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Drug Delivery and Tissue Engineering to Promote Wound Healing in the Immunocompromised Host: Current Challenges and Future Directions

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Abstract

As regenerative medicine matures as a field, more promising technologies are being translated from the benchtop to the clinic. However, many of these strategies are designed with otherwise healthy hosts in mind and validated in animal models without other co-morbidities. In reality, many of the patient populations benefiting from drug delivery and tissue engineering-based devices to enhance wound healing also have significant underlying immunodeficiency. Specifically, patients suffering from diabetes, malignancy, human immunodeficiency virus, post organ transplantation, and other compromised states have significant pleiotropic immune defects that affect wound healing. In this work, we review the role of different immune cells in the regenerative process, highlight the effect of several common immunocompromised states on wound healing, and discuss different drug delivery strategies for overcoming immunodeficiencies.

Graphical Abstract

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Keywords

Tissue engineering; regenerative medicine; immunity; immunosuppression; immunodeficient; infection

1.0 Introduction and Motivation

The immune system plays a key role in both resisting infection as well as wound healing and tissue regeneration. Inevitably, nearly all drug delivery vehicles and scaffolds for tissue engineering purposes will elicit a response from the host immune system *in vivo*. While this response may range from a general foreign body reaction to an antigen-specific cell activation, the interactions between immune system and biomaterials can significantly alter the success of any implanted therapeutic device. Activation of specific immune components can either favorably or adversely affect tissue regeneration [1]. In addition to interacting with the host immune system, drug delivery vehicles and other foreign materials used in tissue engineering may also serve as a nidus for infection even in an otherwise healthy host [2–4]. These considerations (immune response and susceptibility to infection) become even more complicated in immunodeficient populations.

In many cases, the populations that will most benefit from drug delivery and tissue engineering-based strategies for wound healing have immunodeficiencies. For example, diabetic foot ulcers are estimated to represent an annual cost of \$9.1–13.2 billion dollars in the USA [5] and have long been a target for regenerative medicine strategies [6]. However, diabetic-related immunodeficiencies such as decreased macrophage activation, inhibited vascularization and ability for immune cells to access the ulcer, and other complex host factors represent long-standing challenges in diabetic wound healing [7, 8]. Similar immunodeficiency-related challenges are present in strategies to regenerate tissues in populations with large wounds resulting from malignancy and tumor resection, trauma and/or burns, infection, and other clinical scenarios due to either 1) local immunosuppression from disruption of local vascularization or 2) systemic immunosuppression from disease-related factors [9–12]. Furthermore, tissues and organs generated in *ex vivo* bioreactor systems from allogeneic cell sources will require chemically-induced immunosuppression after implantation to prevent rejection by their host, although it is noted that allogeneic mesenchymal stem cells may have immunomodulatory abilities that protect against adverse immune responses [13]. Finally, due to advances in both disease-specific treatment and supportive care, immunocompromised populations such as organ transplant recipients and patients with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) have increased life expectancy and will benefit from wound repair strategies for similar reasons as the immunocompetent population. Ultimately, it is important to understand the role of the immune system in tissue regeneration due to the number of clinical scenarios in which patients with tissue deficiencies will also have co-morbid immune deficiencies.

Given the role of the immune system in wound healing and the specific challenges associated in tissue regeneration in the immunocompromised host, this area is of active

interest to researchers working in the fields of drug delivery and regenerative medicine. In this work, we will discuss 1) the role of various components of the immune system in wound healing; 2) the effect of immunodeficiency in areas relevant to drug delivery/regenerative medicine; and 3) current advancements in harnessing drug delivery strategies to improve outcomes in immunocompromised populations.

2.0 Immune Cells and Wound Healing

There are a variety of important mediators of wound healing and inflammation within the innate and adaptive arms of the immune system. The innate immune system is a nonspecific defense response and includes neutrophils, macrophages, dendritic cells, complement proteins, and natural killer cells. The adaptive immune system is an antigen-specific response and includes B and T lymphocytes. Classically the immune system is studied for its role in protection against infection, involvement in inflammatory and autoimmune disease, and regulatory effects inhibiting the development of cancer. However, the cellular components of the immune system are also instrumental in wound healing. Following tissue damage, the immune system elicits a coordinated and organized response. Wound healing can be divided into four phases: hemostasis (clotting of blood); inflammation (sterilization and debridement); proliferation (expansion of host cells); and tissue remodeling [14]. These phases roughly correlate with the timeline of immune cell entry to the wound bed. Following initial injury and hemostasis, neutrophils enter a wound site after the first 24 hours, followed by macrophages in 48–96 hours, and lymphocytes after 120 hours [1]. Each cell type has a specific role in the promotion of wound healing (Figure 1).

2.1 Neutrophils

Produced in the bone marrow as progenitors of myeloid lineage, neutrophils are one of the initial components of the immune system to reach a wound. In a non-specific fashion, neutrophils eliminate invading pathogens, secrete reactive oxygen species, and clear cellular debris from the wound bed. While these processes are necessary to initiate healing, an exuberant neutrophilic inflammatory response can destroy healthy tissue and impede regeneration [15, 16]. In fact, in certain models, it has been shown that depletion of neutrophils in sterile wounds results in more rapid healing, such as a murine model in which an 85% reduction of neutrophil concentration resulted in significantly greater cutaneous wound re-epithelialization [17, 18]. It should be noted that neutrophil concentration alone may be insufficient to predict effect on healing response. Host conditions such as diabetes can prime neutrophils to become more inflammatory and toxic to wound healing; for example, at baseline, 0.2% of neutrophils in normal hosts produce extracellular traps (cytotoxic proteins) while 2% produce extracellular traps in diabetic hosts [15]. Stimulation by inflammatory cytokines further amplified the difference in expression of cytotoxic secretion by neutrophils in diabetic compare to immunocompetent hosts. Overall, neutrophils are not strictly necessary for wound healing and may even be detrimental depending on recruited concentration and phenotype due to excessive inflammatory response. Despite the possible detriment of neutrophils, it should be noted that most non-surgical wounds are highly susceptible to infection (either from the environment or from the host's microbiome) and infection is highly detrimental to tissue regeneration [19]. From an

evolutionary standpoint, the protective role of neutrophils must outweigh their detrimental effect on healing.

Nevertheless, the presence of neutrophils also activates specific components of the wound healing responses. For example, neutrophil attachment to local endothelial and epithelial populations results in upregulation of *Akt* and β -*catenin* signaling [20] and potent modulators [21]. Knockout of *C-X-C Chemokine Receptor Type 2 (CXCR2)*, a mediator of neutrophil recruitment to the wound bed, inhibits neutrophil entry and resulted in significantly delayed wound healing in a murine model [22]. Disease processes, such as old age and diabetes, in which neutrophil phagocytic ability is compromised has also been shown to delay wound healing [23]. Ultimately, while not necessary for tissue regeneration, neutrophils are important in the sterilization and debridement of the wound and may play a role in priming sites for healing. Recent work has highlighted the complexity of the neutrophil response in reaction to biomaterials and suggested neutrophils play a more nuanced part in host/material interactions [24].

2.2 Monocytes and Macrophages

Monocytes are progenitors of the myeloid lineage produced in the bone marrow and part of the innate immune system and differentiate into macrophages or dendritic cells based on environmental cues. Monocytes will circulate within blood vessels, migrate to a wound site, and then differentiate into specific macrophage populations [25]. The macrophage/monocyte axis is arguably the most important effector immune response following tissue injury in order to elicit and promote wound healing. Both monocytes and macrophages play major roles as major producers of cytokines and growth factors during the inflammatory and early proliferative phases of wound healing [26]. These secreted signaling molecules include factors important for angiogenesis and matrix deposition such as vascular endothelial growth factor (VEGF), insulin-like growth factor-1, transforming growth factor- β , and interleukin-10 [26–28]. In addition, macrophages prevent overgrowth of tissues once repair is complete [29]. However, not all macrophages are the same; macrophage polarization refers to the alteration of macrophage phenotype. M1 macrophages are proinflammatory in nature and phagocytose neutrophils and secrete inflammatory cytokines, whereas M2 macrophages are regarded as regenerative and secrete growth factors and migratory cytokines to promote healing [30]. Uncontrolled proinflammatory M1 polarization leads to chronic wounds rather than successful healing in both human patients and murine models of disease [31]. M1 macrophages inhibit wound healing through mechanisms such as direct tissue damage via secretion of radicals as well as induction of local fibroblasts to destroy rather than produce extracellular matrix [32]. M2 macrophages, on the other hand, play major regulatory roles in tissue remodeling and resolving inflammation [33]. M2 macrophages secrete paracrine factors that upregulate fibroblast proliferation and stimulate matrix production [32]. In addition, M2 macrophages also induce angiogenesis through secretion of growth factors [34]. Macrophages are plastic in nature and polarization has been demonstrated to be reversible under different *in vitro* and *in vivo* conditions [35]. The different factors that drive macrophage polarization during wound healing under different host conditions is an active and complex area of current research.

Unlike neutrophils, macrophages are necessary for tissue healing. Macrophage-specific β -catenin was shown to be necessary to complete wound healing in a murine knockout model [36]. By creating murine knockout models, or animal models where certain monocyte/macrophage-related pathways are selectively inhibited or removed, the importance of these cell lines and their products to wound healing has been demonstrated (Table 1). Specific ablation of macrophages, while leaving monocyte and neutrophil populations intact, also results in impaired wound healing [37].

However, in an unfavorable environment, monocytes and macrophages may be unable to mount a sufficient regenerative response. For example, even extremely low levels of bacterial endotoxin (5 ng/kg body weight injected once every three days for ten days) inhibit the wound healing response of monocytes in a murine cutaneous defect [38]. As arguably the most important immune cell type in tissue repair, there is great interest in developing drug delivery techniques to create favorable environments and locally recruit specific macrophage populations to wound sites to enhance tissue healing in the immunoregenerative biomaterial strategy [39]. Delivery of specific cytokines or use of specific biomaterials can preferentially promote M2 over M1 macrophage phenotype and stimulate wound healing.

2.3 Lymphocytes

Including T and B lymphocytes (adaptive immune cells) and natural killer (NK) cells (innate immune cells), the lymphocytes also have important roles in response to injury. Regulatory T lymphocytes are the predominant lymphocyte present in skin and become activated upon injury [45]. Ablation of regulatory T lymphocytes results in delayed wound healing due to inefficient transition from inflammatory to proliferative phases, suggesting lymphocytes are important modulators of the inflammatory reaction to tissue injury [46]. In addition, T lymphocytes specifically are important regulators of angiogenesis and collagen deposition. Through activation of C-X-C Motif Chemokine Receptor 3 by local secretion of C-X-C Motif Chemokine Ligands 9, 10, and 11 at the wound site, T lymphocytes are involved in the maturation of the angiogenic response and “prune” immature vessels [47]. When challenged with specific antigens, T lymphocytes secrete factors which induce dermal fibroblasts to proliferate and produce collagen [48]. The roles of NK cells and B lymphocytes in wound healing are less clear; both cell types are involved in mitigation of infectious processes.

However, as is the case with neutrophils, it does not appear that lymphocytes are necessary for successful wound healing. In murine models of CD4 deficiency and CD8 deficiency, neutrophil recruitment was altered and cytokine expression was changed but ultimately wound healing was not impaired [49].

2.4 Immune Cells and Angiogenesis

Angiogenesis is key to the regeneration of tissues. As discussed above, immune cells play various roles in initiating and regulating the angiogenic response. Hypoxia, or lack of oxygen within a tissue, recruits immune cells to a damaged site [50]. In addition to sterilizing the wound bed, macrophages drive the initial sprouting of local endothelial cells [51]. As the wound evolves and angiogenesis is initiated, there is a large amount of molecular cross-talk between vascular endothelium and the immune system. For example,

Wnt signaling produced by the endothelium is recognized by Frizzled receptors on T lymphocytes, neutrophils, and macrophages and promotes upregulation of matrix metalloproteases from these immune cells [50], allowing for immune cells to more quickly extravagate to the wound site as well as begin the remodeling process by degrading damaged tissue. As the healing response evolves, T cells are actively engaged to reduce/redirect immature and superfluous vessels as the tissue matures [47]. In wound beds where immune cells exhibit pro-inflammatory phenotypes (such as inflammatory neutrophils and M1 polarized macrophages), angiogenesis has been shown to be inhibited [52]. The interplay between immunity and angiogenesis is an important factor in the wound healing response.

3.0 Diseases of Immunodeficiency and Wound Healing

Not all immunodeficient states are created equal. Even subtle differences in immune populations can drastically alter wound healing. For example, in a study of tissue recovery after limb ischemia, two different strains of mice were studied (inbred Balb/C nude mice versus outbred athymic nude mice) [53]. Despite the same genetic mutation and resulting immune deficit (absent thymus from an autosomal recessive mutation in *Foxn1^{nu}*), Balb/C mice had significantly impaired recovery compared to outbred mice ($50\pm 10\%$ to $75\pm 20\%$). The authors theorized that the outbred mice had greater monocyte activation (possibly from greater genetic heterogeneity), resulting in increased angiogenesis. Genetic background has been shown to significantly affect angiogenesis associated with wound healing among different mouse strains [54]. Therefore, understanding immunodeficiency and its impact on wound healing in the greater context of specific disease states is critical when designing strategies to augment regeneration in immunocompromised hosts.

In addition to complex host disease states, local tissue damage (such as from trauma or combat-related wounds) produces states of local immunosuppression due to compromised vasculature and necrotic tissue. These wound beds are particularly at risk for infection and penetration of therapeutics such as antibiotics is limited [55]. In composite wounds, where multiple tissue types are damaged (i.e. skin, fascia, muscle, nerve, and bone), healing becomes increasingly challenging and the role of the immune system in the repair of these complex wound beds is an area that warrants further research.

In this review, diabetes mellitus, HIV/AIDS, and malignancy will be discussed as examples of immunodeficient states with altered wound healing. However, many other host conditions are associated with immunodeficiency and pose additional specific challenges for regeneration, such as organ transplant recipients on immunosuppressants and patients with inborn genetic immunodeficiencies [56, 57].

3.1 Diabetes Mellitus

In chronic wounds associated with diabetes mellitus, the inflammatory phase is upregulated without progression to the proliferative phase [26]. Recruited neutrophils and macrophages (primarily M1 polarization) secrete more proinflammatory factors and cause additional damage to host tissues [15, 58]. While most likely a multi-factorial issue, diabetes results in the downregulation of Cysteine-rich protein 61 (CCN1), causing decreased neutrophil

efferocytosis (autophagy) and decreased M2 polarization of macrophages; delivery of CCL1 can restore normal wound healing in diabetic mice [59].

In addition to altering the phenotype of immune cells and promoting inflammation, diabetes mellitus is a chronic disease which also results in significant microvascular disease [60]. With decreased blood supply, there is limited access by peripheral immune cells as well as circulating stem cells that can stimulate a healing response. In addition to wound access, diabetes affects stem cells at their source; recent data have shown that diabetes induces epigenetic changes in bone marrow progenitor cells that is then passed down to wound macrophages and drives greater inflammatory interleukin-12 production, resulting in decreased wound healing [61]. Relevant to the field of tissue engineering, it has also been found in a murine model that autologous adipocyte-derived stem cells from diabetic hosts have decreased inherent wound healing capacity compared to allogeneic isolates from non-diabetic animals [62]. In addition to altering immune cell phenotype, inhibiting immune system access to the wound bed, and negatively impacting the signaling between immune cells and stem cells, diabetes also mitigates healing due to the susceptibility of wound to infection. Given the changes in immune phenotype and necrotic tissue from vascular disruption, diabetic wounds are often chronically infected which results in further tissue damage [63]. For these reasons, 1) restoring normal immune phenotype, 2) providing access of immune cells to the wound site through vascularization, 3) artificially delivering growth factors and cytokines normally produced by healthy macrophages and other local tissues to the wound, and 4) releasing antibiotics locally to treat and prevent infection are strategies being pursued within drug delivery for the treatment of diabetic wounds.

3.2 HIV/AIDS

As of 2013, there were estimated to be 1.7–2.1 million new cases of HIV globally per year [64]. With advances in highly active antiretroviral therapy (HAART), patients with HIV/AIDS now have extended life expectancy, suffer from similar medical issues as the general population, and may also benefit from drug delivery/tissue engineering strategies. HIV is a disease in which CD4 cells specifically are targeted and destroyed. As noted previously, T lymphocytes may augment wound healing (such as through enhancement of angiogenesis) but are not necessary. There is controversy regarding the effects of HIV on wound healing. In clinical studies, it does not appear HIV positivity affects healing time after male circumcision, in the healing of tibial fracture, or in the osteointegration of dental implants [65–68]. However, in all of these cases, HIV may increase the likelihood of infection and subsequent inhibition of healing compared to the general population. It is possible that current HAART therapy, the negative effects of HIV infection on wound healing may be masked [67]. In older clinical work published before the widespread use of HAART, it was found that patients with HIV had significantly worsened wound healing after anorectal surgery (attributed to loss of inflammation-modulating CD4 cells) [69].

Therefore, in designing drug delivery strategies to aid in wound healing in populations with HIV/AIDS, it is important to consider the patient's current CD4 levels. Pre-treatment with HAART and adequate antibiotic prophylaxis may be critical in the success of regenerative medicine therapies in the setting of HIV/AIDS.

3.3 Malignancy

Patients suffering from malignancy often have complex tissue defects from either local invasion and destruction by the tumor or due to resection in order to surgically remove the tumor. Leveraging tissue engineering and drug delivery to heal wounds in cancer patients has long been a goal of regenerative medicine [70, 71]. However, cancer and its treatment (chemotherapy and radiation therapy) can be inhibitory to wound healing. For example, cutaneous wounds in a rat model of hepatoma had decreased mechanical integrity compared to healthy animals after being given time to heal, suggesting impaired tissue remodeling [72]. The degree of decreased mechanical integrity correlated with the volume of tumor burden, despite the tumor being ectopic to the injury site. In addition to malnutrition, another possible mechanism contributing to inhibition of wound healing in the setting of malignancy is disruption of the bone marrow. Given that myeloid-derived cells are produced in bone marrow, in addition to other stem cell populations that may be relevant in wound healing [73], tumors that metastasize to the marrow or otherwise exhaust the bone marrow's ability to replenish itself may affect wound healing.

Likewise, the treatments for cancer may be toxic to regenerative processes. Common chemotherapeutics that affect rapidly dividing cells, such as doxorubicin, have been shown to inhibit wound healing in cancer patients [72]. However, even as cancer treatments become molecule-specific, there is still a possibility for a profound effect on wound healing depending on the specific mechanism in question. There are many shared genes that are upregulated in both malignancy and wound healing that may be attractive targets for anti-cancer strategies [74]. For example, the chemotherapeutic bevacuzimab (anti-VEGF monoclonal antibody) increased post-surgical wound healing complication rates in patients with colorectal cancer from 3 to 13% (not statistically significant) [75], as well as has resulted in tissue fistulas and necrosis in the treatment of head and neck cancer [76]. While an attractive target against tumor growth, VEGF is a critical angiogenic growth factor secreted by macrophages and other cells during tissue remodeling. Radiation therapy also locally disrupts wound healing. Through mechanisms that are not entirely understood, radiation causes overexpression of proinflammatory and matrix-producing cytokines by immune cells, resulting in uncontrolled fibrosis [77]. There is also downregulation of matrix metalloproteinases, molecules which help remodel the extracellular matrix. Given the extensive local damage to keratinocytes and fibroblasts, drug delivery strategies may require recruitment of other cell populations to regenerate healthy tissue at the site of radiation. Lastly, check point inhibitors are a new class of therapeutics for cancer treatment. They have been known to induce autoimmune adverse reactions and affect the immune response; however, their effect on wound healing is thus far unexplored and will need to be assessed as clinical trials continue [78]. The effect of autoimmune disease and therapies to treat autoimmune disease draw similar parallels regarding wound healing. Autoimmune diseases, such as systemic lupus erythematosus, rheumatic arthritis, and inflammatory bowel disease result in dysregulation of the immune response which can alter wound healing and treatments for autoimmune disease result in significant immunosuppression that has been demonstrated to negatively affect wound healing [79–81].

4.0 Drug Delivery Strategies in Immunocompromised Hosts

In immunodeficient hosts, drug delivery strategies can be utilized to recruit immune cells, alter cell phenotype, or replace certain functions of deficient immune cells in order to augment wound healing (Fig. 2). In a broad sense, there are few studies designed to investigate the role of the immune system in drug delivery. In one murine experiment, nanoparticle-mediated gene delivery was studied in immunocompetent versus athymic mice. While initial delivery was decreased in immunodeficient mice, the two groups were similar after 24 hours, suggesting that *in vivo* drug delivery kinetics may be unchanged in some immunocompromised models [82]. In diseases of immunodeficiency with significant vascular compromise, such as diabetes, one may expect that drug delivery strategies relying on systemic circulation may be inhibited; therefore, delivery systems featuring local release (such as those included as part of implanted tissue scaffolds) may be of greater benefit in those populations. In general, strategies can be divided as recruitment-based, phenotype-based, or replacement-based.

4.1 Recruitment of Immune Populations

In non-healing wounds in certain immunodeficient populations, cell types such as macrophages and neutrophils are low in number and/or are not being recruited in great enough quantity to drive the wound healing response. Therefore, there have been efforts in leveraging biomaterials to locally deliver chemokines to 1) boost the proliferation of existing immune cells and 2) recruit immune cells locally to the wound site. As described in Figure 1, the wound healing response is divided into phases featuring neutrophil, macrophage, and lymphocyte activity. As a neutrophil/macrophage chemoattractant, the local application of GM-CSF directly to wounds has been shown to promote healing in clinical studies involving chronic non-healing wounds in patients with immunodeficiencies such as diabetes, vascular compromise, and cancer [83]. However, the *in vivo* half-life of GM-CSF is only approximately 1.5–7 hours [84, 85]. Neutrophils and macrophages are heavily involved in the healing response for up to 120 hours (Figure 1). Therefore, there have been efforts for both wound healing as well as augmentation of vaccine efficacy to harness biomaterials as local delivery agents for GM-CSF. Poly(lactide-co-glycolide)(PLGA)/poly(lactide)-based microparticles, for example, were demonstrated to be capable of releasing biologically-active GM-CSF in a murine model at concentrations > 10 ng/mL for at least nine days and these microparticles resulted in local recruitment of neutrophils and macrophages at the site of injection [84]. Chitosan is another biomaterial that has been shown to sustain delivery of GM-CSF within murine models [86]; in addition to its ability to enhance GM-CSF local delivery, chitosan has the added benefit of inherent antimicrobial properties [87, 88]. Further work has been done to create chitosan-based microparticles for further control over delivery kinetics and deliver GM-CSF plasmid DNA [89]. Other explored vehicles include liposomes [90], adenovirus-mediated gene transfer [91], and poly(γ -glutamic acid)-based hydrogels [92].

Biomaterial-based local delivery of GM-CSF is most likely the closest technology to full clinical translation and regulatory approval given that GM-CSF has already been approved by the United States Food and Drug Administration for systemic use following induction of

chemotherapy to shorten time for neutrophil recovery (among other systemic use approvals). However, other experimental agents that attract immune populations to wounds are also being explored. These newer molecules are of interest as they may not only recruit macrophages to wounds but may selectively recruit M2 (pro-regenerative) rather than M1 (pro-inflammatory) macrophages. For example, the controlled local delivery of a macrophage-recruiting chemokine (SEW2871, a sphingosine-1-phosphate agonist) within a gelatin hydrogel selectively recruited M2 macrophages and resulted in increased wound healing in a murine model [93]. The same molecule was also delivered using lactic acid-grafted gelatin micelles within gelatin hydrogels and resulted in greater macrophage presence and improved healing in critically-sized rat ulnar defect [94]. Local delivery of another sphingosine-1-phosphate agonist, FTY720, from a nanofiber scaffold composed of poly(lactide-co-glycolide) and poly(ϵ -caprolactone) promoted increased recruitment of M2 macrophages, vascularization, and wound healing in a murine mandibular defect model [95]. Temporally, if normal neutrophil and/or macrophage activity can be restored to the wound in an immunodeficient host, these cells may be able to initiate angiogenesis [34], tissue remodeling [33], and extracellular deposition [32] and overcome the inhibition of these activities normally seen in immunodeficient hosts.

Of note, wound healing in immunocompromised patients may respond to differently recruitment factor strategies compared to their immunocompetent hosts. For example, IL-6 is a pleotropic macrophage- and keratinocyte-secreted cytokine that aids in immune response such as neutrophil adhesion and has been shown to be important in the wound healing response in mouse models [40]. In steroid-induced immunodeficient disease models as well as IL-6 knockout models, injection of IL-6 (or IL-6 plasmid) promoted increased wound healing [40, 96]. However, these injections delayed wound healing in normal immunocompetent mice [96]. As IL-6 is present in wounds already in healthy animals, the authors of the study speculated that the additional delivered IL-6 may have caused upregulated matrix metalloproteinases that interfered with collagen deposition. These findings highlight the importance of considering host immune status when developing strategies to promote wound healing; the same delivery factors that stimulate regeneration in one host may not be appropriate for another.

4.2. Phenotype Modification

While biomaterials may be conducive as tissue scaffolds to aid in the regenerative response, they also function as foreign bodies that can elicit a pro-inflammatory phenotype from migrating immune cells. In addition, certain immunodeficient host states such as diabetes and malignancy can further predispose recruited macrophages to M1 rather than M2 polarization and impede the wound healing response [33]. As M2 macrophages are critical in the secretion of cytokines for angiogenesis and regulation of inflammation, there has been a great effort in understanding factors influencing macrophage polarization and developing drug delivery systems and biomaterials-based approaches to promote M2 macrophage recruitment.

Interleukin-4 (IL-4) is one such cytokine that is capable of driving macrophages to an M2 phenotype even in the presence of biomaterials that normally elicit M1 polarization, such as

poly(methylmethacrylate) [97]. When injected directly into an implant-associated calvarial model of inflammation, IL-4 resulted in decreased inflammation and osteolysis [98]. For controlled delivery, investigators have studied the use of silk films to as biomaterials to deliver IL-4 locally and promote M2 polarization [99]. In addition to spatially-controlled IL-4 delivery, temporally-controlled IL-4 delivery with respect to macrophage polarization may be important in wound healing. There is some evidence that angiogenesis may be best stimulated by an early M1 response followed by an M2 response. Therefore, there have been efforts to develop scaffold delivery systems with early release of M1-inducing factors (such as interferon gamma) followed by sustained release of M2-inducing factors (such as IL-4). This was achieved by adsorbing interferon gamma onto a decellularized bone-based scaffold for rapid release while simultaneously binding IL-4 via biotin and streptavidin for delayed release [100]. Ultimately, *in vivo* results in a murine subcutaneous implant model did not show differences in M1/M2 polarization; the authors suggested that overlap in the release of both factors may have interfered with affecting macrophage phenotype.

In addition to temporal control, combination approaches of recruitment factors and polarization factors have been explored. For example, MCP-1 and IL-4 were delivered from a multidomain peptide hydrogel scaffold in mouse model to locally recruit macrophages (via MCP-1) and then drive the M2 response (via IL-4) [101]. As a better understanding of the cytokine environment in wound healing is understood, more complex approaches such as these can be designed and implemented. In addition to IL-4, other soluble factors are also being explored for polarization control. Local injections of lipoxin A4 and resolvin D1 caused increased M2 and reduced M1 macrophages within a chitosan scaffold in a mouse model, for example [102]. In contrast to delivering specific cytokines, biomaterials can also be leveraged to capture or scavenge inflammatory cytokines that may be locally detrimental to healing in immunocompromised populations. In a murine diabetic wound model, a glycosaminoglycan-based hydrogel was capable of scavenging inflammatory cytokines and improving wound healing [103].

However, drug delivery is not the only way biomaterials can be leveraged to influence macrophage polarization. The material properties of scaffolds themselves can be designed to drive macrophage phenotype. Pore size [104], fiber diameter [105], surface microstructures [106], and mechanical loading [107] are all physical cues whose modulation has demonstrated to influence polarization. While the focus of tissue engineering has classically been on modulating material properties to optimize migration/differentiation of native cells of the desired tissue type (ie create materials conducive to osteoblasts for bone tissue engineering), maximizing macrophage polarization by scaffold design may be equally important to elicit a regenerative environment within an implant. This may be even more critical in the immunocompromised host, where immune cells that would typically aid in the healing response are further depleted or inhibited.

4.3 Replacement of Immune Cell Functions

Lastly, many efforts within drug delivery and tissue engineering to augment healing in immunocompromised hosts involve replacing the factors produced in wound healing under normal conditions. Replacement of growth factors that are normally secreted or induced by

macrophages in large wounds has been shown to augment healing with local application in clinical studies [108, 109]. For example, initiation and regulation of angiogenesis is a major role of the immune system, a process inhibited in diabetic hosts [27, 47, 110]. However, local delivery of angiogenic growth factors such as basic fibroblast growth factor and VEGF have been shown to enhance wound repair in diabetic animal models, as demonstrated using delivery systems such as chitosan scaffolds [111], fibrin scaffolds (with or without PLGA microparticles) [112], and poly(ethylene glycol)/poly(lactide) electrospun fibers [113]. As macrophages are responsible for inducing endothelial sprouting through growth factor secretion, replacing these growth factors early in the wound healing process may be critical in preventing chronic wound development [32]. In addition to angiogenesis, immune cells also produce growth factors regulating tissue deposition and recruitment of matrix-producing cells, such as epidermal growth factor [114]. In large burn wounds in humans and animal models, considered immunodeficient wound environment [115], local delivery of epidermal growth factor has been shown to enhance wound healing [108, 116]. Drug delivery vehicles such as chitosan gels [117, 118], liposomes within a chitosan matrix [119], fibrin scaffolds with xenogeneic human keratinocytes [120], and hyaluronan [121] have all shown efficacy in improving regeneration in animal models of burn wounds. Other growth factors associated with macrophages have shown similar efficacy in the treatment of large wounds; replacing/augmenting macrophage secretion with local delivery-based strategies have shown benefit using keratinocyte growth factor, platelet derived growth factor, transforming growth factor-beta, and other cytokines in immunocompetent and immunocompromised animal models [122–125].

However, lack of appropriate growth factor secretion is not the only challenged presented wounds in immunodeficient populations. These sites are also inherent to infection which further creates tissue damage and inhibits the healing response. Sterilization of the wound bed is in fact part of the first phase of the wound healing process (Figure 1). To replace the infection-resistant function of the immune system, local delivery of antibiotics to treat or prevent infection has been a longstanding goal within the field with some success in translation of biomaterials-based products to market [126, 127]. Polymer-based delivery of antibiotics as well as the synthesis of inherently antimicrobial polymers and antifouling materials are areas that have been reviewed recently [87, 128, 129]. Emulsions and oils are other vehicles being explored that have inherent antimicrobial activity [130, 131]. In addition to delivering antibiotics via polymer-based delivery vehicles, a recent innovative approach involved loading neutrophil-like cells themselves with antifungal agents *ex vivo*, injection of the cells into an immunocompromised murine model featuring infection, and leveraging the cells as delivery vehicles [132]. In strategies featuring local antibiotic delivery, some caution should be exercised; there is evidence that certain antibiotics have pleotropic effects, including inhibition of neutrophil-generated matrix metalloproteinases necessary for tissue remodeling, that can inhibit the regenerative response [133, 134] although this magnitude of this effect is still under study [135]. Another approach to replace the antimicrobial activity of the immune system in deficient patients has been delivery of antibodies or plasmids for the expression of antibodies to artificially replace B-lymphocyte immunity. For example, local application of polyclonal immunoglobulin to infected burn wounds in a mouse model significantly increased wound healing [136]. If a specific

pathogen is recognized as a threat to wound recovery in a population, there are also strategies to deliver monoclonal antibodies. For example, local delivery of an adeno-associated virus vector was used to deliver anti-influenza antibody and prevented immunocompromised mice from dying of influenza [137]. A similar approach to deliver monoclonal antibodies was shown to prevent respiratory syncytial virus infection [138]. By replacing artificially replacing the immune system's role in promoting angiogenesis, secreting regenerative growth factors, and preventing infection of the wound bed, drug delivery approaches can facilitate healing in the immunocompromised host.

4.4 Considerations for the Immunocompromised Host

As discussed in this work, patient populations featuring immunodeficiencies present unique challenges when designing regenerative medicine strategies. Specific factors relating to the host and drug delivery vehicle that must be taken under consideration are summarized in Table 2.

Regarding the host, the specific immunodeficiency is critical. For example, as diabetics have deficiencies in converting macrophages from M1 to M2, strategies for the release of factors such as IL-4 may be beneficial in treating a chronic ulcer. On the other hand, in a patient with immunodeficiencies due to chemotherapy induction, the challenge to wound healing is not necessarily macrophage polarization but bone marrow suppression and lack of numbers of neutrophils and macrophages. In this example patient, delivery strategies to extend the half-life of GM-CSF as well as to provide local antibiotic wound coverage may be desirable. Another key host factor is the vascularization to the wound site and the host's angiogenic potential. Diabetes, burns, implantation of foreign bodies, and other immunodeficiencies can compromise wound vascularization and deficiencies in macrophages and lymphocytes can inhibit angiogenic potential. In addition, the wounds of immunocompromised patients are at increased risk for fungal infections, which can further inhibit angiogenesis [139, 140]. In addition to inhibiting angiogenesis, pathogens can further disrupt healing by directly damaging surrounding tissue and increasing inflammation within the wound bed. Immunocompromised patients are highly susceptible to infection and often benefit from antibiotic prophylaxis; due to decreased angiogenic potential, these same patients may benefit from local antibiotic delivery strategies due to disruption of systemic vascularization to wound sites. Lastly, many patient populations with immunodeficiencies may suffer from other co-morbidities such as decreased renal or hepatic clearance that may affect drug delivery approaches [141, 142].

In the design of the drug delivery vehicle, there are specific factors that may require special attention in the immunocompromised host. As discussed earlier, as angiogenesis may be decreased due to macrophage and/or lymphocyte deficiencies, systemic approaches (intravenous injection, oral intake, etc) may not result in vehicle or therapeutic appropriately accessing the wound site. In addition, temporal factors such as the duration of release as well as the sequential release of different cytokines has been demonstrated to significantly affect the immune system's ability to augment wound healing [100]. In addition, the need for a tissue scaffold in conjunction with a drug delivery vehicle should be evaluated. In some critically-sized tissue defects, a scaffold may be required for healing to proceed; in others,

delivery of cytokines or growth factors may be sufficient. Finally, the immune response to the delivery device in question must also be considered. As common polymers leveraged in drug delivery have been shown to stimulate M1 polarization without further augmentation [97], these vehicles and/or scaffolds may require modification such as additional loading of M2-inducing cytokines or topographical changes that have been demonstrated to promote M2 polarization. In addition, the increased inflammatory conditions that may be present in the wound of immunodeficient patients may cause increased biodegradation rates of drug delivery vehicles fabricated from commonly used aliphatic polyesters [143, 144], requiring evaluation of release kinetics under different physiologic conditions. Lastly, in immunocompromised patients, wounds are particularly vulnerable to infections which further inhibits the healing response. A new and exciting area of research involves the microbiome (including bacteriome and mycobiome) and its effects on host inflammation and wound healing [145, 146]. Given that many wounds are adjacent to microbiome communities, such as the skin or oral mucosa, this field may hold relevance for treating wounds in immunocompromised populations. The development of drug delivery and tissue engineering-based approaches for the immunocompromised host requires special considerations to optimize success.

5.0 Conclusions

Given the importance of the immune system in initiating and regulating wound healing, patients with immunodeficiencies present unique challenges to the field of regenerative medicine. In this work, we have discussed the roles of specific cells within the immune system, highlighted changes in wound healing in several specific common immunodeficient states that may benefit from drug delivery approaches, and reviewed current strategies in the literature that may benefit immunocompromised populations.

While there has been significant progress in understanding the roles of different cells in the immune system in wound healing, the effect of different cytokines on the regenerative process, and how different immunodeficient states affect tissue repair, there remains much work to be done to understand how these challenges can be overcome using drug delivery strategies. The vast majority of *in vivo* studies are performed in immunocompetent animal models. As has been discussed in this work and demonstrated in the literature, even subtle immunodeficiencies can greatly affect healing. With advances in medicine permitting greater life expectancy in certain immunodeficient populations, such as those with HIV/AIDS, organ transplant recipients, survivors of malignancy, and diabetics, there is a great need to develop and validate strategies in animal models with similar co-morbidities. Biologists and immunologists have developed a plethora of animal models featuring specific immunodeficiencies through chemical or genetic manipulation; many of these models have been validated mechanistically and have well-understood deficiencies in specific immune cell lines. Collaborations between bioengineers, immunologists, materials scientists, veterinarian staff, and physicians may be fruitful in developing physiologically-relevant platforms to evaluate drug delivery strategies for common immunocompromised populations.

Fortunately, there has been a great deal of work done in the development of drug delivery vehicles for wound healing in the immunocompetent host. By understanding the unique challenges in their immunocompromised counterparts, these strategies can be more rapidly modified as required and validated in the appropriate models rather than starting from scratch. There is a real clinical need to enhance wound healing in patient populations with immunodeficiencies. The fields of drug delivery and tissue engineering stand to make a large impact in improving healthcare outcomes by developing strategies to improve wound healing in the immunocompromised host.

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List of Abbreviations

CCN1	cysteine-rich protein 61
CX3CR1	C-X3-C motif chemokine receptor 1
CXCR2	C-X-C motif chemokine receptor type 2
GM-CSF	granulocyte-macrophage colony-stimulating factor
HAART	highly active antiretroviral therapy
HIV/AIDS	human immunodeficiency virus/acquired immunodeficiency syndrome
IL-6	interleukin-6
MCP-1	monocyte chemotactic protein-1
MyD88	myeloid differentiation primary response gene 88
NK	natural killer
PLGA	poly(lactide-co-glycolide)
VEGF	vascular endothelial growth factor

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Figure 1.
Summary of Roles of Immune Cells in Wound Healing

Immune System Cell Populations

Figure 2.

Drug delivery and tissue engineering strategies can promote wound healing by recruitment, phenotype modification, or replacement of immune populations.

Table 1

Examples of Murine Macrophage-Related Knockout Models With Impaired Wound Healing

Factor	Type	Produced by	Effect	Reference
IL-6	Cytokine	Macrophages	Neutrophil adhesion	Gallucci et al. [40]
GM-CSF	Cytokine	Damaged keratinocytes	Neutrophil and macrophage recruitment	Fang et al. [41]
MCP-1	Cytokine	Macrophages	Macrophage phenotype	Low et al. [42]
CX3CR1	Receptor	Macrophages	Macrophage recruitment/phenotype	Ishida et al. [43]
CXCR2	Receptor	Damaged keratinocytes and endothelial cells	Neutrophil and monocyte recruitment	Devalaraja et al. [22]
MyD88	Cytoplasmic adapter	Macrophages	Macrophage phenotype	Macedo et al. [44]

IL-6 = interleukin-6; GM-CSF = granulocyte-macrophage colony-stimulating factor; MCP-1 = monocyte chemotactic protein-1; CX3CR1 = C-X3-C Motif Chemokine Receptor 1; CXCR2 = C-X-C Motif Chemokine Receptor Type 2; MyD88 = myeloid differentiation primary response gene 88

Table 2

Examples of Considerations When Designing Drug Delivery Strategies for Immunocompromised Hosts

Host Factors		Drug Delivery Factors	
Factor	Examples	Factor	Examples
Specific immunodeficiency	-Deficiencies in neutrophils, macrophages, B cells, or T cells -Complex pleiotropic host disease states (diabetes, systemic lupus erythematosus, etc.)	Purpose/goal of delivered therapeutic	-Recruitment of immune cells -Stimulation of specific immune phenotype -Replacement of immune function (antibiotic, antibody angiogenic factor, etc.)
Evolution of host immunodeficiency	-Acute immune insult followed by gradual resolution (burn, trauma, etc.) -Chronic immunodeficiency (diabetes, HIV/AIDs, etc.) -Waxing/waning immunodeficiency (need for intermittent chemotherapy, autoimmune disease, etc.)	Spatial delivery requirements	-Systemic versus local delivery -Concentration gradient -Release from the center of the wound to the periphery versus from the periphery to the center
Angiogenic potential	-Vascularization of the wound site -Ability of the host to initiate angiogenesis	Temporal delivery requirements	-Duration of therapy -Release kinetics (Continuous release, burst release, pulse release, etc.)
Presence of pathogens	-“Clean” versus “dirty” wound (for example, tissue loss from resection of a tumor versus tissue loss from a motor vehicle accident) -Proximity to microbiome (skin, oral cavity, gastrointestinal system, genitourinary system, etc.)	Requirement for tissue scaffold and scaffold considerations	-Large versus small defects -Type of scaffold (synthetic polymer, naturally-derived material, composite, etc.) -Scaffold delivery (injection, self-assembly, surgical insertion, etc.)
Other co-morbidities	-Nutritional status -Genetic conditions -Socioeconomic and psychologic burdens of disease, access to healthcare	Immune response to vehicle/scaffold	-Impact on macrophage polarization -Fibrous capsule formation versus tissue incorporation -Response to and clearance of degradation products