an output of the sum total of interacting, dynamic networks, necessarily related to both disease biomarkers and the ultimate disease phenotype but in a fashion that is not apparent upon cursory examination.

In the setting of inflammation and critical illness, PCA has been used to discern principal characteristics of experimental hemorrhagic shock [15], sepsis [20,21], and wound healing [22], as well as in clinical settings such traumatic brain injury [23] as well as pediatric acute liver failure [18]. This includes a recent demonstration of the potential use of patient-specific PCA followed by hierarchical clustering of circulating inflammation biomarkers to define trauma patient outcome groups [24].

The key conceptual insight is that techniques such as PCA have pointed to different mediators from those inferred as central nodes by dynamic network discovery algorithms. Given our clock analogy, this should not be surprising: we are comparing internal gears (dynamic networks) to external outputs of those gears (the second hand, which we hypothesize might correspond to principal components). PCA helps us focus on those variables/inflammatory mediators that exhibit the most variance: in the clock analogy the second hand moves/varies 60 times as much as the minute hand and 3600 times more than the hour hand. From a biological standpoint, we can hypothesize that PCA is highlighting an intermediate phenomenon between the most proximal control mechanisms (the gears/networks) and the output of the system (hour/minute hand, i.e. biomarkers and clinical phenotype). And yet, the system as a whole is interconnected and changes dynamically.

Mechanistic Computational Modeling Defines an Abstract Inflammatory “Clock”: Patient-Specific Models and *In Silico* Clinical Trials

In parallel to the data-driven modeling work described above, several groups including ours began to pursue an alternative hypothesis, namely that the same inflammatory wiring could drive both an adaptive inflammatory process and a detrimental one, given different circumstances and contexts. Rather than being derived from bioinformatics, this alternative hypothesis mapped more naturally to mechanistic modeling [1,2]. This class of models utilizes multiple formalisms (summarized in Table 1).

Mechanistic modeling efforts started with quite simple, highly abstracted models of inflammation [25-27]; progressed to larger models that could describe inflammation in experimental animals (including defining the impact of perturbations such as gene knockouts and natural processes such as aging) [21,28-31]; and led to the concept of *in silico* clinical

trials in sepsis [32-34], wound healing [35-37], and trauma [38]. More recently, we have demonstrated the potential to generate mechanistic models based on insights derived from dynamic network and PCA models (in this case, in the setting of traumatic brain injury [23]). Furthermore, these models formed increasingly granular variants of a core hypothesis of how inflammation evolved, namely that inflammation induces secondary damage/dysfunction at the cell/tissue/organ/whole organism level, which in turn drives further inflammation [1].

Conclusions: Resetting, not Reinventing, the “Clock”

This key hypothesis has formed the basis of systems-based attempts to modulate inflammation dynamically and in near real-time. We were faced with the realization that inflammation induced by traumatic injury, severe infection, or organ failure progresses extremely rapidly to drive a life or death bifurcation [18,19,23,32,33], and were driven by the aforementioned hypothesis regarding a feed-forward process of inflammation to damage/dysfunction to more inflammation [1]. Thus, the concept of finding an appropriate diagnostic test and administering an individual-specific therapy in a timely fashion is daunting, even given the possibility of mathematically modeling acute inflammation in individuals and populations in order to predict clinical outcomes [24,38].

This realization drove us to envision and then develop the concept of a self-adaptive therapy aimed at subverting the potentially vicious, feed-forward loop. In the context of the clock analogy, we were searching for a means by which to reset the inflammatory clock (or, at the very least, to gum up its gears). To do so, we generated a biohybrid device in which liver cells were stably modified to interrupt the key pro-inflammatory feed forward loop driven by the cytokine tumor necrosis factor- α (TNF- α) [39]. This device produced the natural inhibitor of TNF- α , soluble TNF- α receptor I (sTNFR), and was capable of reprogramming systemic acute inflammation in rats [39].

While the initial device produced sTNFR constitutively [39], more recent generations of the device produce sTNFR in response to systemic TNF- α (unpublished). This raises the potential for a device for patient-specific, self-adaptive diagnosis and therapy for acute inflammation, driven by insights from computational models. Future approaches may focus on key mediators such as IL-17 and related cytokines [19].

The key lesson, however, is not about specific approaches to modulating inflammation, but rather about the power of computational approaches to drive both mechanistic and clinically-relevant advances in complex disease settings [1]. The failures and successes that motivated the computational modeling work described above will hopefully serve as a template for how to approach similarly complex and seemingly intractable biological problems. Our time is too short to waste on reinventing the clock.

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References

1. An, G.V.Y. Translational Systems Biology: Concepts and Practice for the Future of Biomedical Research. Elsevier; 2014.
2. Vodovotz Y, et al. Translational systems biology of inflammation. PLoS Comput Biol. 2008; 4:1–6.
3. Kitano H. Systems biology: a brief overview. Science. 2002; 295(5560):1662–1664. [PubMed: 11872829]
4. Csete ME, Doyle JC. Reverse engineering of biological complexity. Science. 2002; 295(5560): 1664–1669. [PubMed: 11872830]
5. Namas R, et al. Insights into the role of chemokines, damage-associated molecular patterns, and lymphocyte-derived mediators from computational models of trauma-induced inflammation. Antiox Redox Signaling. 2015; 10:1370–1387.
6. Deutschman CS, Tracey KJ. Sepsis: Current Dogma and New Perspectives. Immunity. 2014; 40(4): 463–475. [PubMed: 24745331]
7. Cobb JP, et al. Injury in the era of genomics. Shock. 2001; 15(3):165–170. [PubMed: 11236897]
8. Cuenca AG, et al. The Glue Grant experience: characterizing the post injury genomic response. Eur J Trauma Emerg Surg. 2011; 37(6):549–558. [PubMed: 26815465]
9. Xiao W, et al. A genomic storm in critically injured humans. J Exp Med. 2011; 208(13):2581–2590. [PubMed: 22110166]
10. Janes KA, Yaffe MB. Data-driven modelling of signal-transduction networks. Nat Rev Mol Cell Biol. 2006; 7(11):820–828. [PubMed: 17057752]
11. Hassanien AE, et al. Computational intelligence techniques in bioinformatics. Comput Biol Chem. 2013; 47:37–47. [PubMed: 23891719]
12. Calvano SE, et al. A network-based analysis of systemic inflammation in humans. Nature. 2005; 437:1032–1037. [PubMed: 16136080]
13. Edmonds RD, et al. Transcriptomic response of murine liver to severe injury and hemorrhagic shock: A dual platform microarray analysis. Physiol Genomics. 2011; 43:1170–1183. [PubMed: 21828244]
14. Vodovotz Y, Billiar TR. In Silico Modeling: Methods and applications to trauma and sepsis. Crit Care Med. 2013; 41:2008–2014. [PubMed: 23863232]
15. Mi Q, et al. A dynamic view of trauma/hemorrhage-induced inflammation in mice: Principal drivers and networks. PLoS ONE. 2011; 6:e19424. [PubMed: 21573002]
16. Zaaqoq AM, et al. Inducible protein-10, a potential driver of neurally-controlled IL-10 and morbidity in human blunt trauma. Crit Care Med. 2014; 42:1487–1497. [PubMed: 24584064]
17. Namas RA, et al. Temporal Patterns of Circulating Inflammation Biomarker Networks Differentiate Susceptibility to Nosocomial Infection Following Blunt Trauma in Humans. Ann Surg. 2016; 263:191–198. [PubMed: 25371118]
18. Azhar N, et al. Analysis of serum inflammatory mediators identifies unique dynamic networks associated with death and spontaneous survival in pediatric acute liver failure. PLoS ONE. 2013; 8:e78202. [PubMed: 24244295]
19. Abboud AN, et al. Computational analysis supports an early, type 17 cell-associated divergence of blunt trauma survival and mortality. Crit Care Med. 2016; 44:e1074–e1081. [PubMed: 27513538]
20. Namas R, et al. Hemoadsorption reprograms inflammation in experimental Gram-negative septic fibrin peritonitis: Insights from in vivo and in silico studies. Mol Med. 2012; 18:1366–1374. [PubMed: 22751621]
21. Nieman K, et al. A two-compartment mathematical model of endotoxin-induced inflammatory and physiologic alterations in swine. Crit Care Med. 2012; 40:1052–1063. [PubMed: 22425816]
22. Starzl R, et al. Cardiac arrest disrupts caspase-1 and patterns of inflammatory mediators following localized tissue injury in rats: insights from computational modeling. Frontiers in Immunology. 2015; 6:587. [PubMed: 26635801]
23. Abboud A, et al. Inflammation following traumatic brain injury in humans: Insights from data-driven and mechanistic models into survival and death. Front Pharmacol. 2016; 7:342. [PubMed: 27729864]

24. Namas R, et al. Individual-specific principal component analysis of circulating inflammatory mediators predicts early organ dysfunction in trauma patients. *J Crit Care*. 2016 In Press.
25. Kumar R, et al. The dynamics of acute inflammation. *J Theoretical Biol*. 2004; 230:145–155.
26. Reynolds A, et al. A reduced mathematical model of the acute inflammatory response: I. Derivation of model and analysis of anti-inflammation. *J Theor Biol*. 2006; 242:220–236. [PubMed: 16584750]
27. Day J, et al. A reduced mathematical model of the acute inflammatory response: II. Capturing scenarios of repeated endotoxin administration. *J Theor Biol*. 2006; 242:237–256. [PubMed: 16616206]
28. Chow CC, et al. The acute inflammatory response in diverse shock states. *Shock*. 2005; 24:74–84. [PubMed: 15988324]
29. Prince JM, et al. In silico and in vivo approach to elucidate the inflammatory complexity of CD14-deficient mice. *Mol Med*. 2006; 12:88–96. [PubMed: 16953560]
30. Daun S, et al. An ensemble of models of the acute inflammatory response to bacterial lipopolysaccharide in rats: Results from parameter space reduction. *J Theor Biol*. 2008; 253:843–853. [PubMed: 18550083]
31. Namas RA, et al. Combined in silico, in vivo, and in vitro studies shed insights into the acute inflammatory response in middle-aged mice. *PLoS ONE*. 2013; 8:e67419. [PubMed: 23844008]
32. Clermont G, et al. In silico design of clinical trials: a method coming of age. *Crit Care Med*. 2004; 32:2061–2070. [PubMed: 15483415]
33. Kumar R, et al. A mathematical simulation of the inflammatory response to anthrax infection. *Shock*. 2008; 29:104–111. [PubMed: 18157069]
34. An G. In-silico experiments of existing and hypothetical cytokine-directed clinical trials using agent based modeling. *Crit Care Med*. 2004; 32:2050–2060. [PubMed: 15483414]
35. Mi Q, et al. Agent-based model of inflammation and wound healing: insights into diabetic foot ulcer pathology and the role of transforming growth factor- β 1. *Wound Rep Reg*. 2007; 15:617–682.
36. Solovyev A, et al. Hybrid equation- / agent-based model of ischemia-induced hyperemia and pressure ulcer formation predicts greater propensity to ulcerate in subjects with spinal cord injury. *PLoS Comp Biol*. 2013; 9:e1003070.
37. Ziraldo C, et al. A computational, tissue-realistic model of pressure ulcer formation in Individuals with spinal cord injury. *PLoS Comput Biol*. 2015; 11(6):e1004309. [PubMed: 26111346]
38. Brown D, et al. Trauma in silico: individual-specific mathematical models and virtual clinical populations. *Sci Transl Med*. 2015; 7:285ra261.
39. Namas R, et al. A biohybrid device for the systemic control of acute inflammation. *Disrupt Science & Technol*. 2012; 1:20–27.

Abbreviations

PCA principal component analysis

Highlights

- The acute inflammatory response to injury or severe infection is complex, dynamic, and non-linear, leads to critical illness, and could be thought of as an analog clock whose underlying moving gears (dynamic networks) drive surface phenomena (changes in inflammatory cells and mediators, changes in patient health).
- Reductionist insights are important but can be misleading when attempting to predict the inflammatory responses and associated outcomes of individuals and diverse populations
- Data-driven and mechanistic computational models are likely to be necessary to decipher this complexity, and have already led to novel insights.
- Insights derives from computational modeling could drive rational inflammation reprogramming.

Figure 1. The whole is more than the sum of its parts

(A): A parts list (e.g. a list of inflammatory mediators) does not, in and of itself, elucidate the nature or function of the underlying complex system (the acute inflammatory response).

(B): A complex biological system, such as the acute inflammatory response, can be viewed as an analog clock, whose various parts (panel A) are assembled into a functional whole. We observe only glimpses of this function (the gears showing through, partially, and the current time).

Figure 2. Discerning the function of the inflammatory clock

The disparate parts shown in Figure 1A come together to form the driving mechanism of an analog clock (Figure 1B). These gears move in time.

Table 1
Data-driven and mechanistic modeling methods and their application to inflammation and critical illness

	Modeling Method	Key strengths and weaknesses	Application to inflammation/critical illness
Data-driven Modeling	Hierarchical Clustering	Easy visualization and segregation of complex data in heat maps. Multiple methods for clustering can lead to different results, and the decision regarding how to define sub-groups can be subjective.	Segregating gene transcription signatures [9,12,13]. Segregating sham surgery from hemorrhagic shock in mice based on time courses of circulating inflammatory mediators [15]. Segregating trauma patients based on Principal Component Analysis of circulating inflammatory mediators [24].
	Principal Component Analysis (PCA)	Defines principal characteristics of a dynamic dataset, based on variance. Biological significance of principal components may be difficult to intuit.	Primary systemic inflammatory characteristics of sham surgery vs. hemorrhagic shock in mice [15] and of pediatric acute liver Primary characteristics of pediatric acute liver
	Dynamic Network Analysis (DyNA)	Infers interaction networks among multiple variables in a time-varying dataset, over defined time intervals. Can suggest mechanistic interactions, but is based purely on correlations within each time frame.	Dynamic networks of systemic inflammation of sham surgery vs. hemorrhagic shock in mice [15], of nosocomial infection vs. control in trauma patients [17], and of blunt trauma survivors vs. non- survivors [19].
	Dynamic Bayesian Network Inference (DyBN)	Defines the most likely single network structure that best characterizes the dynamic interactions among multiple variables across time, thereby suggesting likely feedback structures that define central nodes. The networks may suggest possible mechanisms. Dependence on presumed unchanging network structure over time must be validated.	Dynamic networks of systemic inflammation in pediatric acute liver failure patients [18] and trauma patients [19].
Mechanistic Modeling	Differential Equations	Recapitulates mechanism by defining relationships among variables as the summation of positive vs. negative influences; can be constructed in a compartmentalized fashion and calibrated against data. Assumes a well- mixed system (mean field approximation), which may not hold true in certain biological settings. Can be difficult for non-specialists to construct.	Abstracted models of acute inflammation [25-27]. Quantitative models of acute inflammation in animals [21,28-31], trauma patients [38], and traumatic injury patients [23]. <i>In silico</i> clinical trials of sepsis [32,33] and trauma [38].
	Agent-based Models	Recapitulates mechanism by defining stochastic relationships among variables as the summation of positive vs. negative influences; can be constructed in a compartmentalized fashion and calibrated (with greater difficulty than Differential equations) against data. May represent stochastic biological processes better than differential equations. Can be easier for non-specialists to construct as compared to differential equations. Suffers from high computational cost and difficulties in calibrating to data.	Models and <i>in silico</i> trials of sepsis [34] and wound healing [35,37].
	Hybrid Models	A blend of modeling frameworks, often Combining differential equations and agent-based models. May offer the benefits of both modeling formalisms, e.g. combining stochastic micro-scale processes with larger-scale processes for which the mean field approximation holds true. Can be quite difficult to construct, calibrate against data, and run.	Models of wound healing (post-spinal cord pressure ulcer formation and resultant effects on tissue blood flow) [36].