Wound Infection

Subjects: Others

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Wound infection is traditionally defined primarily by visual clinical signs, and secondarily by microbiological analysis of wound samples.

Keywords: wound ; infection ; hard-to-heal ; chronic ; inflammation ; neutrophil

1. Distinguishing Colonization from Infection

Colonization is a term that indicates the presence of an organism without necessarily interfering with wound healing [1]. When or whether colonization leads to infection is often not clear and appears to be dependent on both microbiological and host factors [2]. However, apart from clinical signs, there are few tools for observation and prediction of the transition to infection in a timely manner.

The term “critical colonization” has attracted significant attention and scrutiny in recent years and is often dismissed or regarded as synonymous with local infection [1][3][4]. Nevertheless, the scientific underpinning of the concept of critical colonization lies in the delay of wound healing by microbial factors (e.g., biofilm, or toxins that reduce innate immune response) without the overt and clinically obvious signs of infection. Failure to identify the shift from wound colonization to wound infection (pathogen invasion) impedes timely diagnosis, thus delaying appropriate treatment and wound healing.

2. The Role of Biofilm in Chronic Infection

The term biofilm is widely used to describe surface-associated microbial communities, comprising various organisms and growth forms within a three-dimensional matrix of extracellular polymeric substances (EPS). EPS provides the organisms with protection from external threats such as cellular and chemical antimicrobial agents. Biofilm plays a significant role in the inability of chronic, hard-to-heal wounds to progress towards healing [5][6][7]. Alteration of gene expression and gene products within biofilm are responsible for persistent inflammation [8], antibiotic tolerance [7][9][10] and evasion of host adaptive and innate immunity [11]. However, no individual gene or technology can be used to identify the biofilm mode of growth. Furthermore, persister cells arise within biofilm, owing to a state of metabolic dormancy [12][13]. Persister cells in biofilm appear to contribute to the recalcitrance of chronic infections in that their metabolic quiescence protects them from antimicrobial substances but allows resumption of activity once antimicrobials have been discontinued [14].

One of the most significant barriers to effective biofilm management lies in the most commonly used diagnostic tool for wound infection, namely culture and viable counts. This is ill-suited to the detection of biofilm due to issues of sampling, separation, mutualism, and metabolic dormancy, rendering biofilm cells difficult to culture via traditional methods [15]. This has caused a paradigm shift within clinical wound management to account for the presence of biofilm, although in many cases this manifests as an observational “trial and error” approach [6]. While the link between pathogenic microorganisms and infection has been understood for over a century, the link between wound infection and biofilm has only recently been understood [16][17][18][19].

3. Current Approaches in the Diagnosis of Wound Infection Status

3.1. Clinical Observation

Visible signs of infection-induced inflammation are familiar and can be directly related to underlying immune processes. Acute infection occurs when virulence factors in one or more microorganisms neutralize or evade the patient’s innate and adaptive immune systems. Subsequently, invasion and dissemination of metabolically active (planktonic) microorganisms in viable tissue provoke a series of local and systemic host responses that manifest as heat, pain, edema and erythema

[20][21]. The qualitative diagnosis of wound infection frequently involves the identification of such clinical signs; hence its early detection relies heavily on the skill and experience of the health care providers (HCPs).

The key conceptual issue that emerges is that wound infection is still broadly considered to be a state in which the wound is visibly infected, namely, that inflammation, suppuration, and pain are so advanced as to be obvious to patient and HCP alike [20]. In reality, and particularly in chronic wound infections, the total time period of a wound infection is likely to be longer than is visibly recognized because clinical signs and symptoms take time to become apparent. Thus, the current "infection" may be more accurately considered a severe or established infection. While acute wound infections tend to develop more rapidly with obvious signs of infection-induced inflammation, chronic wound infections manifest very differently [22]. Because biofilm is the root of the problem, as a foreign body it induces the infiltration of neutrophils as occurs in acute wounds. However, since biofilm matrix protects associated bacteria, neutrophils accumulate around the biofilm, becoming “frustrated” in their inability to thwart microbial onslaught. Neutrophil activity around the biofilm results in the release of antimicrobial oxygen metabolites and enzymes, that ultimately destroys host tissue, and providing an additional nutrient source for the evolving biofilm [22]. Wound biofilm thus enjoys a parasitic relationship with the host, taking control of host inflammation and using it to its benefit [22]. Biofilm-induced chronic wounds consequently manifest as a persistent hyper-inflammatory condition, with subtle clinical signs including sullen/dark granulation tissue, friable granulation tissue, malodor, and delayed healing [22]. Any delay in diagnosis is partly because these signs are subjective, and often require examination of the wound and patient over a prolonged period to observe changes.

Despite the subjectivity, most practitioners rely on clinical signs and symptoms to diagnose wound infection (98% of the time), followed by patient-reported symptoms (88% of the time) [23], yet these HCPs still commonly use wound cultures in an attempt to confirm infection status [24].

3.2. Microbiological Investigation

The current approach to confirming infection by enumerating and/or identifying organisms is based on the concept that infection is associated with the abundance of microorganisms or the presence of specific pathogens. However, enumeration does not correlate with infection status [25] and does not address the relative pathogenicity of isolates [26], nor the position of organisms in the wound profile (potentially confounding opportunists at the surface interface with potential pathogens in the wound bed) [27][28]. Punch biopsy partly mitigates these issues, but it is too invasive and time-consuming for routine monitoring and has the potential to spread infection and cause pain [20]. There is also evidence that punch biopsy and surface swab are similar in terms of recovery/organism specification [16].

Whilst culture is often justified with the argument that knowing the causative organism will aid in the selection of an appropriate treatment pathway, this is less relevant in general wound therapy practice where first- and second-line approaches are usually pre-defined [29], and most infections are initially polymicrobial [18]. In addition, causative organisms are often anaerobic bacteria that are notoriously difficult to culture in vitro, thus often overlooked despite their significant contribution to microbial biomass and pathogenicity [30]. Enumeration also under-represents other unculturable or hard-to-culture organisms [31] which is often associated with biofilm [15].

Consequently, microbial culture is only weakly predictive in practice, providing results that are easily interpreted only when the infection is already clinically obvious (and where a significant over-growth of one organism is apparent) [3][20]. Therefore, culture is often, at best, only weakly confirmatory and rarely yields a clear causation-treatment nexus. Indeed, given that it can often take several days to obtain results from microbiology culture, most first-line antibiotic therapy is applied before any microbiology results are available.

In early, local, polymicrobial infections without a dominant pathogen, the application of broad-spectrum antimicrobials is combined with a “wait-and-see” approach. Since antibiotic therapy often selects for the emergence of a dominant pathogen, one value of microbiological culture then lies in the evaluation of antibiotic susceptibility profiles, providing useful information to prescribe the most efficient antibiotic treatment [32].

3.3. PCR and Sequencing-Based Technologies

Acute bacterial infections in general medicine often involve single species [20][33]; thus, identification of the causative organism can be helpful in selecting therapy, as it is likely that similar symptoms are associated with the same pathogen in a given area and time. Taking community-acquired pneumonia as an example, the identification of causative organisms can trigger the use of defined treatment protocols [34]. However, chronic wound infection differs in that colonizing microorganisms originate from a variety of sources including surrounding skin, mouth, gut, and the environment, and consequently, this rarely leads to a single pathogen dominating the infection [25].

Given this complexity, total sample DNA sequencing is a potentially unbiased means to enumerate and classify a microbial community. It has been widely applied at the research level in studies of the gut [35], and initial data sets from wound infection sites have been obtained [36][37]. These data show significant diversity but provide indications that certain classes of organisms such as enterobacteria and facultative anaerobes in general are associated with non-healing wounds [38]. Polymerase chain reaction (PCR)-based diagnostics and mixed primer panels (e.g., for 16S variants) have the potential to identify and quantify organisms present with good sensitivity [31][39][40]. In ideal cases, PCR can also detect known resistance genes of common organisms [36]. These detailed outcomes are based on a degree of supposition of likely pathogens and strains. Whilst it would be theoretically possible to create diagnostic primers for most organisms and strains typically found in wounds, it remains more practical to use defined primer panels for hypothesis-driven identification of pathogens present. Sequencing and PCR avoid the bias against anaerobes and fastidious organisms observed in culturing techniques and provide more realistic indications of microbial diversity and abundance. Nonetheless, they are resource intensive, and their use is still only justified once a clear case for infection exists. Establishing this case in complex hard-to-heal wounds remains the key problem to solve.

The advantages of these techniques are unfortunately outweighed by several disadvantages. These systems require clean samples and can be affected by patient DNA (which can be in significant excess over that of the microorganisms in wound samples). They cannot distinguish between viable and non-viable pathogens and require expensive equipment that is still unsuited to point-of-care (POC) use. Additionally, sequence databases are often biased towards pathogenic organisms, thus resulting in a significant underestimation of the true species diversity within a wound [41][42]. As such, their uptake into practice has been limited and they are used less in monitoring or routine screening, but rather as an investigative tool in clinically obvious acute infection [43]. Thus, the role of PCR is currently confirmatory rather than predictive. If, in the future, POC molecular techniques to detect infection become available, then this confirmatory role may become increasingly useful. However, further technological and automation improvements to reduce cost and time would be required to make this feasible or to use it in routine screens to detect incipient infection [44].

3.4. Existing Biomarkers and Uses

An alternative, evolving approach to determine the presence of infection is the measurement of patient biomarkers of the immune system in response to incipient infection. Existing host-derived biomarkers of infection include C-Reactive Protein (CRP) [45], procalcitonin [46], hematologic markers [47], and more recently, the proposal to monitor lipocalin release from *N*-formyl-methionyl-leucyl-phenylalanine stimulated whole blood neutrophils [48]. All these markers are usually measured from blood or plasma samples and reflect systemic inflammatory status. Elevation associated with a local infection may suggest some systemic spread and the need for appropriate action (including intravenous antibiotic therapy). These markers are less useful in the early phases of local wound infections since the local markers that reach plasma are too dilute, and there has been no activation of significant systemic response, until the infection is, again, obvious at its source. Thus, local sampling of the wound itself is likely to yield sufficient biomass of the relevant host cells and cell products that otherwise would be highly diluted in blood samples.

The discussion of blood versus wound sampling highlights a general issue in diagnostics, namely that of sensitivity and timing during the development of the target condition. The ideal in all diagnostic approaches is to detect changes as early as possible and this means both sensitivity to small amounts of marker and avoiding dilution or contamination in sampling. In the context of early detection of wound infection, local surface sampling is usually both convenient and non-dilutive. In contrast, systemic sampling appears more relevant for deep undrained surgical wounds for pragmatic reasons. The local biomarkers may not be the same as the systemic biomarkers, thus site and sampling should not be separated from the consideration of which biomarkers to evaluate. In this regard, wound infection diagnosis has a major advantage in that in most cases, the source of the sample is easily accessed.

3.5. Electronic Noses and Imaging

The importance of anaerobes can lead to changes in volatile compounds emerging from the wound [49] and thus the potential for detection via electronic noses and similar technology. The advantage of such approaches is that they are non-invasive, potentially suitable for continuous monitoring and if sufficiently sensitive, potentially able to provide predictive data for incipient infections. Many such applications have been demonstrated using in vitro models [50] but the approach remains more difficult to apply in the clinical setting both in terms of sample acquisition and location of apparatus. These devices are generally not yet portable or suitable for point-of-care use. As the technology is driven by alternative uses, it is likely that improvements in sensors and portability will find their way to wound care applications in the coming decade. An alternative non-invasive approach is imaging, either thermal or ultraviolet. Multi-spectral analysis has the potential to track size, general biochemical markers, and fluorescent metabolites [51]. Imaging relies on powerful

fluor- and chromophores produced by infecting organisms. These include porphyrins and pyocyanins, which can be distinguished from host autofluorescence. While fluorescence is capable of detecting a wide variety of porphyrin-producing wound bacteria (red fluorescence) and pyocyanin-producing *Pseudomonas aeruginosa* (cyan fluorescence), it is dependent on operator experience to distinguish the many sources of autofluorescence in wounds. This approach can be of significant benefit in locating bacterial “hotspots” in a wound to guide debridement and effective bioburden/biofilm removal, but it does not necessarily detect incipient infection. An extension of this approach is to stain the wound using materials that are specifically bound by biofilm components. Reports include the use of dyes used for plaque staining for teeth to stain biofilm in wounds. While elegant, these approaches serve a similar purpose as fluorescence techniques in detecting bioburden/biofilm to guide effective debridement, without facilitating the determination of infection status.

Such advances in microbial detection technologies and devices highlight the significance and progress that is being made in this field. In terms of ideal clinical requirements, related devices would be non-invasive and simple to use (by practitioners at all levels of expertise), would identify any potential foci of infection (including biofilm), would be sufficiently sensitive to detect incipient (early, non-obvious) infections, and would provide immediate outputs that guide a practitioner in providing optimal wound care such as effective local wound hygiene. While not all of these criteria are presently met, progress continues, and new approaches continue to evolve including a host-directed infection detection technology that is described in these contents.

4. Wound Healing: An Overview

4.1. The Role of Inflammation in Wound Healing and Chronic Wounds

These observations suggest that a common aspect of wound stasis is sustained inflammation that persists because resolution is not initiated due to constant stimulus. Biofilm is now recognized as a constant stimulus, provoking a hyper-inflammatory state that prevents wound healing. Inflammation is an essential, innate immune response involving pathogen clearance as well as tissue breakdown and removal of cellular, extra-cellular and pathogenic debris. The inflammatory phase of wound healing involves a complex and overlapping cascade of molecular signals that ultimately facilitate leukocyte (monocyte and neutrophils) infiltration of the wound bed to mount a rapid and robust antimicrobial response [52]. During the inflammatory phase, platelet aggregation is followed by infiltration of leukocytes into the wound site, which are then found throughout the wound in varying degrees of vitality. Similarly, invading microorganisms can be found both within tissue, outside the confines of the wound bed, and in the wound dressing. Depending on the number and virulence of microorganisms encountered, the immune cells are either active and attracted to sites of infection, inactivated by pathogens, or are engulfing and lysing pathogens [53].

Once pathogens are cleared, immune cells orchestrate remodeling primarily through tissue degradation and formation through the activation of fibroblasts and endothelium. As such, an imbalance (excessive or reduced numbers) of inflammatory cells may have profound effects on downstream cell migration, proliferation, differentiation, and ultimately, the quality and duration of the overall healing response. Crucially, successful tissue repair requires the resolution of the inflammatory response for healing to progress to the proliferative stage [54][55]. The lack of resolution should be an indicator of persistent pro-inflammatory signaling or an imbalance in the regulation of immune cells at the site. Persistent organisms, biofilms or repeated injury can provide this pro-inflammatory stimulus, while the lysis of immune cells and the cleavage of signals and growth factors is one cause of the dysregulation of the cellular response to healing. A key source of the destructive inflammatory proteases is lysed neutrophils.

4.2. The Role of Neutrophils in the Inflammatory Phase

Neutrophils are polymorphonuclear, phagocytic leukocytes that are part of the early host immune response against invading pathogens. They are recruited from peripheral blood initially, and later from bone from marrow in response to “find me” signals including damage-associated molecular patterns (DAMPs), hydrogen peroxide, lipid mediators, adenosine, and chemokines released from regions of injury or infection [56]. Neutrophils, like other myeloid cells, are not homogeneous and even more phenotypes are being recognized, which are related to tissue, age and phase of inflammation.

Neutrophils represent the most abundant inflammatory cells to infiltrate a wound in the early inflammatory phase of healing, where their primary function is to clear microorganisms to prevent infection and remove debris via a variety of mechanisms including phagocytosis, the release of toxic granules (degranulation), or the release of neutrophil extracellular traps (NETs) [57].

Whilst neutrophils play a crucial role in re-establishing tissue homeostasis via pathogen phagocytosis and macrophage recruitment, excessive neutrophil activity may lead to an overproduction of reactive oxygen species (ROS) and release of hydrolytic enzymes, causing extra cellular matrix (ECM) and cell membrane damage, ultimately resulting in premature cell senescence. The presence of ROS may also activate proteases (matrix metalloproteinases (MMPs) and serine proteases) and simultaneously inactivate protease inhibitors. Most of these effects are due to NETosis and neutrophil lysis, both of which release granules to the extracellular space. Both phenomena are associated with stimuli such as biofilm which are not susceptible to intracellular processing. The effect of granule release is to degrade ECM and growth factors which cause wounds to become chronic (or static) due to lack of structure, growth stimulus, and sustained immune activity [58].

Clearance of neutrophils begins with their apoptosis and subsequent engulfment by macrophages; a process known as efferocytosis [59]. This is critical because neutrophil contents are particularly potent in tissue degradation and their ordered destruction is important to homeostasis. Failure to activate neutrophil efferocytosis can lead to secondary necrosis where the neutrophils lyse, resulting in the release of pro-inflammatory cytotoxic molecules and proteases that increase tissue damage [52]. However, not all neutrophils are cleared by macrophages. Recent studies have shown that a subset of neutrophils leave the wound site through interstitial migration, or re-entry into the vasculature via the process of “reverse neutrophil migration” [60]. The purpose may be, amongst others, to transport captured pathogen cells to central immune organs such as the lymph nodes and the marginal zone of the spleen for antigen presentation [61].

Timely clearance of neutrophils is critical because it precedes resolution of inflammation. Neutrophil persistence, often itself is a response to microbial biofilm persistence, leads to a prolonged inflammatory state and thus non-healing wounds, due in part to the abundance of antimicrobial enzymes and peptides that degrade tissue and stall healing [52].

4.3. Neutrophil Granules: A Rich Source of Proteases and Peroxidases

Neutrophilic granules are located in the cytoplasm as small packages encapsulated by a lipid bilayer membrane. They contain multi-functional assemblages of proteins able to perform intracellular translocation, rapid alteration of neutrophil plasma membrane composition, extracellular discharge, cell–cell communication, and deployment of antimicrobial functionalities. Granules are classified based on the time at which they are formed during granulopoiesis, protein markers and dye affinity. Specifically, there are three types of neutrophil granule: (i) primary or azurophilic (markers include: myeloperoxidase (MPO), human neutrophil elastase (HNE), Cathepsin G (CatG), azurocidin); (ii) secondary or specific granules (lipocalin 2, lactoferrin); and (iii) tertiary or gelatinase granules (matrix metalloproteinase-9 (MMP-9), neutrophil collagenase) [62][63]. Lysozyme is found in primary, secondary (co-located with lactoferrin [64]) and tertiary granules. The complement of proteases carried by neutrophils and other myeloid cells has multiple purposes. At one level it is to lyse pathogens, at another it is to allow these cells to pass through tissue or degrade intracellular proteins, or indeed other cells. The terms gelatinase, collagenase or indeed elastase are over-simplifications in that these enzymes are rarely truly specific and are almost always present in mixtures.

Neutrophils contain proteolytic enzymes (including serine proteases) which, along with MPO, define the primary granules. As pre-stored agents, neutrophil serine proteases can be quickly deployed in reaction to microbial challenge, to degrade internalized microbes, or upon release from activated neutrophils. Serine proteases are important contributors to the physiological response to infection, both as antimicrobial agents and as immunomodulators [65].

Uncontrolled HNE is known to be responsible for tissue loss and degeneration. Well-known examples include chronic lung diseases such as cystic fibrosis or chronic obstructive pulmonary disease. In wounds, proteolysis from host-derived enzymes fulfills a similar role in that it reverses or halts regenerative processes and degrades growth factors [66]. These factors further increase the total protease activity within the wound and exacerbate the host tissue damage. HNE thus impedes keratinocyte migration causing delayed healing [67].

5. Scenarios of Wound Healing

5.1. Scenario 1: The Healing Wound

The innate immune system is activated immediately following injury or tissue damage, setting in motion a local inflammatory response that includes the recruitment of inflammatory cells from the circulation. Neutrophils promptly accumulate at the site of tissue injury, where their principal role is to phagocytose pathogens [63]. During physiological wound repair, neutrophils undergo apoptosis after completion of their various functions and are then subject to local macrophage uptake to trigger the transition out of the inflammatory phase.

When a neutrophil encounters a microorganism, phagocytosis stimulates the maturation of the phagolysosome. Digestive antimicrobial enzymes which are held in granules are then recruited to the phagolysosome and their contents are transferred to it via fusion. These enzymes have exposed amines on their surfaces and are normally held in granules in an inactive form via electrostatic interactions with the anionic sulfated proteoglycan granule matrix (heparin-like) core of the granule [63]. Upon release into the phagolysosome, the presence of hypertonic potassium ions (K^+) allows the release and activation of the enzymes. Other control measures such as elastase inhibitory peptides are also removed [68][69]. The action of the granular enzymes at the bacterial surface is accentuated by the phagosomal membrane conforming tightly to the bacterial surface, forcing granule contraction, which potentiates local pore-forming action. Subsequent acidification acts via the pore to ensure the loss of bacterial cytoplasmic pH control, elevating pH to a level optimal for neutral proteases, which are also activated by K^+ driven into the vacuole to compensate the charge across the membrane [70][71].

In a successful interaction, the neutrophil with its dead bacterial contents becomes apoptotic and is cleared by a macrophage [58]. Digestive enzymes in the macrophage inactivate neutrophil contents, and most importantly, their lytic enzymes. In processes that are still poorly understood, the immune system is able to select the degree of digestion such that either the antigens are partially preserved and presented to the adaptive immune system (e.g., via dendritic cells), or all contents are maximally digested via the necrotic pathway with minimal antigen preservation [72].

5.2. Scenario 2: Acute (Early Onset) Infection

Pathogens may defeat neutrophils at various stages, either by permeabilizing membranes to prevent the formation of gradients or pH change, or by interrupting granule recruitment or maturation [28]. Mechanisms of evasion are many and have been widely studied in models involving *Mycobacterium tuberculosis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. In this scenario, opportunistic commensals or pathogens are metabolically active in the planktonic form and multiply as saprophytes initially in wound debris before actively invading the wound bed. If perfusion to the wound bed is inadequate, neutrophil recruitment at the site will be limited. When these neutrophils encounter microorganisms and engulf them, the microorganisms are not efficiently killed, as they disable the phagosome and continue multiplication, drawing nutrients from the neutrophil. As microbial numbers increase, the phagosome is breached and the neutrophil lysed, releasing both microbial and neutrophil cell contents into the wound milieu [73].

5.3. Scenario 3: Chronic (Prolonged, Local) Infection

The major determinant of the onset and outcome of microbial infection is the ability of the infecting organisms to overcome host innate defenses. This is dictated by the number of organisms, their virulence expression, their protection in biofilm communities or their ability to disable/evade immune response [1][74][75][76][77]. Hypoxia, devitalized tissue, biofilm, microbial toxins, viral co-infection, cancer, cancer treatments, obesity, diabetes, or foreign matter can weaken local innate immune cells and hamper the killing of phagocytosed organisms. Similarly, impairment of immune response through inadequate blood supply, or immune suppression reduces the ratio of neutrophils and other immune cells to pathogens and thus the probability of clearance [78]. When pathogens gain advantage either through abundance, pathogenicity or host weakness, immune cells become ineffective. This is particularly true where biofilm dominates within a wound environment, provoking a hyper-inflammatory state where neutrophil toxins and enzymes are unable to inactivate bacteria within the biofilm, and instead destroy host tissue and provide additional nutrition for mature biofilm [22]

The presence of biofilm poses a very different challenge to immune cells [6]. The main aspect of this is the size of the microbial community and its essential insolubility due to its matrix of extracellular polymeric substances (EPS). Biofilm communities may be many times larger than immune cells which means that there is no way for neutrophils to engulf the biofilm-protected microorganisms. Phagocytosis or engulfment works well for planktonic or isolated bacteria (i.e., once released from biofilm) that are typically one-tenth or less the diameter of the immune cell. Where the target is approximately the size of a mammalian cell, adhesion and cell–cell pore formation is used to kill the cell, followed by injection of digestive enzymes such as granzyme (e.g., natural killer cells and t-cells use this mechanism with tumor cells). In contrast, biofilm communities are larger and resemble a macro-parasite, yet the response of the immune system is similar in terms of physical cell disposition. Namely, attraction to the surface and the release of granules with lytic enzymes at the surface [79].

This is apparent in the neutrophil NETosis response. Neutrophil extracellular traps are structures that become apparent where the well-known engulfment processes do not function [79][80][81]. These appear to be a coordinated set of processes, resulting in the neutrophil lysing in such a way that its DNA strands form a large network that distributes the lytic granules over a wider area [82]. Local microorganisms can be caught in these strands and the granules brought into contact with the microbial surface. NET formation is an aspect of hyper-inflammation (also referred to as “frustrated phagocytosis”) and represents a form of last resort response to an evasive pathogen [83].

In the context of biofilm, the NETosis response likely reflects the fact that the biofilm structure is too large to be engulfed yet persists in emitting stimulatory signals leading to both neutrophil attraction and the NETosis response [84]. This is perpetuated, in that the biofilm structures and organisms within are tolerant to the enzymes released by neutrophils, thus successive waves of neutrophils are lost in this way. Furthermore, the DNA released by neutrophils is often incorporated into the biofilm and is not degraded by the DNAse that neutrophils also release. Thus, the NETosis response is often ineffective and may also help build biofilm via the incorporation of the resulting debris into biofilm EPS [82][85].

Both the biofilm response and the lytic response to planktonic or isolated pathogens are associated with the release of neutrophil contents [86]. With increasing infection, more neutrophils are attracted, thus more are lysed or NETosed. Thus, there is a positive correlation between infection progression and the number of lysed neutrophils [87].

Wound progression towards macroscopically detectable infection is characterized by initial phases in which microorganism numbers are low. Either the outgrowth of opportunists, or the presence of organisms with virulence factors, initiates tissue injury and neutrophil activation. Should these immune cells fail to contain this initial insult, cell lysis begins along with tissue injury and stimulus. This subsequently attracts more immune cells, and an “incipient infection” condition is present. Ultimately, if the immune response fails, tissue injury, excess dead immune cells and microorganisms combine to form pus and other exudates, which are macroscopically recognizable as an infection (where current practice leads to intervention) [28].

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