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SYMPOSIUM

Implant Healing in Experimental Animal Models of Diabetes

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Abstract

Diabetes mellitus is becoming increasingly prevalent worldwide. Additionally, there is an increasing number of patients receiving implantable devices such as glucose sensors and orthopedic implants. Thus, it is likely that the number of diabetic patients receiving these devices will also increase. Even though implantable medical devices are considered biocompatible by the Food and Drug Administration, the adverse tissue healing that occurs adjacent to these foreign objects is a leading cause of their failure. This foreign body response leads to fibrosis, encapsulation of the device, and a reduction or cessation of device performance. A second adverse event is microbial infection of implanted devices, which can lead to persistent local and systemic infections and also exacerbates the fibrotic response. Nearly half of all nosocomial infections are associated with the presence of an indwelling medical device. Events associated with both the foreign body response and implant infection can necessitate device removal and may lead to amputation, which is associated with significant morbidity and cost. Diabetes mellitus is generally indicated as a risk factor for the infection of a variety of implants such as prosthetic joints, pacemakers, implantable cardioverter defibrillators, penile implants, and urinary catheters. Implant infection rates in diabetic patients vary depending upon the implant and the microorganism, however, for example, diabetes was found to be a significant variable associated with a nearly 7.2% infection rate for implantable cardioverter defibrillators by the microorganism *Candida albicans*. While research has elucidated many of the altered mechanisms of diabetic cutaneous wound healing, the internal healing adjacent to indwelling medical devices in a diabetic model has rarely been studied. Understanding this healing process is crucial to facilitating improved device design. The purpose of this article is to summarize the physiologic factors that influence wound healing and infection in diabetic patients, to review research concerning diabetes and biomedical implants and device infection, and to critically analyze which diabetic animal model might be advantageous for assessing internal healing adjacent to implanted devices.

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Abbreviations: (BB) biobreeding, (ECM) extracellular matrix, (eNOS) endothelial nitric oxide synthase, (EPC) endothelial progenitor cell, (FGF) fibroblast growth factor, (GK) Goto-Kakizaki, (IL) interleukin, (KK) Kyoji Kondo, (MCP-1) monocyte chemotactic protein 1, (MHC) major histocompatibility complex, (MIP-2) macrophage inflammatory protein 2, (MMP) matrix metalloproteinase, (NO) nitric oxide, (NOD) nonobese diabetic, (PDGF) platelet-derived growth factor, (PMNL) polymorphonuclear leukocyte, (TGF-β) transforming growth factor β, (TNF-α) tumor necrosis factor α, (VEGF) vascular endothelial growth factor

Keywords: biomedical implants, foreign body response, sensors, wound healing

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Introduction

In 2007, the American Diabetes Association estimated nearly 25 million people were affected by diabetes in the United States.[1] A considerable number of these individuals will require some sort of indwelling medical device in their lifetime as the use of devices such as orthopedic implants, breast implants, and glucose sensors is rapidly expanding. In 2004, 600,000 joint prostheses and 2,000,000 fracture-fixation devices were implanted in the United States. Breast implants accounted for 130,000 implanted medical devices (**Table 1**), while statistics estimated that nearly 800,000 dental implants were done in the United States in 2004.[3] As the percentage of the population affected by diabetes increases, a larger fraction of these implanted devices will be placed in diabetes patients. Specifically, percutaneous glucose sensors are designed for use in diabetic patients. Medtronic, Inc., which manufactures the MiniMed glucose sensor, has sold over one million sensors as of 2008, which have been implanted in patients worldwide.[4]

Performance of biomedical implants has been hindered by fibrosis, infections, and deficient tissue integration due in part to the body's foreign body response. Implant-associated infections account for half of the nearly 2 million nosocomial infections in the United States yearly.[5] While most of these infections involve catheters, infections involving surgical implants are generally more difficult to manage because they usually require a longer period of antibiotic therapy and repeated surgical procedures.[2,6,7] Concerning glucose sensors, while there is no reported research on the infection rates of such devices, infection is a potential concern due to the tract available for bacterial migration. Some groups have engineered coatings for glucose sensors to prevent or mediate bacterial infection. The Schoenfisch group has coated glucose sensors with nitric oxide-releasing xerogels, which have antibacterial properties.[8] In addition, device function and performance can be adversely affected if the foreign body response elicited by the device culminates in fibrotic encapsulation.

Additionally, common complications of diabetes are altered healing of wounds and a higher susceptibility to infections.[9,10] These deficiencies can complicate the body's acceptance of an implant and lead to greater rates of rejection. While some research has investigated the effect of diabetes on the body's healing ability, most particularly the case of diabetic foot ulcers, these findings have not been applied to understanding a diabetic patient's response to an indwelling implant. Few examples discuss the prevalence of infection in diabetic patients with indwelling implants or even the ability of a diabetic patient to receive such implants. However, the prevalence of diabetes and the increasing use of indwelling medical devices warrant such investigation.

Many physiologic factors contribute to wound healing deficiencies in diabetic patients. Many studies have implicated a decreased inflammatory response consisting of decreased or impaired growth factor production and decreased neutrophil and macrophage function. During later stages of wound repair, diabetic patients exhibit decreased and less organized granulation tissue formation, poor angiogenic response, and altered collagen

Table 1.
Clinical and Economic Consequences of Infections Associated with Surgical Implants[a]

Implant type	Implants inserted in the US anually	Projected infections of implants annually	% first implant infection rate	Preferred no. of stages for surgical replacement	Estimated cost of each med + surgical treatment
Cardiovascular					
Vascular grafts	450,000	16,000	4	1	$40,000
Pacemaker	300,000	12,000	4	2	$35,000
Orthopedic					
Joint prostheses	600,000	12,000	2	2	$30,000
Fracture-fixation device	2,000,000	100,000	5	1 or 2	$15,000
Plastic – breast (pair)	130,000	2,600	2	2	$20,000

[a] Only >100,000 implants; adapted from *New England Journal of Medicine*[2]

deposition and organization.[11] Also, diabetic wounds are characterized by nitric oxide (NO) deficiency, which has negative effects on the wound healing response as well as the response to infection.[12]

Little research has focused on how diabetes alters the internal wound healing around an implant. In normal tissues, the presence of an implant leads to the foreign body reaction and eventual fibrotic encapsulation. Few studies have attempted to determine if a similar response occurs in diabetic patients. Studies that focus solely on the diabetic response to a biomedical implant are essential to improving device performance in these patients.

Diabetic patients are generally more susceptible to infections, which presents a significant obstacle to implant integration. Infections result in considerable morbidity and mortality in diabetic patients even in the absence of an indwelling medical device. In a study conducted by Bertoni and colleagues, 9,208 adults aged 30–74 years in 1976–1980 were followed over a period of 12–16 years. Thirty-six infection-related deaths occurred among 533 diabetic adults versus 265 deaths in 8,675 adults without diabetes (4.7 vs 1.5 per 1,000 person-years, $p < .001$), suggesting that diabetic adults are at greater risk for infection-related mortality.[13] In the presence of an implant, several large-scale retrospective studies have found diabetes patients have increased rates of surgical site infections when compared to healthy individuals.[14–17] In diabetic patients undergoing spondylolithesis, the rate of infection was 10.3% compared to 0.7% in nondiabetic patients.[15] Extensive research has been done to elicit the differences in infection response in diabetic and nondiabetic patients, with most studies suggesting defects in cellular innate immunity as being primarily responsible for the decreased ability to fight infection. Additionally, some studies have shown certain microorganisms exhibit an increased adherence to diabetic cells.[10,18] Understanding the diabetic response to infection can be useful in improving implanted device longevity.

Many therapies have been developed to address the deficiencies present in the diabetic wound. Treatments include NO supplementation in the diabetic wound and growth factor therapy. Molsidomine, an orally active, long-acting vasodilating drug, spontaneously releases NO and was found to increase wound breaking strength in diabetic wounds.[12] Treatment with recombinant human growth factors such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF) has been shown to increase fibroblasts and capillaries at the wound site and promote greater closure of diabetic wounds.[19] Additionally, vascular endothelial growth factor (VEGF) production in a diabetic wound can be stimulated by administering simvastatin (ZOCOR®), a drug typically used to reduce cholesterol. Simvastatin-treated wounds showed greater amounts of VEGF protein expression and increased angiogenesis.[20]

Animal research has been instrumental in understanding the mechanisms and complications of diabetic wound healing. Several models exist that accurately reflect aspects of both type 1 and type 2 diabetes. Certain drugs can induce type 1 diabetes by damaging pancreatic beta cells. Alternatively, genetic models are produced by selective inbreeding to develop hyperglycemia and other related traits such as obesity, immune deficiency, or insulin resistance. Owing to the complexity of diabetes, new models are continually being developed and studies involving transgenic and humanized models are becoming more prevalent.

Molecular Pathogenesis of Wound Healing in Diabetes Patients

Over 100 physiologic factors have been discovered that contribute to wound healing deficiencies in diabetic patients.[11] Such factors include impaired growth factor production,[21–23] angiogenic response,[23,24] macrophage function,[25] collagen accumulation, quantity of granulation tissue,[23] fibroblast migration and proliferation, number of epidermal nerves,[26] and balance between extracellular matrix (ECM) component accumulation and remodeling by matrix metalloproteinases (MMPs).[27] In normal tissues, wound healing is characterized by efficient inflammatory cell recruitment in response to a variety of chemokines, including macrophage inflammatory protein 2 (MIP-2) and monocyte chemotactic protein 1 (MCP-1). Chemokine and cytokine signaling stimulate the production of growth factors that promote matrix formation, angiogenesis, and reepithelialization (**Figure 1**). In diabetic wounds, however, decreased chemokine expression results in decreased growth factor production and delayed inflammatory cell infiltration.[28] Alterations in many chemokines and growth factors in diabetic wounds lead to impairments in angiogenesis, matrix formation, and reepithelialization (**Table 2**), deficiencies that are detrimental not only to cutaneous wounds but also to internal wounds adjacent to surgical implants.[28,29] During later stages of diabetic wound healing, persistence of inflammatory cells within injured tissue results in continued damage and turnover due to increased expression of interleukin-1b (IL-1b), tumor necrosis factor a (TNF-a), and MMPs.[29]

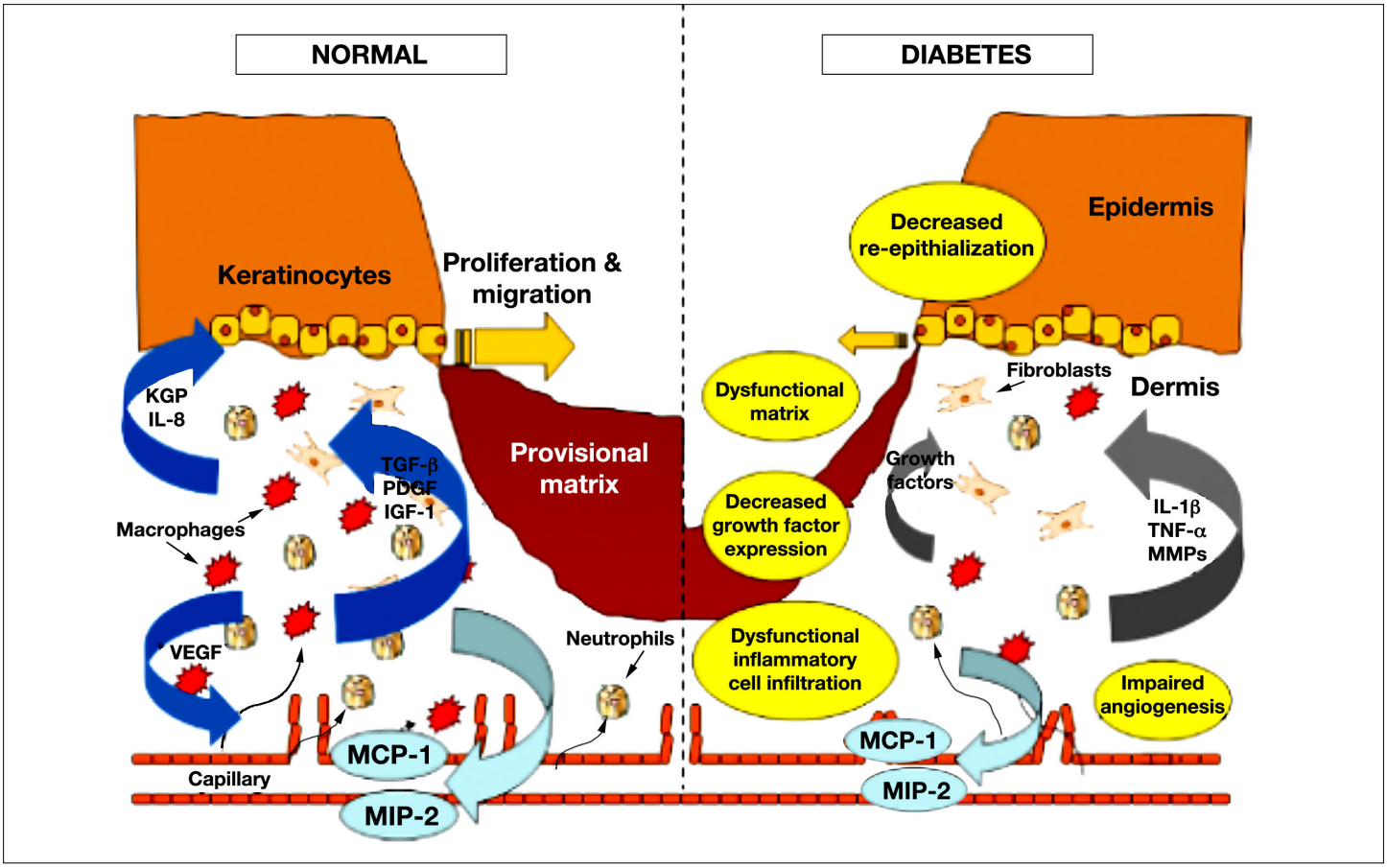

Figure 1. Normal wound healing and alterations in diabetic wounds. IGF-1, insulin-like growth factor 1; KGF, keratinocyte growth factor. Reprinted with permission from *Vascular*.[28]

Table 2.
Changes in Expression of Cytokines and Growth Factors in Diabetic Wound Healing[a]

Cytokine and growth factors	Normal role in wound healing	Expression In diabetic wound healing	Reference
IGF-1	Promotion of reepithelialisation Keratinocyte and fibroblast proliferation Endothelial cell activation	Decreased	(58–60)
TGF-β1	Chemoattractant (keratinocytes, fibroblasts, inflamamtory cells) ECM deposition Promotes angiogenesis	Decreased	(62,65–68)
PDGF	Fibroblast activation Promotes angiogenesis ECM deposition MMP synthesis	Decreased	(69)
EGF	ECM deposition Keratinocyte migration and proliferation	Decreased	(70)
IL-8	Keratinocyte proliferation Mactrophage chemotaxis Neutrophil chemotaxis	Decreased	(71)
Angiopoietin-2	Disrupts blood vessel formation	Increased	(72)

EGF, endothelial growth factor; IGF-1, insulin-like growth factor-1; IL-8, interleukin-8; MMP, matrix metalloprotease; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor-β1.

[a] Reprinted with permission from *International Wound Journal*[29]

Other aspects of diabetic wound healing include reduced cell proliferation in the wound tissue, slowed onset of myofibroblast differentiation, and increased levels of apoptosis during inappropriate stages of the healing process.[30] Keratinocytes show an absence of migration, hyperproliferation, and incomplete differentiation while fibroblasts exhibit decreased migration and proliferation.

Some of these factors are particularly of significance in the tissue integration of an indwelling medical device. Decreased amounts of VEGF and other growth factors influence the development of blood vessels crucial to proper wound healing; poor angiogenesis results in poor implant integration with surrounding tissue.[31–33] Decreased capillary concentration around an implant results in poorly perfused tissue that is inconsistent with healthy tissue. Additionally, diabetic wound sites were found to be stiffer due to greater collagen accumulation and cross-linking, and decreased amounts of growth factors such as PDGF and epidermal growth factor result in poor matrix formation.[29,31] Finally, NO, an important mediator in wound healing, is deficient in diabetic wounds.[12] In addition to its role in wound healing, NO has antibacterial properties that can partly assist in combating implant-associated infections.[34]

Mechanisms of Wound Healing in Healthy People vs People with Diabetes

Wound healing is a complex cellular response to injury and involves the activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. These cells release growth factors, cytokines, and chemokines that coordinate and maintain healing in healthy individuals. Shortly after injury, hypoxia is induced and VEGF released by macrophages, fibroblasts, and epithelial cells initiates the phosphorylation and activation of endothelial nitric oxide synthase (eNOS) in the bone marrow. Rising NO levels initiate the mobilization of bone marrow endothelial progenitor cells (EPCs) to the circulation. Chemokines direct these EPCs to the wound site where they participate in vasculogenesis.

According to a murine model of diabetes,[11] eNOS phosphorylation in the bone marrow is impaired as evidenced by significantly limited EPC migration from the bone marrow into the circulation. In addition to deficient activation of eNOS, expression of key chemokines is decreased in epithelial cells and myofibroblasts of the diabetic model. EPCs do not receive the signals necessary to relocate to the wound site, and healing is impaired due to decreased angiogenesis (**Figure 2**).

Diabetes and the Time Course of Wound Healing

Much of what is known about diabetic wound healing has been derived from experimental wounds in animals. The biological and molecular events occurring after cutaneous injury are generally divided into four phases of repair: coagulation, inflammation, migration-proliferation and matrix deposition, and remodeling (**Figures 3** and **4**). The phases overlap considerably but healthy wounds exhibit linear progression. Conversely, chronic nonhealing wounds such as those often seen in diabetic patients have a disorganized progression of wound healing, with some wound areas present in different phases simultaneously.

The Course of Normal Wound Healing

During coagulation, the first phase of wound healing, a fibrin plug forms shortly after injury and inflammatory cells quickly migrate to the wound. Platelets within the plug release growth factors such as PDGF and transforming growth factor b (TGF-b) that are responsible for cell recruitment. Although hypoxia results from damage to blood vessels, beneficial effects include increased keratinocyte migration, early angiogenesis, proliferation and expansion of fibroblasts, and transcription and synthesis of necessary growth factors and cytokines. These processes occur within hours of injury.

During inflammation, which takes place within about 2–3 days after injury, neutrophils and monocytes arrive and assist in wound debridement and release growth factors. Several other inflammatory and dermal cells follow including macrophages, fibroblasts, and endothelial cells. Granulation tissue begins to form.

Occurring over several days after injury, the migration-proliferation phase is marked by epidermal resurfacing, fibroplasia, angiogenesis, ECM deposition, and initial wound contraction. Contraction is promoted by the formation and organization of granulation tissue and the presence of myofibroblasts. Angiogenesis supplies the wound area with oxygen and nutrients. In the weeks and months after injury, a scar is formed at the wound site that is remodeled over time. ECM degrades and further contraction occurs.[23,35]

In diabetic patients, all stages of the wound healing cascade are affected. Many studies have shown a decreased inflammatory response in diabetic wounds, including decreased chemotaxis, bacterial killing and phagocytosis, and antioxidant levels resulting from the impaired function of neutrophils and macrophages.[12,36–38]

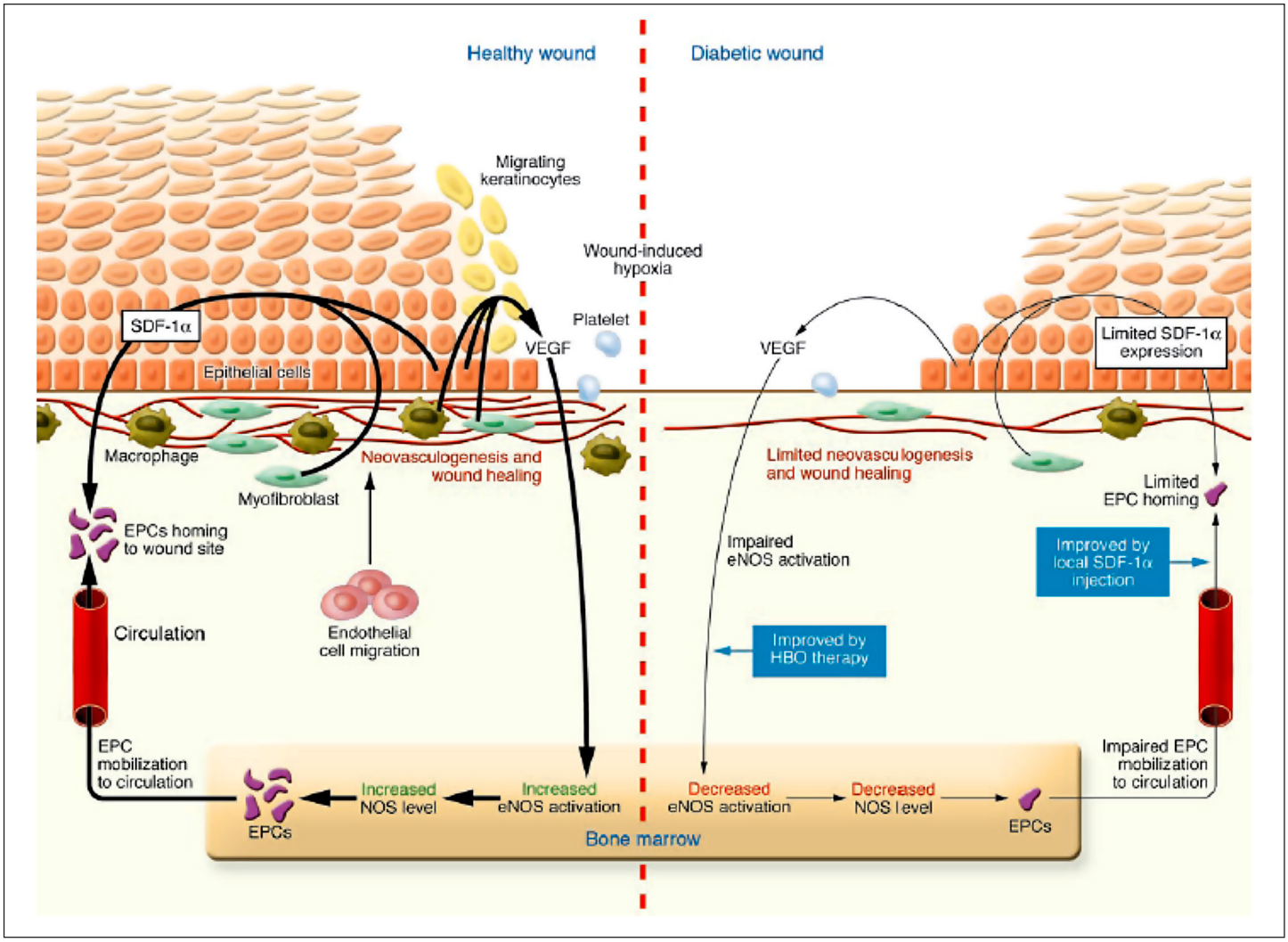

Figure 2. Mechanisms of wound healing in healthy people versus people with diabetes. HBO, hyperbaric oxygen therapy; SDF-1, stromal cell-derived factor-1. Reprinted with permission from *The Journal of Clinical Investigation*.[11]

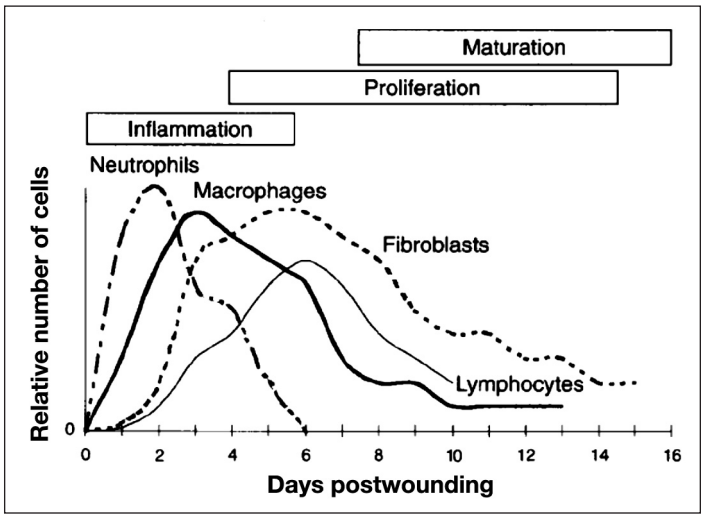

Figure 3. The time course of the different cells appearing in the wound during the healing process. Macrophages and neutrophils are predominant during inflammation, whereas lymphocytes peak somewhat later and fibroblasts are predominant during the proliferative phase. Reprinted with permission from *Surgical Clinics of North America*.[35]

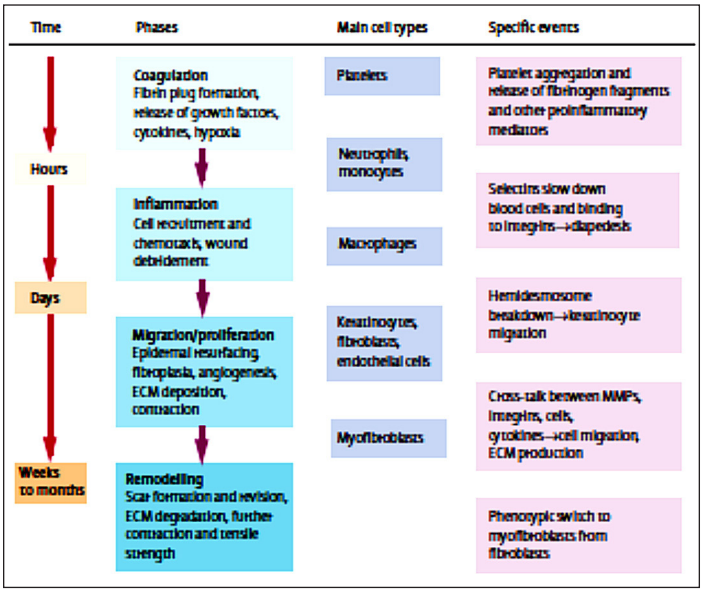

Figure 4. Phases of wound healing, major types of cells involved in each phase, and selected specific events. Reprinted with permission from *Lancet*.[23]

During later stages of healing, diabetic wounds show growth factor depletion,[39,40] raised glucocorticoid concentrations,[41] diminished cell proliferation,[42,43] and increased apoptosis.[30] Several days after injury, neovascularization is decreased and granulation tissue is poorly developed.[44] The alterations in cell recruitment and proliferation seen in diabetes may complicate integration of implanted devices; decreased granulation tissue formation likely results in an unstable environment for a device. Additionally, poor blood vessel development and perfusion may result in the formation of an avascular tissue barrier around a device when optimally the area around an implant should be identical to uninjured tissue. In particular, decreased neovascularization is a considerable impediment to the function of glucose sensors, as distance from the sensor to glucose in the bloodstream can affect the accuracy of glucose measurements.

Diabetes and Biomedical Implants

In normal tissues, the presence of an implant can lead to the foreign body reaction and eventual fibrotic encapsulation, which can severely limit the device's performance. For example, fibrotic encapsulation of glucose sensors prevents accurate measurements of blood glucose levels because glucose must diffuse through an abnormal tissue barrier before its concentration can be read by the sensor. The ensuing lag time results in different concentrations in the blood and at the sensor surface. It is unknown if a similar foreign body response occurs in diabetic patients.

Considerable research has focused on dental and orthopedic implants in diabetic patients, however, these implants are atypical of soft tissue implants. With regards to soft tissue implants, subcutaneous and percutaneous devices of varying materials have been implanted in diabetic animal models to study the wound healing response—not necessarily to the implanted device. Additionally, implants releasing therapeutic agents such as NO have been reported[12] but these studies emphasize techniques to enhance wound healing in diabetic patients. Therefore, it is crucial to study implant healing in diabetic patients because understanding how the foreign body reaction is regulated in diabetic patients will allow for the development of new therapies to reduce fibrosis and increase the utility of implanted devices.

Dental and Orthopedic Implants

Several experimental models have investigated the effect of diabetes on the osseointegration of dental and orthopedic implants.[45] Periodontal disease is a frequent complication of diabetes. The failure rate of bone implants is higher in diabetic patients, particularly if the disease is poorly controlled. Studies have shown that diabetes induces an alteration in the bone remodeling processes. Deficient mineralization then results in decreased osseointegration of the implant. Reduction in contact between the bone and the implant is present despite comparable bone formation in diabetic and control animal models and infection of oral implants is a continual danger. Antibiotics are often recommended for diabetic patients because of their greater susceptibility to infection.

In the orthopedic literature, research examining differential healing in diabetic animal models has focused primarily on bone fractures because, clinically, diabetes has been shown to alter bone composition, reduce bone mass, and impair fracture healing in humans.[46] The mechanism of delayed bone healing in diabetic animal models has yet to be fully elucidated, but impaired osteoblast[47] and osteoclast[48] function as well as altered chondrogenesis[49] have been proposed.

Subcutaneous Implants

One study[31] investigated wound healing in a diabetic baboon model using a polystyrene implant. Drum devices were implanted subcutaneously in the thighs of long-term diabetic baboons. Implants were removed after 2 and 4 weeks and analyzed for granulation tissue and inflammatory cell migration into the drums. Granulation tissue was reduced at both time points in diabetic baboons and vessel lumen areas were greater at 4 weeks compared to control animals. Fewer macrophages were present in diabetic tissue while neutrophils were prominent. Lastly, after 4 weeks, diabetic wound tissue exhibited less connective tissue ingrowth, resulting in a coarser, more disorganized tissue structure. There was no mention of fibrotic capsule formation.

Percutaneous Implants

Gerritsen and colleagues[50] investigated the soft tissue response to subcutaneous and percutaneous devices composed of titanium fiber mesh in diabetic and non-diabetic rabbits. The percutaneous device consisted of a subcutaneous component of titanium fibers and a percutaneous segment that was attached via a threaded hole. Titanium mesh was also implanted subcutaneously to examine the difference in tissue response to subcutaneous and percutaneous implants. The group found a greater number of infectious complications around percutaneous implants in severely diabetic animals.

Severe diabetes adversely affected matrix maturation and delayed neovascularization in both implant types. Inflammatory cells were present in higher numbers around percutaneous devices in severely diabetic rabbits; a nearly 40% increase in inflammatory cells was seen around these devices when compared to the subcutaneous devices.

Diabetes and Infection

Patients with diabetes are more susceptible to infections. Infections are responsible for considerable morbidity and mortality in diabetic patients and thus understanding the deficiencies that lead to infection susceptibility is a crucial area of research. Many causes may play a role, including defects in immunity, an increased adherence of microorganisms to diabetic cells, and the elevated number of medical interventions among diabetic patients.[10,18] The risk of infection in diabetic patients is a significant obstacle to proper integration of a medical device. Nearly half of all nosocomial infections in the United States are associated with an indwelling device; approximately 200,000 of these infections will involve surgical implants as opposed to indwelling catheters.[4,5] By investigating the diabetic response to infection in the absence and presence of an indwelling implant, factors may be identified that contribute to increased infection susceptibility and guide improved implant integration.

The Normal Immune Response to Infection

The normal immune response in wound healing can be divided into the innate and the adaptive immune response. The repeated observations in other publications suggest that only the innate response is adversely altered in diabetic foreign body reaction.

In the innate response, cells such as T cells, natural killer cells, neutrophils, mast cells and macrophages immediately recognize an antigen and attack the carrier in a nonspecific manner. The response is short-lived and does not confer lasting, protective immunity to the host.[48] Innate immunity provides immediate defense against infection by recruiting immune cells via cytokine signaling, assisting in the activation of the complement cascade, identifying and removing foreign substances, and activating the adaptive immune system through antigen presentation. Many studies have implicated the following constituent factors of the innate immune system as being most responsible for the alterations in diabetic healing: defects in polymorphonuclear leukocytes (PMNLs), altered monocytes and mast macrophages, and increased adherence of microorganisms to diabetic cells.

The adaptive immune system consists of specialized systemic cells and processes that eliminate or prevent pathogenic challenges. The adaptive immune system is activated by the innate immune system and provides the ability to recognize and remember specific pathogens. Future infection by those pathogens will result in a stronger immune response. The humoral aspect of the adaptive immune system is mediated by secreted antibodies produced by B cells. Antibodies bind to antigen markers on the surfaces of invading microbes such as viruses or bacteria, which flags them for destruction.

The adaptive humoral immune response is the main defense against invasion of extracellular bacteria. Production of antibodies assists in the removal of bacteria and the inactivation of bacterial toxins. Antibodies that bind to available antigens on the surface of bacteria assist in activating the complement cascade, resulting in increased phagocytosis, clearance, and localized production of immune effector molecules that assist in the inflammatory response. Effector molecules such as C3a and C5a function as anaphylatoxins to promote local mast-cell degranulation, vasodilation, and extravasation of lymphocytes and neutrophils from the blood into the tissue. Other complement products act as chemotactic factors for neutrophils and macrophages, increasing the number of phagocytic cells at the infection site.

Immune Deficiencies in a Diabetic Patient

Deficiencies in diabetic patients appear to occur primarily within the innate immune system. For instance, adaptive humoral immunity in diabetic patients expresses normal levels of serum antibodies and normal response to vaccinations.[18,51–53] In adaptive cellular immunity, the proliferative response of lymphocytes in diabetic patients is inhibited with some stimuli, such as *Staphylococcus aureus* and phytohemagglutinin, and normal with others.[10] However this alteration does not appear to enhance infection susceptibility in diabetic patients.

Defects in Cellular Innate Immunity

Cellular Innate Immunity: Defects in PMNLs

Studies involving PMNLs in diabetic patients have reported abnormalities in the adherence, chemotaxis, phagocytosis, oxidative properties, and intracellular killing of these cells.[10] Reportedly, PMNL chemotaxis is significantly lower in diabetic patients even after stimulation when compared to controls.[54] In addition, PMNL phagocytotic and killing capacity has been found to be lower in diabetic patients (**Table 3**), leading to a poorer ability to fight off

infection. The pathogenesis of these abnormalities is not entirely known, and conflicting studies have emerged. It also seems that proper glucose control can rectify these immune deficiencies so the true influence of PMNL defects on infection susceptibility is still uncertain.[18]

Cellular Innate Immunity: Defects in Monocytes/Macrophages

Diabetic monocytes exhibit both impaired chemotaxis and phagocytosis most likely resulting from an intrinsic monocyte defect. One study showed that children with type 1 diabetes had a lower immune response to intradermal administration of hepatitis B vaccine when compared with control children. In this case, defective macrophage function appeared to be responsible.[53]

Adherence of Microorganisms to Diabetic Cells

Microorganism adherence to mucosal or epithelial cells is an early step in the pathogenesis of infections. *C. albicans* is an infectious microorganism commonly found in diabetic patients. Researchers determined several risk factors that could increase the risk of contracting the infection in diabetic patients, including a lower age, higher hemoglobin A1c level, the presence of glucosuria, and cigarette smoking. It is theorized that poor regulation of high blood glucose levels and other factors lead to altered receptors on the patient's cells that exhibit greater adherence for infectious organisms.[18]

Immune Dysfunction and Infection Response in Diabetic Animal Models

Animal models have played a key role in the attempt to elucidate the mechanism of immune dysfunction in diabetic patients. Although these findings are not broadly applicable, if taken into proper context, they can provide useful insight into the underlying physiology. Leukocyte dysfunction in diabetic animals has been studied extensively, and the decreased inflammatory response observed in diabetic rodents has been attributed to many factors including a decreased microvascular response to histamine and bradykinin, reduced mast cell degranulation, and reduced levels of TNF-α and IL-1.[55] Additionally, diabetic rodents infected with *S. aureus* have shown altered neutrophil chemotaxis and decreased production of reactive oxygen species.[56] Macrophage dysfunction in streptozotocin-induced diabetic rats has also been studied, showing that mice with prolonged uncontrolled diabetes have decreased cytokine production, antigen presentation, and phagocytosis.[57]

Enhancing Wound Healing in Diabetes: Experimental Therapies

Many therapies have been developed to address deficiencies present in the diabetic wound. Most experimental healing therapies have been applied to skin incisions of varying thickness; studies have investigated healing of these incisions in a variety of animal models from mice to baboons.[20,31] Other studies involve subcutaneous implants that can release healing promoters such as NO.[12] These various treatments have shown promise in some animal models but not all have been attempted in human patients.

Nitric Oxide Supplementation in the Diabetic Wound

Diabetic wounds are characterized by NO deficiency. One group[12] developed a method for delivering NO donor to the wound site. Polyvinyl alcohol sponges were implanted subcutaneously through dorsal skin incisions created in diabetic and nondiabetic rats. Half of the implants were treated with the NO donor molsidomine. The study found that wound breaking strength and MMP-2 activity were significantly increased by exogenous NO. Nitric oxide treatment, however, had no effect on the delayed inflammatory reaction in diabetes. The results

Table 3.
Summary of the Different Immune Dysfunctions Found in Diabetic Patients[a]

	Humoral		Cellular	
Innate	Complement	↓	PMNs	↓ =
	Cytokines without stimulation	↑	Monocytes/macrophages	↓
	Cytokines after stimulation	↓ =		
Adaptive	Immunoglobulins	=	T lymphocytes	↓
Adherence		↑		

↓ means that this function is decreased, = means that this function is the same and ↑ means that this function is increased in diabetic patients compared with nondiabetic controls.

[a] Reprinted with permission from *FEMS Immunology and Medical Microbiology*[18]

suggested NO therapy can partially enhance the wound healing response in a diabetic animal model.

Growth Factor Therapy Stimulates Wound Healing

Concentrations of many growth factors are decreased in diabetic wounds. In one model,[19] full-thickness skin wounds were made in the backs of genetically diabetic mice. These wounds were characterized by delayed infiltration of inflammatory cells, poor granulation tissue formation, and delayed wound closure when compared to wounds in nondiabetic littermates. Diabetic wounds were treated with recombinant human PDGF and basic FGF individually and in combination. Treatment with the growth factors resulted in increased fibroblasts and capillaries at the wound site and significantly greater wound closure after 21 days.

Enhancing VEGF Production in the Diabetic Wound

Enhancing VEGF production in a diabetic wound can promote angiogenesis. One group[20] used simvastatin, a drug typically used to reduce cholestorol, to stimulate angiogenesis in a diabetic mouse model. Simvastatin was administered daily to diabetic and nondiabetic mice with incisional skin wounds. Treatment with simvastatin increased VEGF mRNA and protein expression in diabetic mice and enhanced NO production, successfully restoring wound healing capabilities.

Animal Models of Diabetes Mellitus

Many models exist that accurately portray aspects of both type 1 and type 2 diabetes. Type 1 diabetes mellitus results from the specific autoimmune destruction of the insulin-producing pancreatic beta cells. Hyperglycemia then results because there is a lack of or a reduced amount of insulin. Type 2 diabetes encompasses a heterogeneous group of disorders characterized by insulin resistance and impaired insulin secretion. A number of toxins can induce type 1 diabetes by damaging pancreatic beta cells. Additionally, selective inbreeding has resulted in strains that are fairly reasonable models of the disease states of type 1 and 2 diabetes and even obesity and insulin resistance. Animal models have been instrumental in the study of the pathogenesis of diabetes and its complications as well as the testing of new diabetic treatments before they can be considered for clinical use.[58]

Animal Models of Type 1 Diabetes Mellitus

Type 1 diabetes mellitus results from the specific autoimmune destruction of the insulin-producing pancreatic beta cells. Subsequently, hyperglycemia results due to the lack or reduced amount of insulin.

Surgically and Chemically Induced Type 1 Diabetic Animal Models

The effects of hyperglycemia can be studied most simply in animals by partial or complete removal of the pancreas. Hyperglycemia can also be induced nonsurgically by administering toxins such as streptozotocin and alloxan that damage the pancreas.[59,60] Streptozotocin is a broad-spectrum antibiotic that has alkylating properties and thus modifies biological macromolecules, fragments DNA, and destroys the beta cells causing a state of insulin-dependent diabetes. The drug can be administered intravenously or intraperitoneally in a single, large dose or smaller, repeated doses over a period of days. A single large dose (60 mg/kg) of streptozotocin is sufficient to produce diabetes in rodents, although repeated smaller doses are equally effective and in fact, may produce consistent models more reliably. Both surgically and chemically induced pancreatic damage are useful in investigating what complications arise because of hyperglycemia. For example, the multiple low-dose streptozotocin model has been used to investigate the immunological pathways that lead to insulitis and beta cell death, and both methods performed on female animals are helpful in studying the effect of gestational diabetes on offspring.[58]

Spontaneous Animal Models of Type 1 Diabetes

Certain animal strains spontaneously develop diseases that are similar to type 1 diabetes. These models are produced by selectively inbreeding for generations based on hyperglycemia. Inbreeding results in the enrichment of a variety of genes and phenotypes, which may differ in relevancy to the pathophysiology of diabetes in animals or humans. Two of the most common spontaneous models for type 1 diabetes are the nonobese diabetic (NOD) mouse and the biobreeding (BB) rat because they spontaneously develop diabetes similarly to humans.[58] Nevertheless, new animal models are continuously being developed because of the inability of a single model to completely represent the human disease.

The NOD Mouse

Diabetes in NOD mice develops rapidly; inflammation of beta cells due to infiltration of mononuclear cells occurs at approximately 4–5 weeks of age. Ensuing destruction of the beta cells and decreasing insulin concentrations lead to the presentation of frank diabetes within 12 to 30 weeks. The NOD mouse model has been used extensively in diabetes research because similarly to humans, the mouse major histocompatibility complex (MHC) region largely influences the development of

disease.[58] As an indication of the importance of genetic heterogeneity, the NOD model consists of many genes related to susceptibility to autoimmunity.[61] However, the model differs from human type 1 diabetes in that ketoacidosis is generally mild in NOD mice. While these mice can survive for extended periods of time without the administration of insulin, ketoacidosis is a serious condition in humans that can result in diabetic coma or death if left untreated. Additionally, the gender difference of animals developing diabetes does not reflect the findings of human studies.[58] Studies involving NOD mice have focused on cell apoptosis in diabetic wounds and the immunological cascade consisting of T-helper type 2 cells and effector cells and the contribution of cytokines.[30,58]

The BB Rat

Several strains of the BB rat exist, although only some are diabetes prone. For strains that develop diabetes, weight loss, hyperglycemia, and other diabetic complications occur at approximately 12 weeks of age. Similarly to NOD mice, inflammation of pancreatic beta cells induces an autoimmune response and recruitment of T cells, B cells, macrophages, and natural killer cells. More in common with humans, however, ketoacidosis in the BB rat is severe and fatal in the absence of administered insulin. The BB rat has been used in diabetic research to investigate the role of diet and a variety of viruses as possible environmental stimuli for the disease state.[58]

Animal Models with Specific Known Mutations or Pathway Defects

In certain cases, specific known mutations or pathway defects influence the development of type 1 diabetes and other autoimmune diseases. Animal models with such defects can be used to better understand pathways in human disease. Animals that exhibit specific defects in genes encoding important tolerance mediators, for example, can give insight into the role of these genes in humans.[61]

Humanized Mouse Models

Humanized mouse models of type 1 diabetes are produced by introducing genes encoding MHC, T-cell antigen receptors, and costimulatory molecules from humans. Specific aims of such models include modeling disease initiation and evaluating *in vivo* immunomodulation by specific antigens presented in the context of humanized MHC and other genes.[61–63] Unfortunately, humanized mouse models struggle to reproduce human diabetes effectively in the animal, particularly when human gene products interact with ECM and tissues that are not humanized.[61]

Animal Models of Type 2 Diabetes Mellitus

Type 2 diabetes encompasses a heterogeneous group of disorders characterized by insulin resistance and impaired insulin secretion. This heterogeneity has led to a variety of animal models attempting to address the many facets of a complex disease. While some animal models emphasize insulin resistance, others predominately exhibit beta cell failure resulting in impaired insulin secretion. Different models will be more or less clinically relevant and extrapolation of results to the clinical setting must be undertaken cautiously.

Spontaneous Animal Models of Type 2 Diabetes

The Kyoji Kondo (KK) Mouse

Originally bred for large body size, this animal strain develops obesity and insulin resistance that is followed by mild hyperglycemia. The severity of the diabetic phenotype is highly dependent on food intake, and the model is useful in researching human obesity. However, multiple strains of KK mice exist because of specific inbreeding or spontaneous mutations that are genetically and phenotypically different.[58]

The Goto-Kakizaki (GK) Rat

The GK rat exhibits both insulin resistance and impaired insulin secretion having developed relatively stable hyperglycemia upon reaching adulthood. This model is useful for investigating some complications of diabetes as the rats develop renal lesions,[62] structural changes in peripheral nerves,[63] and retinal abnormalities[64] similarly to humans.[58]

Single Gene Mutation Animal Models of Type 2 Diabetes

The Zucker (fa/fa) Rat

Obesity is generally a fair predictor of type 2 diabetes in humans. Thus, animal models of obesity have been developed in hopes of gaining insight into the disease. The Zucker rat is an obese strain that maintains normal blood glucose levels by counteracting insulin resistance with increased insulin secretion from pancreatic beta islet cells.[58]

The db/db Mouse

In contrast to the Zucker rat, the db/db mouse strain fails to maintain the levels of insulin secretion necessary for normal blood glucose levels and thus quickly develops hyperglycemia.[58] The db/db mouse exhibits excessive eating, obesity, and elevated blood glucoses of 300–500 mg/dl caused by a defect in the leptin receptor. Similar to the human condition, the mouse has a reduced

ability to heal standard skin wounds in addition to decreased epidermal nerves.[65]

Animal Models Resulting from Gene Targeting and TransgenicTechniques

Molecular biological techniques such as gene targeting have been used to develop new animal models. Knockout animals are produced by disrupting single genes within an embryonic stem cell. These defects are then transmitted along the germ cell line. Additionally, transgenic animals are formed when modified genes are incorporated into a host genome. Researchers have produced models by manipulating various genes, including those that encode for insulin receptors or glucokinase. Resulting phenotypes include animals that exhibit varying degrees of insulin resistance and sensitivity and animals with varying blood glucose levels. Complications of these techniques arise when gene manipulation produces unexpected results. Many genes have different functions at various points in an animal's life and may also influence physiological processes not involved in the disease process.

Selection of a Diabetic Animal Model

With the variety of animal models representing both type 1 and type 2 diabetes, choosing an appropriate model for an experimental study requires careful consideration. To investigate internal wound healing adjacent to medical devices, it should first be determined which subset of diabetes is the investigative focus. While both type 1 and type 2 diabetic patients will need identical implants such as glucose sensors and orthopedic implants, the mechanisms leading to the disease state and hyperglycemia differ. Type 1 diabetes is most easily produced and reproduced by the administration of toxins. The disease state requires less time to achieve and dosage can be customized to each individual animal. Animals generally express diabetes within several days of the last dose of drug. Type 2 diabetes is affected by a variety of factors such as insulin resistance, impaired insulin secretion, obesity, and genetic and environmental factors.[58,59,61] Because of this variety, investigations often begin with type 1 animal models. Optimally, studies should be performed in a variety of diabetic animal models—representing both type 1 and type 2 diabetes—for the purpose of comparison and confirmation of data.

Summary

The increasing use of indwelling medical devices and the growing population of diabetic patients warrants investigation into diabetic wound healing around implants. Two of the main reasons for implant failure are deficient tissue integration leading to foreign body encapsulation and poor tissue integration caused by infection. These modes of failure have not been widely investigated in diabetic models, and little is known of the diabetic response to an indwelling medical device. Much of our understanding about wound healing in diabetic patients comes from animal experiments involving cutaneous wounds and patients with diabetic foot ulcers; such information can serve as the basis of the investigation of internal diabetic wound healing around an implant and the response to implant infection in a diabetic model. By quantifying the diabetic response to soft tissue implants and infection, diabetic wound healing can be better understood and guide improvements in implanted device performance.

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