Chapter

Modulation of Inflammatory Dynamics by Insulin to Promote Wound Recovery of Diabetic Ulcers

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Abstract

About 5% of the world population is diabetic and are at a risk of slow non-recoverable wound formation. Estimated 15–25% of diabetic patients develop foot ulcers, 6% among them needing clinical attention among which 15–20% will need an amputation. This counts for around 50% of all traumatic amputation. Wound leads to activation of dynamic inflammatory cascade responsible for the healing process. But in diabetes, a persistent rise of pro-inflammatory cytokines and low anti-inflammatory cytokines blocks the dynamic cascade. Wounding induces various pro-inflammatory cytokines such as IL-1, IL-6, IL-12, IL-18, IFN-γ, and TNFs causing accumulation of free radicals leading to inflammation which become persistent in diabetes. Inhibition of proinflammatory cytokines drives the equilibrium towards the expression of anti-inflammatory cytokines such as IL4, IL-10, IL-11, IL-13, IFN-α, and TGF-β, which is necessary for the wound recovery process. Here in this chapter, the inflammatory modulatory roles of different drugs/formulations have been discussed to unravel their significance to promote wound recovery.

Keywords: diabetic wound, tissue inflammation, pro-inflammatory cytokines, anti-inflammatory cytokines, nanoformulation for wound recovery

1. Introduction

Over the last 25 years, there has been found a four-fold increase in the number of diabetes mellitus cases commonly called diabetes [1]. 422 million people worldwide in 2016 have been reported to have diabetes mellitus. Diabetes in the year 2012 was a cause of 1.5 million deaths worldwide; according to WHO (World Health Organization), diabetes becomes the 8th leading cause of death [2]. Diabetes mellitus is mainly identified by the presence free glucose at high or the chronic level in the body fluids like sweat, urine, blood, etc. [3]. The major reason for diabetes mellitus among others was the hormone-mediated metabolism regulation failure. Hormones like glucagon and insulin play an important role in regulating the level of blood sugar or maintaining its balance [4]. The sugar balance in blood is important for perfect functioning of human body [5]. High/chronic level of glucose in body fluids is responsible for different pathological conditions like infection susceptibility, leading to various diseases such as arthritis, hypertension, cardiovascular problems, cataract,

retinopathy, neuropathy, damage of kidneys, damaging of blood vesicles, wound healing delay, etc. (**Figure 1**) [5, 6]. Due to the linkage of diabetes mellitus with other different diseases, the International Diabetes Foundation (IDF), in 2014, recorded that the 4.9 million lives loss and ~1.25% dead were diabetic patients, either directly by diabetes mellitus or indirectly through other diseases [7]. All these diseases are linked causing various effects on different body organs with various pathways; pathological/hyperglycemic conditions are linked with the inflammation of tissue [8]. Diabetes is responsible for lower gradation inflammation in a systemic way and leads to the promotion of different diseases like arthritis, retinopathy, etc. [9, 10].

One of the major problems associated with diabetes mellitus is inflammation in wounds and results in wound recovery delay [11]. The chronic wounds in diabetes mainly show the persistent increase in the level of pro-inflammatory cytokine and the absence of the signals, which are responsible for signals in the damaged tissues [12]. The treatments used in diabetes mellitus are also helpful in controlling the level of glucose blood and helps in delaying the further progression of other diseases linked with diabetes mellitus, like retinopathy, contract, arthritis, neuropathy, retinopathy, etc., but very less is known in the literature about diabetes mellitus treatment's effects on diabetic wound recovery [13]. Wound results in release of the pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-1, IL-12, IL-18, and tumor necrosis factors (TNFs) and interferon-gamma (IFN-γ), which results in inflammation of tissues [14]. Pro-inflammatory cytokines like IL-6 and IL-1β are released from the macrophages and monocytes in the wound and result in pain responses by signaling the neurons [15]. IFN-γ, IL-1β, and TNF-α induce apoptosis and pyroptosis mediated by the activation of innate immunity and oxidative stress [16]. IFN-γ is an activator; it activates macrophages by stimulating STAT1 expression in order to activate the defense mechanism against the pathogens in the infected area [17]. IL-12 stimulates TNF-α and IFN-γ production and reduces the expression of IL-4, an anti-inflammatory cytokine, and negatively controls the expression of IFN-γ; IL-4 also through the activation of STAT-3 signaling inhibits IFN- γ [18]. IL-18 for defense against pathogens activates T cells and natural killer

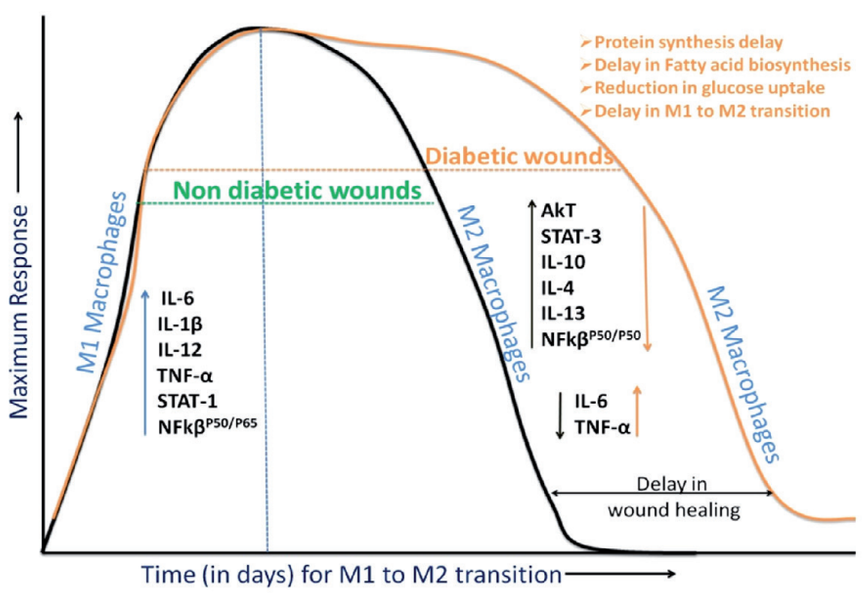

Figure 1.
M1 macrophages (also known as classically activated), such as IL-12, IL-1β, IL-10, STAT1, TNF-α, and NFkβP50/P65, are leads to wound inflammation. Alternatively, active, that is, M2 macrophages, such as STAT3, HIF-α, PKC, NFkβP50/P50, etc., help in healing of the wound by decreasing inflammation. People having diabetes show prolonged M1 macrophage expression in wounds in comparison to nondiabetic wounds that delay M1 to M2 macrophage transition.

(NK) cells and promotes the expression of of IFN-γ cytokines at the wound site [19]. However, the prolonged expression of inflammatory cytokines leads the damage of tissues, which results in a delay in the repairing process of wounds. IL-1 is a TNF activator and is responsible for the damaging of cells. IL-1β overproduction is responsible for neuronal tissue inflammation, leads to damage of neuro-muscular junctions and ultimately leads to delaying in wound healing [20]. In the presence of pathogens, the macrophages secrete IL-6 which in turn increases Toll like receptor expression (TLR)-9 response-mediated defense for killing foreign particles. In case of prokaryotes, unmethylated DNA activates the TLR-9 pathway and helps in killing the pathogen at the wound site; mitochondrial DNA spillage, essentially the unmethylated DNA, triggers similar kind of responses in the tissue of wound [21]. In tissues, the angiogenesis is inhibited by IL-12 by overexpression of IFN-γ-mediated interferon-gamma-induced protein 10 (CXCL-10 or IP-10) [22]. Vascular endothelial growth factor (VEGF) expression is negatively controlled by IL-18, essential for the development of new blood vessels at the wound site and is essential for the growth and repair at wound tissue [23].

To start and control the wound healing mechanism, the inflammation is most important. In the nondiabetic wounds, anti-inflammatory cytokines like IL-4, IL-10, IL-13, IL-11, and transforming growth factor-beta (TGF-β) and interferon-alpha (IFN-α) play an important role in the wound healing process [24]. At the initial stage of wound recovery, TLR-9 induces the instant expression of pro-inflammatory signals like TNF-α through the increase in expression of mitogen-activated protein kinase (MAPK)/p38 and c-Jun N-terminal kinase (JNK) pathway [25]. In non-diabetic wounds, MAPK activation is prolonged and leads to MAPK phosphatase enzyme activation, which works as a negative regulator of JNK and MAPK/p38 pathways, resulting in the negative regulation of TNF-α production. The de-phosphorylation of MAPK/p38 leads to more expression of anti-inflammatory cytokines such as IL-10 a homodimeric cytokine, which is produced by the macrophages, monocytes and induce signaling of TGF-β, and can enhance the division of cells [26]. Cytokines like IL-4, IL-13, and IL-10 can stimulate extracellular matrix and fibrinogen, mainly collagen synthesis. IL-4, cytokines secreted by macrophages, mast cells and inflamed T cells activate the Janus kinase/signal transducer and transcription-6 (Jak/STAT6) pathway activator which promotes the wound repairing [27]. IL-4 is responsible for the extracellular matrix synthesis, mainly collagen which gives the physical support for the healing of the wound [28]. Another kind of cytokine, L-1RA, secreted from immune cells, adipocytes cells and cells of epithelia, leads the inhibition of pro-inflammatory IL1β cytokine effect by binding with the (IL-1R) interleukin-1 receptor. On the other hand, deregulation of TNF-α and IL-1β prolongs the phase of inflammation phase and leads to delay in wound healing [29]. IL-11 released from cells of bone marrow expresses anti-inflammatory effect. IL-11 inhibits the synthesis of IL-1 and TNF-α synthesis by the inhibition of NFkβP50/P65 with increasing the expression of inhibitory NFkβP50/P50 synthesis in monocytes/macrophage cells [30]. Transition between cytokines of pro and anti-inflammatory is balanced in nondiabetic wounds but in case of diabetic wounds, it gets impaired (**Figure 1**).

For the type-1 diabetes treatment or insulin-dependent diabetes, insulin is administrated systematically.

2. Activation of anti-inflammatory cytokines and increased differentiation of cells by signaling through insulin

Insulin, released from pancreatic gland produced in its beta cells of the islets of Langerhans is a peptide hormone. Insulin precursor is proinsulin in humans, is

encoded by the INS gene and is a single polypeptide; after processing of proinsulin, two secretory proteins are produced, one chain having two chains namely A (21 amino acids) and B (30 amino acids), which forms mature insulin, and the second is C-chain known as C-peptide having 31 amino acids [31, 32]. Chain "A" is more compact having (2 small) α-helix region; on other hand, B chain has 1 such region. Two disulfide bonds between A20-B19 and A7-B7 hold chain A and chain B together; in addition to this, there is a disulfide bridge between A7-A11 cys amino acids of chain A. In the presence of Zn^{2+} and at ~6.0 favorable insulin pH, it folds to hexameric forms and is stored in pancreas. After diffusion of insulin in blood, with change in the pH, the hexameric insulin form changes to its monomeric form and shows binding with the insulin receptor [33]. The insulin binding with receptors depends on the regions present in the insulin monomeric form. The binding regions are present on the surface of insulin receptors; the changes or mutations in the binding regions reduce the insulin binding affinity [34]. The regions are located at TyrA19, AsnA21, CysA20, on the "A" chain C-terminus, IleA2, GlyA1, GluA4, ValA3, on the N terminus and at PheB24, GlyB23, TyrB26, and PheB25 at B" chain C-terminus (**Figure 2**) [35].

The insulin is found only in the humans, but peptides which are like insulin are also present in invertebrates like insects and molasses. Insulin-like peptides are having growth-related functions, and it indicates that the insulin is not only involved in metabolism of glucose but has other functions as well [37]. Drugs which can balance between the pro-inflammatory and anti-inflammatory cytokines can also be helpful for the treatment of other insulin-independent or dependent diabetes mellitus and its linked disease conditions. Using insulin as a wound-healing agent, very few studies have been found.

The anti-inflammatory effect by insulin is shown by activating the cytokine expression that can decrease the inflammation and help in recovery of the wound. Through metabolism and synthesis activities, insulin shows its effect on the differentiation and survival of cells. Insulin promotes NF-kβP50/P50 upregulation by the suppression of TNF-α and p65 expression. NF-kβP50/P65 expression suppression leads to decrease in expression of proinflammatory cytokines like IL-12, IL-1β, IL-6, and TNF-α cytokines at the site of wound [38]. Proinflammatory cytokine inhibition shifts the equilibrium towards the anti-inflammatory cytokine expression, like

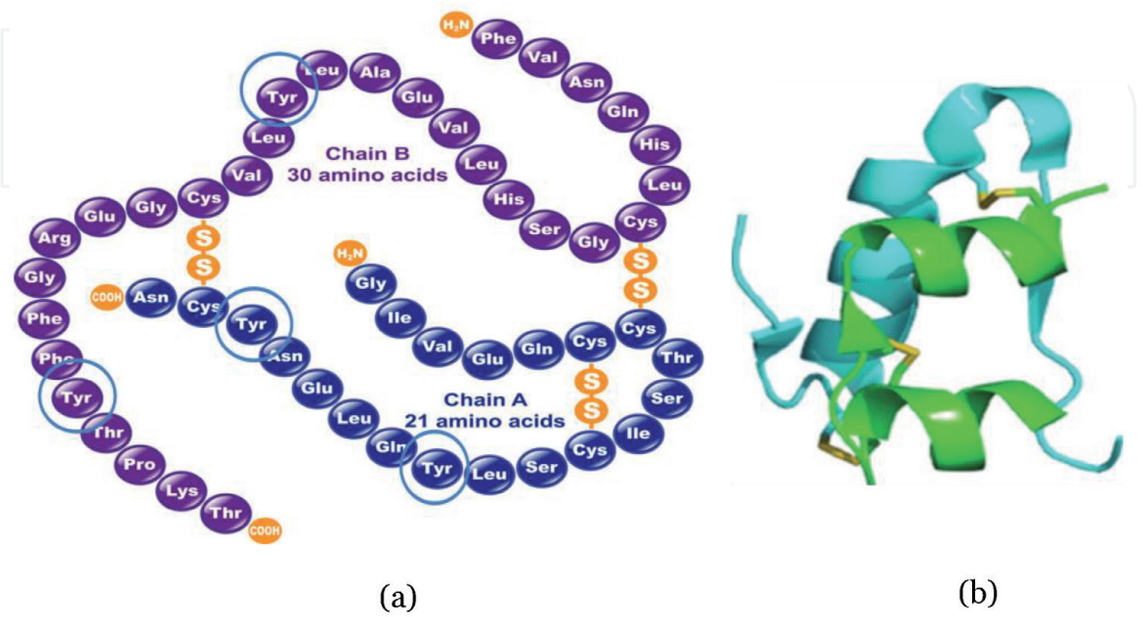

Figure 2.
Insulin structure (a) showing the sequence of amino acids present in insulin protein (b) showing the 3-D model of insulin [36].

IL-4, IL-10, and VEGF, etc., inhibits the apoptosis of cells, and increases proliferation of cell similarly like IGF [39]. Below sections show the regulation of cytokine dynamics by the insulin: (a) Inactivation of NFkβp50/p65 by insulin results in decrease in inflammation by inducing uptake of glucose uptake, (b) biosynthesis of fatty acid induction by insulin and inactivation through TNF-α, (c) role of insulin in cell differentiation and growth by synthesis of protein and inhibition of proteolysis by inactivation of FOXO to promote the survival of cell, (d) insulin functions like IGF and activates the same signaling pathway and reduces inflammation, and (e) anti-inflammatory action of insulin by reduction in proinflammatory cytokines and increased expression of the anti-inflammatory cytokines (**Figure 3**).

2.1 Role of insulin to promote wound recovery

2.1.1 Inactivation of NFkβp50/p65 by insulin results in decrease in inflammation by inducing uptake of glucose

The presence of high concentration of glucose at the wound site promotes microbial growth and leads to inflammatory signaling activation. The main function of insulin in the body is regulation of blood glucose level. It helps in the utilization of the glucose present in the blood through activation of glucose transporters and stored in glycogen form in the cells. The glycogen stored in the tissue of muscles behaves as a source of energy and gets used aerobically [40]. Wounds mainly in peripheral nerves, renal cortex, and retina are results from microcirculatory damage mainly due to increment in consumption of wound by the inflammatory cells, which leads to switch from aerobic glycolytic to anaerobic glycolytic [41]. The direct result of this is the lactic acid formation as the end glycolysis product. In addition to this, other resources of anaerobic glycolysis are the wound-proliferating cells, which are showing anaerobic respiration in the muscle cells [42]. In the blood, the lactic acid gets used in the liver to form glucose.

Lactate converts into pyruvate and nicotinamide adenine dinucleotide (NADH); NADH behaves as a substrate for nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and helps in the formation of reactive species of oxygen (ROS) induced by lactate [43]. Due to more NADPH synthesis, NADPH to NAD+ ratio

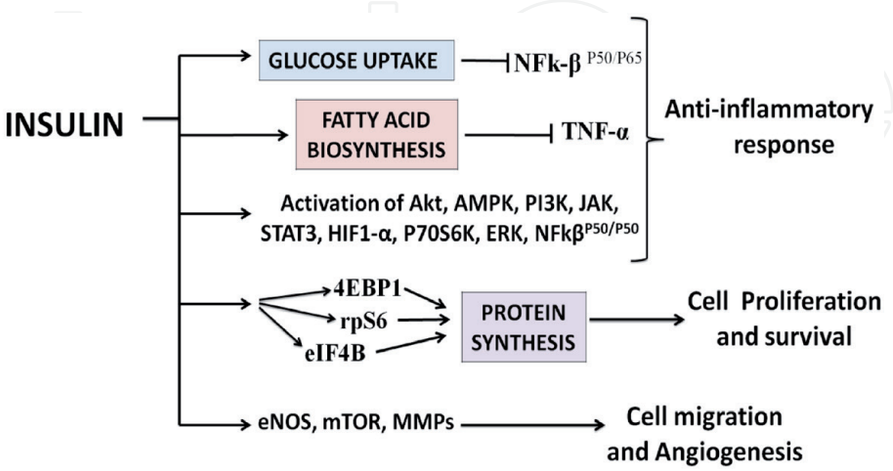

Figure 3.
Insulin plays an important role as an anti-inflammatory agent and helps in the survival of cells by synthesis and metabolic pathways. Glucose metabolism activates NFk-β, and biosynthesis of fatty acids leads to TNF-a activation which can inactivate the inflammatory signaling. By this signaling, insulin helps in the survival of cells and synthesis of protein. Along with this, the insulin activates the Akt pathway and can increase mTOR, MMPs, and eNOS expression leading to the formation of new blood vessels. Insulin can also decrease the NFkβP50/P65 expressions by the ERK and MEK pathways like the pathway for glucose uptake.

reduces, which leads to the VEGF activation and angiogenesis. NADPH and pyruvate, both lead to the formation of new blood vessels and collagen through the inactivation of prolyl hydroxylase hypoxia inducible factor (HIF PHD) [44].

HIF causes the damage of tissue and inflammation at the wound [45]. Hypoxia is also responsible for the damage of peripheral blood vessels and also causes activates NADP oxidase (NOX) to generate oxidative stress in the wound, regulating key factor in the process of wound recovery and leading to inter-cellularly ROS overexpression [46]. High ROS level induces oxidation of protein and peroxidation of lipid, which causes apoptosis of cells [47]. The production of ROS leads to accumulation of NFkβP50/ P65 and inhibits HIF1 α and mTOR expression. In addition to mTOR and HIF1 inhibition, NFkβp50/NFkβp65 induces resistin expression, and both are responsible for intercellular insulin resistance [48]. The resistin leads to the activation of vicious cycle through p65 overexpression [49]. P65 activation shifts the equilibrium from NFκβp50/p50 to NFκβp50/p65 and results in insulin resistance generation [50]. It also induces mTOR and HIF1, by activation of the AKT pathway and inhibition of TNFα [51]. HIF1 activation shifts back to NFκβp50/p65 to NFκβp50/p50 equilibrium. The blood glucose normalization is possible with insulin proper functioning and also by the effective reduction of NFkβP50/P65 expression [52]. NFkβP50/P50 activation reduces expression of proinflammatory cytokines like IL-6 and IL-1β, induces high anti-inflammatory cytokine expression, leads to reduction in inflammation stage, and enhances repairing of tissue [50].

Pyruvate and NADPH inactivate the expression of HIF PHDs, by the oxidation of ascorbic acid and Fe (II). HIF PHDs are the dioxygenase and are 2-oxoglutarate and Fe (II)-dependent and require ascorbic acid. In lactate presence, ascorbic acid and Fe (II) get oxidized and inhibit damage of tissue and increase IL-8release, basal fibroblast growth factor (bFGF) and NF-kβP50/P50 activation [53]. Lactate also upregulates the expression of NF-kβP50/P50 by suppressing the formation of NF-kβP50/P65 and results in reduction of expression of IL-12, IL-1β, TNF-α, and IL-6 cytokines. This pathway ultimately results in more cell viability. Also, ROS-dependent IkBα expression inhibition and VEGF receptors are responsible for synthesis of collagen and angiogenesis [54]. IkBα helps in NFkβ translocation from nucleus, and p65 gene expression in turn is responsible for inflammation [55]. Along with this, NF-kβP50/P65 expression suppression happens through the phosphorylation of ERK through signaling of insulin [56]. In contrast to these findings, the lactate formed in skeletal muscles impairs the signaling of insulin and results in glucose metabolism inhibition [57]. Glucose metabolism signaling of insulin occurs through 6-phosphofructo-1-kinase (PFK-1), which in turn is formed by pyruvate dehydrogenase (PDH) and fructose-2, 6-biphosphate and used for the conversion pyruvate to oxaloacetate. This signaling of insulin is inhibited by lactate through the production of more citrate and reducing fructose-2, 6-biphosphate, and inhibits and promotes PFK-1 expression, respectively. Inhibition of PDH by rising ratio of NADH to NAD ultimately stops the pyruvate to oxaloacetate transformation [58]. This negative effect of lactate on glucose metabolism shows that it acts as an glycolysis inhibitor and results in increase in concentration of glucose in the blood serum [59]. The glucose high concentration in the blood leads to long-time expression of the inflammation cytokines at the wound site (**Figure 4**).

2.1.2 Fatty acid biosynthesis induction by insulin and inactivation through TNF-α pathway

Insulin also plays different other functions like it can stimulate the synthesis of protein and lipogenesis, as well as differentiation and growth of cells [60]. The lipogenesis is the fatty acid synthesis process which converts acetyl-CoA

to triglycerides [61]. Lipogenesis is stimulated by insulin through two types of enzyme activation, PDH (pyruvate dehydrogenase), responsible for pyruvate conversion to acetyl CoA and another acetyl CoA carboxylase helps in conversion of acetyl to malonyl CoA. In the cytoplasm, Malonyl CoA gives 2-C building blocks, used for larger fatty acid synthesis [62]. Transportation of acetyl CoA from mitochondria to cytoplasm occurs by tricarboxylate translocase enzyme, after formation of citrate by reaction with oxaloacetate. The glucose shows a role in increasing the release of both citrate and insulin [63].

Fatty acids, mainly polyunsaturated, play an important role in the formation of cell membrane. Cell membrane composition affects the absorption of enzymes which are responsible for cell phosphatidylinositol 4-kinase (PI4K) proper functioning; membrane associated phosphatidylinositol kinase shows an important role in signaling of cell [64]. The fat metabolism products activate PI4K, which in turn regulates the Protein Kinase C (PKC) functioning and controls proinflammatory cytokine TNF-α signaling [65]. PKC also induces inflammation through increasing the NFkβ and p38MAPK expression. In PI4K presence, the activity of PKC is inhibited, which leads to the reduction of proinflammatory cytokine (TNF-α) release [66]. The free fatty acid component plays an important role in the wound recovery process (**Figure 4**).

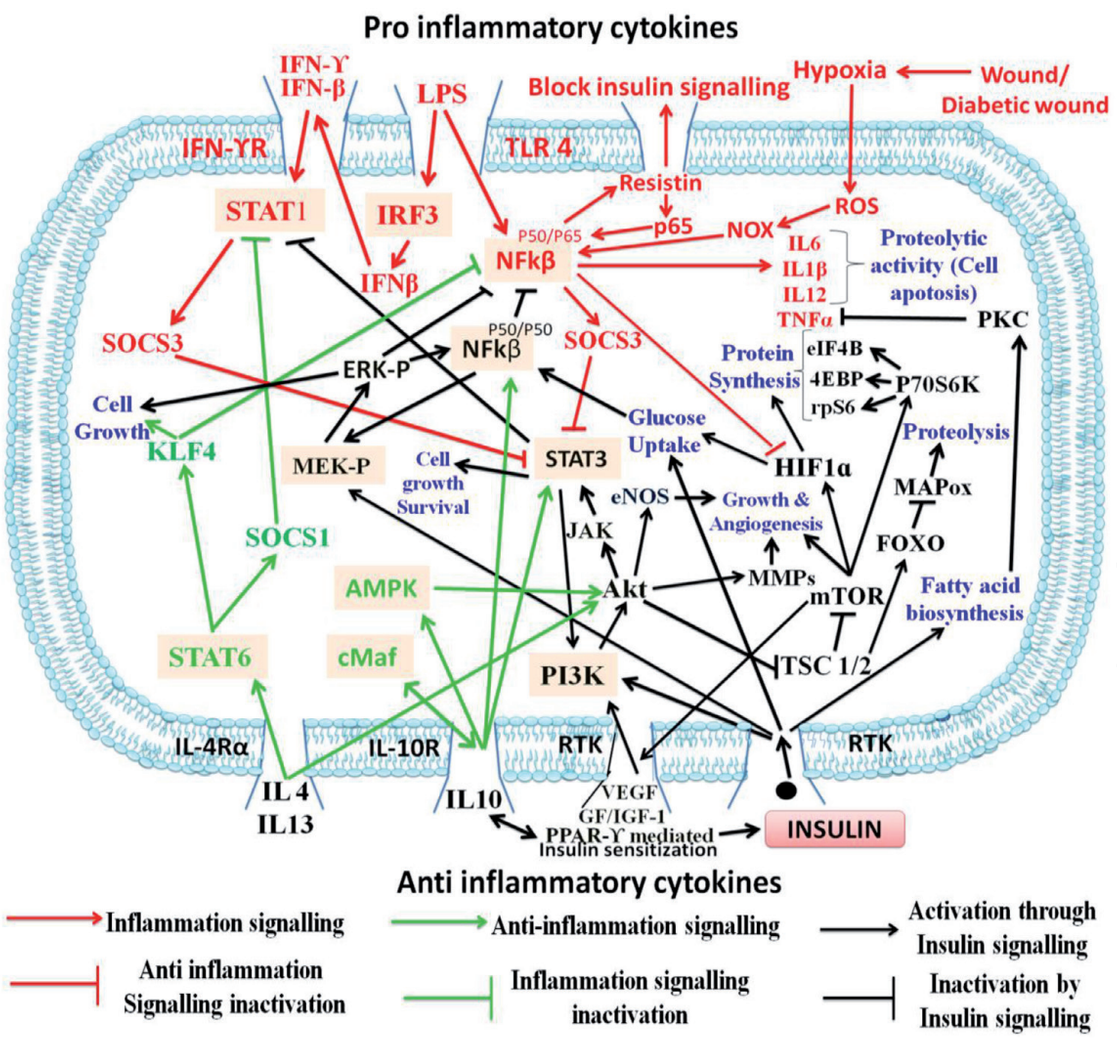

Figure 4.
Transition pathway from M1 to M2 macrophages. TNF α and IFN ϒ activate NFk β, STAT1, and IRF-3 at the wound site and help in the release of IL-10, IL-12, NFkβP50/P65, TNF-α, IL-1β, and STAT-1 leading to inflammation. M1 to M2 transition is important for wound recovery. IL-10, IL-4, IL-13, VEGF, insulin, and IGF can activate HIF-α, STAT3, and NFkβP50/P50 cytokines to activate the anti-inflammatory action.

2.1.3 Role of insulin in cell differentiation and growth by synthesis of protein and proteolysis inhibition by the inactivation of FOXO to enhance the survival of the cell and tissue

Insulin stimulates the synthesis of protein in different cells and tissues. In muscle tissues, insulin affects the flow of blood and amino acids uptake by the tissues of muscle and helps in anabolism in the muscles [67]. It has been studied that insulin systematic uptake loses the muscle volume, which mainly happens due to insulin systematic infusion and results in the reduction of the amount of free amino acids in blood and plays an important role in the anabolism in muscles [68]. The deficiency of insulin can be overcome by systematically giving insulin exogenously [69]. Essential protein formation is stimulated by promoting the concentration of RNA contents in the cells and tissues by the insulin functioning pathway through the translocation of the mRNA by the phosphoinositide-3-kinase (PI3K) pathway [70]. By the PI3K pathway, an Akt inhibits the tuberous sclerosis protein ½ (TSC1/2) functioning and behaves like an inhibitor of the mechanistic target of rapamycin (mTOR) and in the end by phosphorylation of 4E Binding protein (4EBP-1leads it activates the eukaryotic initiation factor (eIF4B)). The eukaryotic 2^0 structured mRNA 5'end eIF4B binds. During the synthesis of protein, eIF4B binds with subunits of eIF4A and eIF4G, further binding with ribosome 40S, and it has RNA helicase absent or low results in impairment in the synthesis of protein. The mTOR activation leads to proteolysis inhibition by MAPox activation [71]. High concentration of insulin in the muscle cells inhibits the protein degradation and leads to muscle cell and tissue expansion [72]. Due to inhibition of protein degradation, the insulin ultimately leads to reduce the blood amino acids concentration [73]. This amino acid concentration regulation of insulin clearly indicates that insulin plays a very important part in the diabetic wound healing condition when the patients are on systemic treatment of insulin (**Figure 4**).

2.1.4 Insulin behaves the same as insulin-like growth factor and can also activate similar pathway to decrease the inflammation

Insulin-like growth factor (IGF) is composed of two IGF ligands such as IGF-I and II. At the time of embryogenesis, IGF are the proteins that regulate development and growth, tissue differentiation in adults and show anti-inflammatory actions through the activation of anti-inflammatory cytokines. Insulin shows anti-inflammatory effect by increasing release of IL-10 and IL-13/4. By decreasing pro-inflammatory cytokines release (IFN-ϒ) [74]. IGFs show binding with the insulin receptor (IR), insulin-related receptor, and IGF receptor (IGF-1 and IGF-2). Main functions of IGF-I and II are mediated by Insulin Growth Factor Insulin receptor (IGF-IR) [75]. IGF-I is an important growth factor produced by the macrophages, fibroblast keratinocyte, and platelets. It enhances endothelial cell migration into the wound site. It enhances the mitosis and fibroblast cell proliferation for the new blood vessel formation and extracellular matrix and activation of protein kinase B signaling. In addition to this, it also enhances the synthesis of protein and blocks the muscles atrophy in order to skeletal hypertrophy catalysis [76].

Upon binding with receptor, the IGF-I activates the insulin receptor substrate-1 (IRS-1) which in turn by phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) phosphorylates the protein kinase B (Akt). Phosphorylated Akt activates the mTOR; PI3K-related kinase controls the proliferation of the cells [77]. Also, IGF-I enhances cell growth by activating the mitogen-activated protein kinase/extracellular signal regulated kinase/MAPK/ERK pathway through RAF/RAS kinase phosphorylation of [78]. Along with this, IGF-I binding of receptor enhances the

secretion of anti-inflammatory cytokine such as IL-10 activates Akt by AMPK signaling. Likewise, such as IL-4 and IL-10 can also bind with Akt and plays role in M2 macrophages infiltration at the site of wound (**Figure 4**).

2.1.5 The anti-inflammatory action of insulin through a reduction in pro-inflammatory cytokine expression enhances the formation of anti-inflammatory cytokines

The decrease in action of insulin may be due to resistance of insulin or due to the insufficient insulin release, and it ultimately results in diabetes mellitus. The functioning of insulin either reduces due to the β-cells functioning loss or due to the improper functioning of insulin receptors or due to the kidney disease [79]. The insulin treatment systemically is already taken by 6 million people of America, and it keeps on increasing to control high blood glucose condition. High blood glucose concentration leads to the tissue damage by the oxidative stress through increasing flux of other sugars and glucose by the polyol pathway and also enhances the expression end products of advanced glycation and it's activating ligand receptors and through the overexpression of the pathway of hexosamine and activation of protein kinase. The mechanisms mainly take place by the overexpression of mitochondrial ROS [80]. In the polyol pathway, due to more NADPH consumption in the glucose transport pathway, more redox stress is generated and remains insufficient to form the scavengers of ROS that is GSH reduced form advanced glycinated product precursor formation modifies the proteins of plasma that can bind with the receptors of the advanced glycination product present on the surface of macrophages, smooth cells and vascular endothelial cells. This activation of NFkβ transcription factor, in turns activates HIF-α and results in hypoxia stimulated chemokines production through the ROS production [81]. In the presence of high glucose, the protein kinase enzyme shows hyperactivity and stimulates the expression of eNOS in the smooth muscles cells and leads to the destruction of tissue. Increased ROS expression shows the activation of different proinflammation pathways and helps in generating the epigenetic changes, which can result in the prolonged expression of the proinflammatory genes during the wound recovery. Matrix metalloproteinase (MMP-2, 4) excessive production impairs the recovery process of wound and results in extracellular matrix protein breakdown such as vitronectin and fibronectin [82].

In nondiabetic wounds, the wound healing process involves the activation of the series of different physiological events for wound recovery like inflammation at wound site, cell proliferation, epithelisation of cells, vascularisation, maturation, and re-modeling at the site of wound [83]. Macrophages play an important role in the whole healing process. At early wound phase, in the wound recovery process, macrophages function by the cytokine release and activation of leucocytes, which leads to the production of inflammatory response at the site of the wound [84].

The infiltration of the macrophage at the wound site takes place by the effect of chemotaxis which induces the factors like Toll-like receptor (TLR) ligand, PAMP (pathogen-associated molecular patterns), LPS (lipopolysaccharide), PDGF, and IFN-gamma (IFN-ϒ) [85]. M1 macrophages lead to high level secretion of STAT1 and expression of TNF-α or IFN-β. By the activation of the Akt/PI3 pathway, insulin stimulates STAT3, which inhibits STAT1 formation and activates the transition from M1 macrophages to M2 macrophages for the repairing of wound and tissue repairing. M2 macrophages can help in the production of polyamines and ornithine by the pathway of arginase enzyme and anti-inflammatory pathway IL-10, IL-13and IL-4 cytokines [86]. Insulin along with M2 macrophages activates the anti-inflammatory cytokines by Akt, or IP3K pathway activates biosynthesis of protein to induce fatty acid and blood vessel formation and division and migration of cells, to increase

wound recovery. With resistance of insulin in diabetic condition, there is constant increase in concentration of proinflammatory cytokines TNFα and IL-6 have been shown in the figure. In the non-diabetic/normal glycemic condition, cytokines are produced by adipocytes such as IL-13, which can promote the M2 activation or alternative macrophages. Alternatively, M2 or activated macrophages are important for the expression of anti-inflammatory cytokine secretion such as IL-10 and PPAR-ϒ (Peroxisome Proliferator-Activated Receptor Gamma) and insulin-sensitizing factors, forming a vicious circle for the functionality of insulin. PPAR-ϒ also activates anti-inflammatory IL-10 cytokine [87] (**Figure 4**).

In the glycemic condition, there is an excessive proinflammatory macrophage expression of cytokines like TNF-α and IL-1β leading to impaired wound recovery. Overactivation of cytokines like IL-17, TNF-α or IL-1β reduces expression of inflammatory cytokines and upregulates genes responsible for wound healing and increases the healing process [86]. In the blood and adipose tissue, the high TNF-α cytokine concentration and TNF-α neutralization improve the insulin sensitivity in the humans or animals. High glucose condition stimulates changes in the gene expression and adipocyte metabolism and lipolysis increment and synthesis of fatty acids (FFAs) and proinflammatory cytokines induces the expressions of macrophages, like tumor necrosis factor α (TNF-α) and monocyte chemotactic protein-1 (MCP-1). M1 macrophage activation produces an excessive concentration of cytokines responsible for inflammation such as TNFα, resistin, and IL-1β, which can act on the cells of adipocyte to make them insulin-resistant. This signaling pathway forms the feedback loop which can increase the resistance to insulin and inflammation [88].

TNF-α, an inflammatory cytokine, performs role in the healing of nondiabetic wound process, but activation of TNF-α for a long time leads to enhancing enzymatic activity of protease enzyme. In human diabetic wounds, MMPs are found in very high amount. In chronic or diabetic wounds, there is imbalance in the expression of cytokines causes proinflammation and their proteases, inhibitors, and their ant protease expression [89].

The switching of macrophages in the high glucose condition gets delayed due to MMPs, IL-1β, IL-6, and ROS cytokine oxidative stress (**Figures 5** and **6**). This leads to delay in M1 to M2 transition and is responsible for the inflammation for long time and leads to delay in the wound recovery [90, 91]. The insulin role in the switching from inflammatory state to anti-inflammatory state is shown in **Figure 5**.

2.1.6 The insulin-like activity of C-peptide

C-peptide consists of only 31 amino acids, is a short peptide and has glycine amino acid-rich regions and behaves like a linker between the two peptides of proinsulin A and B [93]. By ERK1/2 activation and Akt phosphorylation, C-peptide shows angiogenesis. The angiogenesis signaling pathway shows similarity with the VEGF pathway and leads to the formation of nitric oxide by eNOS activation. C-peptide plays a curious role in the cell mitogenesis like insulin, by the same signaling pathway as the insulin protein [94]. C-peptide shows binding to the insulin receptor (IR) and results in intracellular substrate phosphorylation in Ras/MAPK. The PI3K/Akt signaling results in cell division and mitogenesis. Along with the abovementioned two functions, C-peptide also shows its anti-inflammatory effect. C-peptide shows MIP-1α, IL-8, MIP-1β, and IL-6 expression inhibition and other pro-inflammatory cytokine expression [95]. Like insulin, C-peptide can also reduce the problems linked with diabetes, such as vascular inflammation, neuropathy, and nephropathy, in diabetes case especially type 1 diabetes case [96].

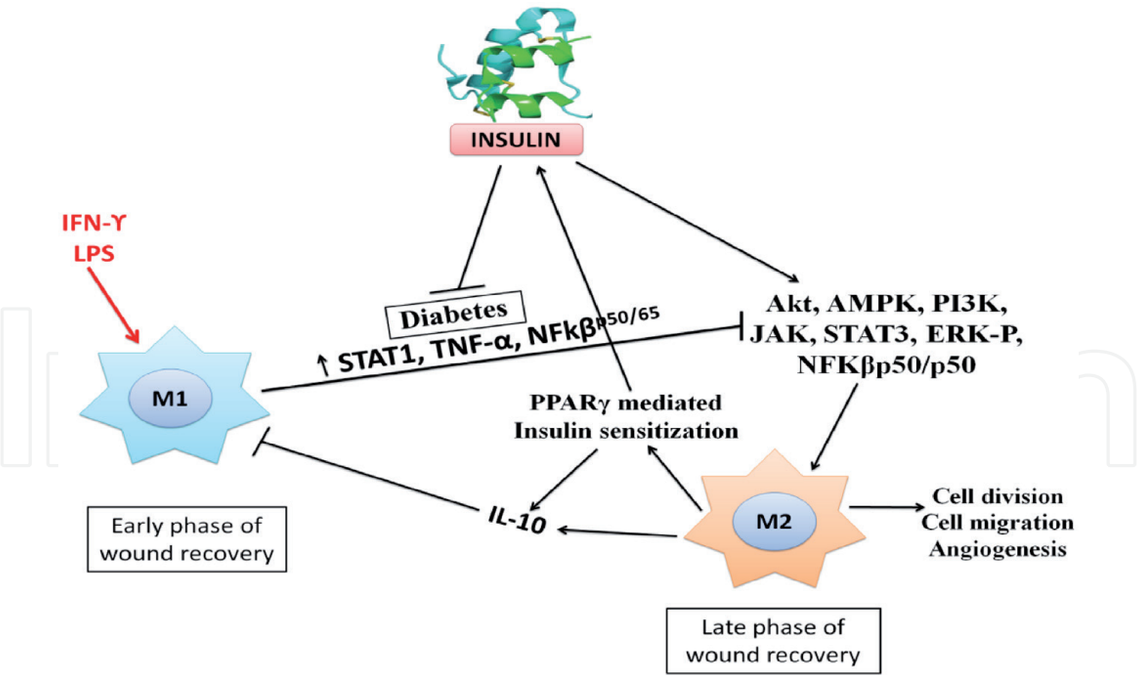

Figure 5.
Effect of insulin on switching of M1 to M2 macrophages. In insulin presence, (AMPK, Akt, STAT3, PKC, HIF-α, PI3K, NFkβP50/P50, and ERK) M2 macrophage expression increases show an anti-inflammatory effect and help in wound healing.

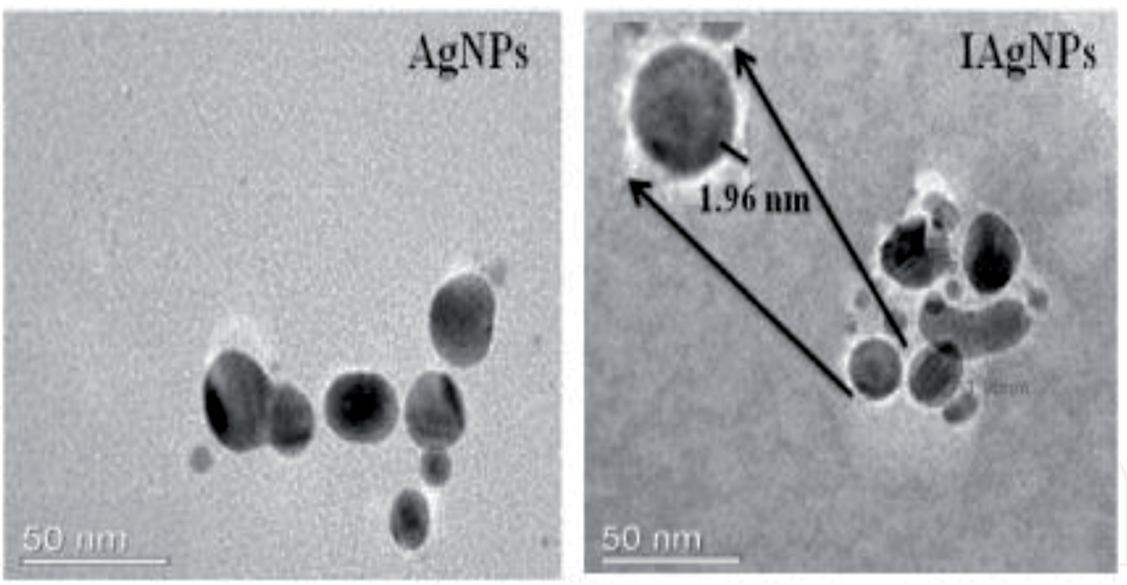

Figure 6.
TEM micrographs of AgNPs and IAgNPs shown with insulin protein coating; the size of AgNPs shifts from around 22 ± 2 to 1.96 ± 0.1 nm. Scale bar: 50 nm [40].

The C-peptide level rises in the blood during diabetic mellitus type 2, which is due to resistance of insulin [97]. At this time, endothelial dysfunction initiated led by the C-peptide deposition in the blood vessel intima walls. The C-peptide deposition causes more inflammation in the blood vessels of the aortic arch and promotes atherosclerotic lesions. The inflammation effect of C-peptide is shown due to C-peptide chemotactic behaviour towards macrophages responsible for inflammation. Monocytes/T-lymphocytes/macrophages migrate through the vessel walls and then release TNF-α, IL-6, and MIF etc., pro-inflammatory cytokines and chemokines and nitric oxide, and activates intracellular signaling pathway [98].

3. Insulin encapsulated mettalic nanoformulations for wound recovery

Nanoparticles of metals such as silver nanoparticles can be used for the delivery of insulin at the site of a wound. Silver nanoparticles have clinical applications due to its antibacterial, anti-inflammatory, and wound healing role. Due to the presence of charge on the surface of metal nanoparticle surface, they are highly reactive and can be surfaces modified by adsorbing different molecules or drugs. The drugs having thiol or amine can easily adsorb on the surface of silver nanoparticles, and due to this, it can help as a drug delivery agent. The anti-inflammatory effect and wound healing (non-diabetic and diabetic) efficiency of silver nanoparticles can be improved by encapsulating the insulin with silver nanoparticles [99, 100].

3.1 Synthesis and characterization of metal insulin nanoparticles

It is very easy to synthesize silver nanoparticles by using reducing and stabilizing agents. Plant extracts like tulsi leaf extract may be used as both reducing and stabilizing agents. The tulsi (*Ocimum tenuiflorum*) aqueous extract of leaves (ATE) was extracted by boiling 3 g of tulsi leaves in water (100 ml for 2 h). After extraction, the extract was allowed to cool and filtered, and the pH of the extract was adjusted to 7.4, and the pH adjusted extract was stored at 4°C for further use. AgNP synthesis was performed by using ATE as a reducing and capping agent. $AgNO_3$ 240 μM was added in ATE (5000 μl) and for 10 min was kept under sunlight. The solution color changed from faint light yellow to reddish brown in the presence of sunlight. After this, AgNPs were incubated with insulin at physiological conditions, the temperature being 37°C for an hour in an incubator in order to produce insulin-protected AgNPs (IAgNPs).

Surface plasmon of nanoparticles with or without protein was monitored using a UV–visible spectrophotometer equipped with Peltier, which showed a resonance peak observed at 352 nm due to the silver nitrate reduction by ATE in sunlight. After incubation with insulin, a blue shift (3 nm) with almost peak intensity double was observed, and λmax was obtained at 349 nm due to the formation of monodispersed IAgNPs. The hydrodynamic size of 22 ± 2 and 42 ± 2 nm (approximately diameter) are observed for AgNPs and IAgNPs, respectively. The Zeta potential showed an increment in the potential values from −12.4 to −15.1 mV due to the conversion of AgNPs to IAgNPs. TEM micrographs showed that both AgNPs and IAgNPs are similar in shape (spherical in shape). AgNP have a size ranging between 20 ± 4 nm, and further, it received a cap of 2 ± 0.5 nm when coated with insulin (IAgNPs) as shown in **Figure 6**.

3.2 Metallic insulin nanoformulation wound healing and anti-inflammatory effect

Wound recovery is promoted by both insulin and IAgNPs in hyperglycemic/diabetic and normal/nondiabetic animal conditions. In both in vivo and in vitro cases, the insulin promotes the wound healing in hyperglycemic and normal conditions. With IAgNPs 12 and 20%, faster wound recovery on treatment's 5th day of the wound was found for nondiabetic and diabetic rats in comparison to the untreated control. Whereas in relation to the IAgNPs, faster wound recovery was shown by free insulin with lesser efficiency with an enhanced rate of 7.27 and 4.67%, respectively, for the normoglycemic and diabetic rate in comparison to untreated rats. The % was 60.0 and 73.33% and with IAgNPs and 33.33 and 40% with only insulin in nondiabetic and diabetic models, respectively, on the 11th day, in comparison to the untreated controls as shown in **Figure 7**.

The quantification of serum showed an increase in anti-inflammatory cytokine percentage and reduction in the expression of inflammatory cytokines in diabetic and normal/normoglycemic animals after treatment with insulin and IAgNPs in comparison to their respective controls. On 5th day, in diabetic rats, the IL-6 concentration was 25%, and TNF-α is in double higher concentration than the normoglycemic control. With treatment of IAgNPs, 50% inhibition of expression of cytokine is much higher than free insulin in both the groups. On the 11th day, IL-6 expression and TNF-α was 30% and 50%, respectively, in control than in normal models, which reduces to 45% in both hyperglycemic and normal animals after treatment with free insulin, and with IAgNPs, the inhibition was around 30% in TNF-α and 40% in IL-6. In addition to reduction in inflammatory cytokine expression, the anti-inflammatory cytokine percentage (IL-10) increases after treating with free insulin and IAgNPs. On the 5th day, IAgNP-treated rats showed that IL-10 increased 50% in diabetic rats and 70% in normal and similarly showed increment in IL-10 concentration of 30% and 45% in diabetic and normal models, respectively, in free insulin-treated groups in comparison to control. On 11th day with IAgNPs anti-inflammatory cytokine concentration was increased by 50 and 65% and with insulin slightly less in both hyperglycemic and normal animals models, respectively. On the 5th and 11th days, the histological evaluations significantly decreased leukocyte infiltration level; faster collagen deposition and fast re-epithelization were observed with insulin and IAgNPs in relation with sub-group (**Figure 8**).

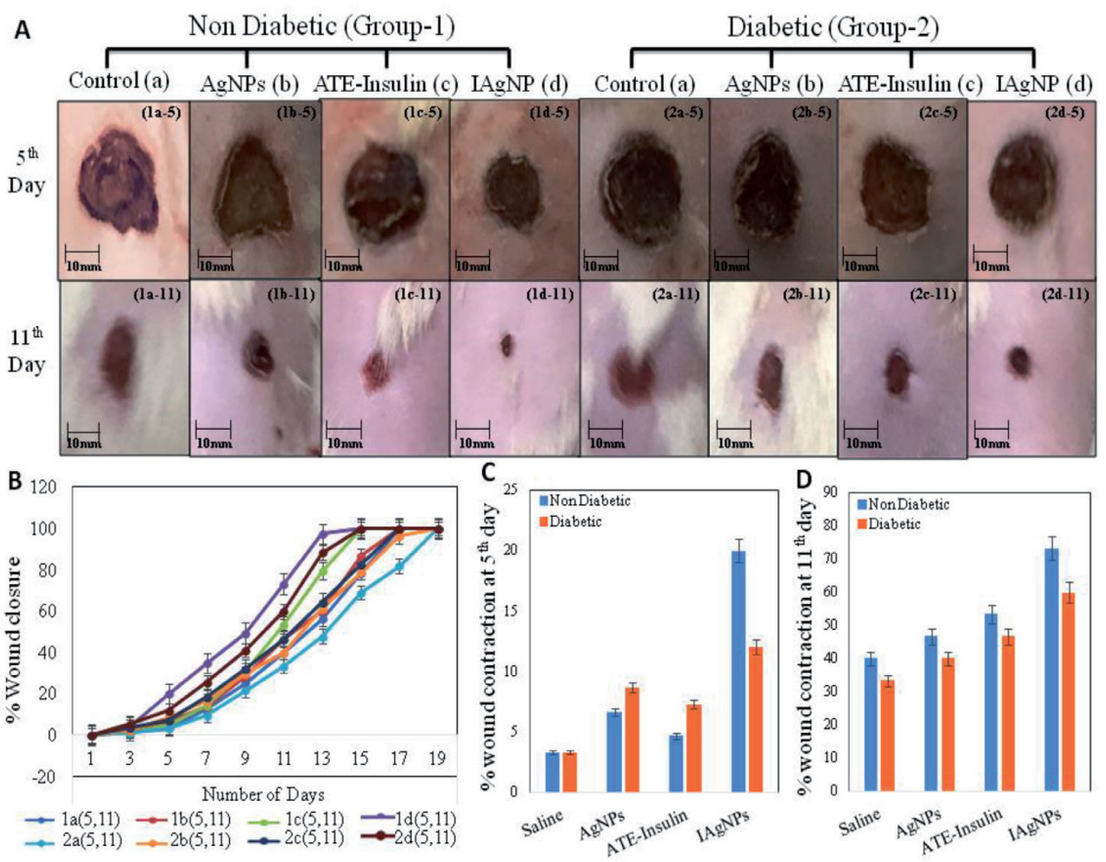

Figure 7.
Wound healing rate of AgNP, ATE-insulin and IAgNP treatment in both hyperglycemic and normal animals on 5th day and 11th day. (A) Wound contraction physical observation in various treatment and control groups. (B) Percentage of closure of wound in different treated groups (AgNPs, ATE-insulin, and IAgNPs) and respective controls of hyperglycemic and normal animals until complete healing of wound takes place. (C) Percentage of contraction of wound in four subgroups of hyperglycemic and normal animals on 5th day. (D) Percentage of contraction of wound in all the four subgroups of hyperglycemic and normal animals on the 11th day [40].

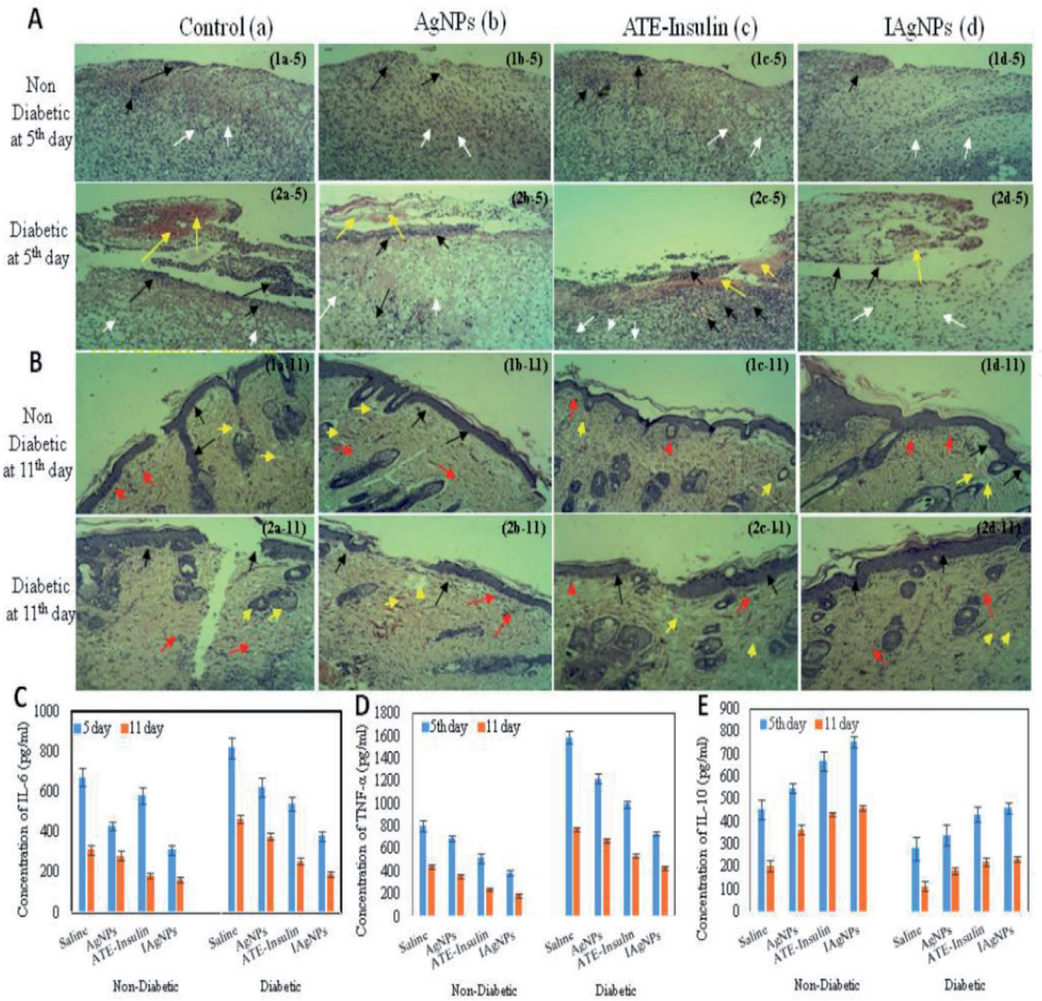

Figure 8.
Histological evaluation (40×) wound site of different groups (a and B, respectively). On the 5th and 11th day of post-treatment, infiltration of leukocyte, formation of exudates and deposition of collagen are denoted by red, yellow and white arrows respectively. Each micrograph represents an overall pattern of a 6-rats group. (C–E). Pro-inflammatory cytokines such as IL-6 and TNF-α and the anti-inflammatory cytokines like IL-10 concentration in all sub-groups of hyperglycemic/and normal animals at 5th and 11th day. TNF-α and IL-6 results show a significant reduction and IL-10 increment by IAgNP treatment in comparison to control, AgNPs, and ATE-insulin-treated animals of both sets on the 5th and 11th day, respectively. Values are shown by the average ± SD of group of six rats [40].

4. Conclusions

Insulin, a hormone which shows the various multiple functions in the body like controlling the inflammation, enhancing the differentiation of cells, biosynthesis of protein and lipid, etc., in addition to controlling the level of glucose in blood through metabolism of glucose. By the metabolism of glucose, the NFkβP50/P50 and IL-8 get activated, causing an inactivation of the IL-1β, TNF-α, NFkβP50/P65, IL-6, resistin IFN- ϒ, and NOX pro-inflammatory cytokines. The metabolism of fat by insulin through inactivating TNF-α mediated pathway also inactivates the pro-inflammatory cytokines. The synthesis of protein gets induced by insulin through Akt; PI3K pathway helps in survival of the cell through the formation of 4EBPI, ribosomal protein S6 (rpS6). This indicates that along with maintaining the blood glucose level, the insulin also shows its anti-inflammatory effect, though the mechanistic aspects of the insulin's anti-inflammatory role is still remained to be elucidated and understood. In addition to biosynthesis and metabolism, insulin pathways have the similarity in structure with IGF-I, can also bind with receptor of IGF and can shows anti-inflammatory activity through PI3K and Akt signaling pathway, which leads to the activation of the

pro-inflammatory cytokines such as STAT-3 and can activate Akt again and promote the formation of blood vessels and increases the eNOS production. Likewise, due to similarity in structure, insulin can bind with the receptors of IGF and activate the same pathway as GF/IGF-I, necessitating further studies on insulin, IGFs and their role in anti- response of inflammation (**Figure 5**). About 5% of the world population is diabetic and are in the risk of nonrecoverable or slow wound recovery. The insulin can increase the recovery of wound by inflammatory dynamics modulating, therefore insulin-like inflammatory modulators (such as IGF) or insulin novel formulations based on and have a huge potential for the different clinical applications such as including the diabetic care and should be explored for the beneficiary purposes.

5. Future perspective

Inflammatory regulation is one of the most important factors for wound recovery which caught attention lately. Here in this chapter, the authors have discussed the role of inflammatory regulators in controlling wound recovery taking insulin as an example and model drug for diabetes treatment where wound recovery get delayed to prolonged inflammation. Macrophages in the wound tissue play a critical role in controlling the wound recovery process. Macrophage plasticity is curtailed in the initiation of tissue regeneration, tissue remodeling, and epithelization. Anti-inflammatory activators which can promote M1 to M2 Macrophage transition have a great influence in the promotion of wound recovery. Therefore, anti-inflammatory molecules can be of great virtue for designing advanced wound recovery agents in the future.

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Conflict of interest

The authors declare that there is no conflict of interest.

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