Topical Insulin Delivery

Subjects: Polymer Science | Biochemistry & Molecular Biology | Medicine, Research & Experimental
Contributor: Pedro Fonte

Insulin is one of the cheapest growth factors in the market able to accelerate the re-epithelialization and stimulate angiogenesis and cell migration. However, the effectiveness of topical insulin in wound healing is hampered by the proteases in the wound bed. The encapsulation into nanoparticles improves its stability in the wound, providing adhesion to the mucosal surface and allowing its sustained release.

Keywords: insulin ; wound healing ; nanomedicine ; topical delivery ; polymer nanoparticle ; lipid nanoparticle ; inorganic nanoparticle

1. Introduction

Wound healing is a biological and dynamic process, in which the skin begins to self-repair after an injury. The prevalence of wounds has been increasing, becoming a significant public health issue with high morbidity and mortality rates among the population. About 100 million people across the globe suffer from acute wounds and 300 million from chronic wounds, representing a major cost for health-care systems. Populations with high-risk of incidence of chronic wounds, such as diabetics, obese, and elderly people, have a high probability of experiencing it during their lifetime [1]. While the treatment of acute and superficial wounds is highly efficacious, treating chronic wounds is challenging [2]. The approach for chronic wound healing has been in reducing the inflammation and topical application of exogenous growth factors. However, ongoing therapies lack efficacy, leading to long-term recovery due to excessive proteases at the wound site [3]. Insulin, a peptide hormone used in diabetic patients to manage blood glucose levels is also used as a growth factor, being able to mitigate the compromised skin by triggering cell migration and proliferation in order to coalesce and heal the wound. Insulin stimulates the migration of keratinocytes, influences the proliferation of fibroblasts and the production of extracellular matrix proteins, and modulates the release of inflammatory cytokines [4]. Moreover, insulin is also a low-cost growth factor and a compatible wound dressing, turning the inclusion of this peptide hormone into dressing matrices beneficial for wound treatment efficacy [5].

Despite the benefits of insulin in wound healing, its lack of stability in the wound bed due to the proteases action hampers its therapeutic benefits. In past years, research in the field of nanomedicine has progressed toward improving nanomaterials features, such as large surface area to volume ratio, antimicrobial activity, or electric conductivity of small-scale particles. Many biological processes occur in the nanometer range, causing the nanoparticles (NPs) to be singular instruments for drug delivery [6]. Thus, the encapsulation of insulin into nanoparticles is an excellent strategy to improve the stability of the protein in the wound bed and deliver it in a controlled manner, improving the therapeutic outcome. Different materials have been explored to produce insulin-loaded nanocarriers, mainly from lipid, polymer, or inorganic sources.

2. Effect of Insulin in Wound Healing

2.1. Diabetic Wounds

Patients suffering from diabetes mellitus manifest several complications such as nephropathy, neuropathy, or retinopathy. In wound healing, cell migration, growth and differentiation are also absent among diabetic patients. The low levels of growth factors, fibroblast dysfunction, increased proteolytic activity, and impairment in collagen assembly are manifested in diabetic wounds. Moreover, diabetic patients show increased levels of TNF-α and IL-6, leading to severe inflammation and insulin resistance [7][8].

Peripheral neuropathy causes chronic wounds in the limbs, commonly referred to as diabetic foot ulcer, lower extremity arterial disease, and foot deformity [9]. It is prevalent in about 7% of diabetic patients and is more recurrent among the elderly. Diabetic foot ulcer care involves a multifaceted procedure, consisting of surgical removal of the necrotic tissue, prevention or treatment of infections, limb elevation, and compression as well as revascularization [10].

In 1923, William Thalhimer was the first to describe the insulin activity in postoperative acidosis [11]. In parallel, Foster showed improvement in postoperative recovery among 20 diabetic patients receiving between 30 and 75 of insulin units intravenously. Wound healing among the diabetic patients was ameliorated and the number of deaths due to infection decreased approximately 30% in type I diabetic patients [12]. In a case study in 1965, a 56-year-old woman with diabetes was amputated below her knee due to gangrene on her foot and leg. The amputated stump became infected with *Streptococcus pyogenes*. She was administrated both cloxacillin and erythromycin, yet no improvement was observed. Thus, the researchers topically administered 20 IU of soluble insulin in gauze covered by a bandage, changing it twice a day. Four days later, the mitigation of the bacterial infection was observed [10]. Weringer tested the influence of insulin on wound recovery using diabetic and non-diabetic C57B1/6 mice treated and non-treated with insulin. The mice ears were perforated with a 0.1 cm dermal trephine and insulin was administered 40 h later. The ear wounds were evaluated by light and electron microscopy and fibroblasts, capillaries, polymorphonuclear leukocytes (PMN), collagen, and interstitial oedema were quantified by lineal point analysis. The untreated diabetic mice showed reduced capillaries, fibroblasts, collagen and PMN of total percent wound volume, in contrast to the similar response of both insulin-treated diabetic mice and control mice. The total percent wound volume for oedema was 44.0 ± 1.2% in the case of untreated diabetic mice, while insulin treated diabetic mice and control showed 13.5% ± 0.7 and 10.5 ± 1.3%, respectively. These results show that insulin treatment induced neovascularization, fibroblast proliferation, synthesis of collagen, and edema reduction in diabetic rats compared to placebo-treated diabetic and healthy rats [13].

Insulin pathways activation were analyzed in rats and diabetic patients with the administration of topical insulin cream [14]. The study compared male Wistar induced diabetic rats treated with and without topical insulin cream, along with non-diabetic rats. Wounds of diabetic rats were treated with 0.5 U/100 g of insulin cream or placebo, applied directly after skin excision and then every day until the end of the study. The authors observed an abrupt cellular response in diabetic animals and faster wound healing concomitant with an increase of protein levels, such as nitric oxide synthase (NOS) and vascular endothelial growth factor (VEGF), than the wounded tissue of normal rats. Conversely, the expression of insulin receptor substrate (IRS), protein kinase B (AKT), and extracellular signal–regulated kinases (ERK) was reduced in the wounded skin of diabetic rats, compared to the wounded skin of normal rats, indicating an increase of wound recovery period. Immunoblotting results showed approximately 25% of IRS-1 protein levels in the wounded skin of diabetic rats, in contrast to 270% of IRS-1 in the wounded skin of normal rats. AKT levels were approximately 50% less in the wounded skin of diabetic rats compared to the wounded skin of normal rats. The extracellular signal-regulated kinase (ERK) pathway was stimulated in the wound healing tissue on healthy rats, in contrast with undamaged skin, which highlights the major contribution that insulin signal transduction occupies in wound healing. In the clinical study, 22 diabetic patients with uncured lacerations for at least 3 months were randomized to assess the effect of topical insulin in a double-blind, placebo-controlled clinical trial for 8 weeks. Changes in the laceration length, width, and depth were observed. After 8 weeks, the patients that received the insulin cream showed a significant recovery in contrast to the patients that received the placebo cream. The treatment using insulin cream continued for both groups, and by the week 15, all patients showed total recovery.

Li and co-workers proposed an effective topical insulin delivery system to protect the insulin's vulnerable structure by encapsulation into microparticles. Later, insulin microparticles were incorporated in a silk fibroin sponge. The authors were able to maintain the molecular structure of insulin and an extended release for 30 days. Moreover, the in vivo restorative effect of the matrix wound dressing was assessed in full-thickness diabetic wounds of Sprague-Dawley rats. After 3 weeks, histological analyses revealed strong cell relocation, collagen deposition, and epidermis, compared to control [15]. Ribeiro et al. prepared chitosan nanoparticles loaded with insulin embedded in a hydrogel and evaluated their therapeutic activity for wound healing in diabetic rats. The chitosan nanoparticles had a size below 200 nm, a positive potential zeta (33.7 ± 4.88 mV), and an AE of approximately 97%. In vivo studies showed the emergence of large blood vessels at day 7 for the animals treated with insulin-chitosan nanoparticles, although, empty and insulin-loaded chitosan nanoparticles were able to stimulate inflammatory cell proliferation and angiogenesis.

2.2. Non-Diabetic Wounds

Non-diabetic wounds are caused by trauma, pressure, inflammation, or cardiovascular disease without a diabetic origin. In comparison with diabetic wounds, non-diabetic wounds have shown less recurrence, lower incidence of infections, and less wound appearance in the lower limbs [16].

In 1930, the Barnet Joseph published a brief communication describing the effect of 10 IU of insulin administered once daily in five patients suffering from non-diabetic pressure ulcers. After 14 days, considerable improvement was observed in skin recovery [17]. Rosenthal, in 1968, used albino Wistar rats to evaluate the stimulation of wound healing caused by insulin. A laceration was performed on the abdomen of rats, and suspensions of protamine-zinc insulin were administered

3 days before the incision and every day thereafter. Insulin-treated animals expressed an increase in body weight and, in parallel, a 20% increase in skin toughness at the wound site [10].

Udupa and colleagues studied the histological aspects of insulin in skin wounds in rats. Albino rats, having an average weight of 110 g, were given 0.02 units of protamine zinc insulin per g bodyweight subcutaneously once per day through a period of 3 weeks. The trial was composed of 25 insulin-treated rats and 25 controls. Incision wounds in the abdominal of 50 mm in length were sutured with cotton stitches and the effects of wound reopening were observed. The insulin treated rats showed increased wound bursting strength in contrast to the control group, demonstrating a significant difference in the 7th and 12th days ($p < 0.01$). Histological evaluation showed accelerated collagen production and higher collagen structure organization, compared to the control group [18]. Additional studies observed the positive effect of insulin on wound recovery. Wilson and co-workers reported an 80-year-old patient suffering from a chronic postoperative abdominal wall wound who underwent negative-pressure wound therapy (NPWT) to improve wound closure, re-epithelialization, and humidity by promoting granulation tissue formation. However, after 21 days of NPWT, the chronic postoperative wound did not heal. The wound was washed with 2 IU/20 mL of human soluble insulin every day for 1 week. There was also no evidence of side effects regarding the use of insulin solution [19].

Zhang et al. (2007) questioned the efficacy of local insulin application in contrast with intravenous therapy concerning wound-healing rate, dropping of blood sugar, and potassium levels, as these signs appear in intravenous insulin administration. Insulin-zinc solution was injected locally around the wound and every other day into white male New Zealand rabbits. The authors noticed a higher healing rate among animals with localized insulin therapy compared to intravenous treatment cohorts [20]. Two years later, Liu et al. also studied the influence of insulin on keratinocyte migration and on PI3K-AKT signal transduction pathway activation in non-diabetic wounds. An in vitro study scratch assay was performed in cultured human keratinocytes, in which insulin treatment stimulated human keratinocyte migration and differentiation. The immunoblot test showed increased AKT activation levels after insulin application for at least 1 h. Furthermore, the in vivo study was composed of C57BL/6J mice with a 0.7 cm punch excision wound. Insulin was topically administered and animal lacerations exhibited a significant epithelium restoration compared to the control group [21].

2.3. Burns

The pathophysiology of burns differs from the biological mechanism for healing incisional and excisional wounds. The frequency of episodes of hyperglycemia and insulin resistance has been observed in patients with severe burns, decreasing the probability of wound healing. Studies have been conducted to obtain more information about the chemical processes involved during burning and on the effectiveness of its treatment with insulin [22]. In 1995, Sakurai et al. administered insulin intravenously to nine patients suffering from severe burns for 1 week to assess the variation in muscle protein levels. The novel amino acids production, their relocation from the blood, and a significant increase in muscle mass was observed due to the presence of insulin. Although the results were variable among patients, the authors observed that insulin treatment caused an increase of nearly 50% in protein synthesis at the wound site [23]. Later, in 1999, Zhang and collaborators considered the effect of insulin and growth hormone on protein synthesis in muscle and wounds. L-[ring-13C6]phenylalanine was used to determine protein anabolism. Rabbits which had undergone thermal ear burns were randomly divided into four groups: growth hormone, high-dose insulin, low-dose insulin, and blank control groups. Both high and low doses of insulin significantly decreased protein breakdown ($p < 0.01$), stimulating protein synthesis and inhibiting proteolysis, compared to the growth hormone group in which there was no change in protein balance [24]. Zhang and collaborators analyzed the protein levels in partial-thickness wounds through the introduction of insulin and several amino acids. Male New Zealand White rabbit ears were subjected to 72 °C water for 3 s. The scalded ears were used as an arteriovenous unit to measure the response of protein kinetics in the wound. The authors found increased wound protein production with both an exogenous amino acid mixture and high dose of insulin infusion administration, moving from 7 ± 4 μmol.100 $g^{-1}.h^{-1}$ to 1 ± 5 μmol.100 $g^{-1}.h^{-1}$ in the control group [25].

In 2001, Van den Berghe and co-workers evaluated the recovery rate of patients who received insulin at the surgical intensive care unit for one year. The authors conducted a prospective, randomized, controlled clinical trial where patients with assisted ventilation were divided into two groups. One group received a common insulin infusion therapy while the other experienced exhaustive insulin treatment. In the common insulin infusion group, glycemia was kept between 180 and 200 mg/dL, and insulin was only administered if the blood glucose level passed 215 mg/dL. In the exhaustive insulin therapy group, the glycemia was kept between 80 and 110 mg/dL. The authors verified that exhaustive insulin infusion reduced mortality by 8%, in contrast to 4.5% mortality reduction in the common insulin infusion group. Furthermore, patients who received exhaustive insulin treatment did not need extended assisted ventilation [26]. It has been suggested that severe burns cause insulin resistance along with impaired insulin signal. Following a severe burn, an acute phase

reaction occurs in the liver, stimulating an inflammatory response and the immune system. In 2007, Jeschke et al. studied the impact of insulin therapy on severely burned hepatic-failure patients. The researchers observed improvement in liver function in burned patients treated with insulin by finding reduced levels of transcription factors and inflammatory cytokines. Insulin therapy increased recovery chances by minimizing sepsis in critically burned patients [27]. In 2010, Jeschke and collaborators explored the consequence of insulin administration on death rates in severely burned pediatric patients having burns above 30% of TBSA. The mortality rate for the control group was 11%, whereas the mortality rate for the pediatric insulin patients group was lower by 7% [28].

In 2012, Jeschke et al. developed a clinical study in severely burned children over 15 months to estimate the correlation between hyperglycemia and insulin resistance within unfolded protein response (UPR). UPR is a cellular stress response associated with endoplasmic reticulum (ER) stress. Researchers concluded that during patient rehabilitation, biochemical processes linked to the cell cycle, swelling, sarcoplasmic reticulum stress, and insulin resistance were altered [29]. Later, in 2013, Vural's team examined the link between insulin and liver. The study was developed in severely burned patients who were receiving insulin. The authors found increased prealbumin and albumin levels, and decreased triglycerides and pro-inflammatory proteins levels with insulin therapy [30].

In 2010, Fram and co-workers found that insulin administration was safe and efficient in pediatric burn patients due to decrease of insulin resistance and improvement of metabolic hemostasis. A randomized clinical trial was conducted in burned pediatric patients whose burns exceeded 40% of TBSA. Two groups were created: conventional insulin treatment and intensive insulin treatment. The patients in the first group received an average insulin concentration of 55 µU/mL and had their blood glucose values below 215 mg/dL, while the second group received 105 µU/mL of insulin and had their blood glucose values between 80 and 110 mg/dL. Reduction of hepatic glucose secretion was lower in the conventional insulin treatment, in contrast to intensive insulin treatment, being 2.5 ± 0.6 vs. 5.0 ± 0.9 mg/kg·min, respectively. Intensive insulin treatment significantly improved mitochondrial oxidation of palmitate [31]. Tuvdendorj et al. performed skin transplants on burned children with more than 40% TBSA and examined the effect of insulin on the accelerating healing rate. In the first days after surgery, the authors observed an increase in the fractional synthetic rate (FSR) of the donor site wound protein, leading to increased collagen and laminin levels [32].

Azevedo et al. (2015) studied the effects of topical insulin application on wound healing in rats subjected to second-degree burns on the increase of collagen retention, stimulation of the microvascular network, and depression of the inflammatory phase. The animals were divided into four groups: diabetic rats receiving topical insulin cream, diabetic rats receiving placebo, healthy rats receiving topical insulin cream, and healthy rats receiving placebo. Histological examination showed increased Type III collagen levels between days 1 and 7 in healthy and diabetic wounds treated with insulin cream, compared to diabetic wounds treated with placebo. Angiogenesis and inflammatory responses were significantly higher at 2 weeks postburn in wounds treated with insulin cream to levels related to those of healthy rats receiving topical insulin cream and placebo [33]. Dhall et al. improved insulin stability required for wound healing by developing an alginate dressing containing insulin encapsulated in PLGA microparticles and showing sustained release for more than 3 weeks. The in vivo study was performed on the dorsum of Sprague–Dawley rats that had partial-thickness burn wounds made with a 15 mm diameter brass cylinder warmed up to 80 °C. Alginate dressings having 0.04 mg insulin/cm^2 were then applied once every 3 days for 9 days, promoting higher levels of neutrophils and M1 macrophages. Histological examination showed increased collagen levels with fibers deposited neatly on the skin and a lower rate of necrotic tissue [34].

References

1. Frykberg, R.G.; Banks, J. Challenges in the treatment of chronic wounds. Adv. Wound Care 2015, 4, 560–582.
2. Menke, N.B.; Ward, K.R.; Witten, T.M.; Bonchev, D.G.; Diegelmann, R.F. Impaired wound healing. Clin. Dermatol. 2007, 25, 19–25.
3. Besson, J.C.F.; Hernandes, L.; Campos, J.M.; Morikawa, K.A.; Bersani-Amado, C.A.; Matioli, G. Insulin complexed with cyclodextrins stimulates epithelialization and neovascularization of skin wound healing in rats. Injury 2017, 48, 2417–2425.
4. Lai-Cheong, J.E.; McGrath, J.A. Structure and function of skin, hair and nails. Medicine 2013, 41, 317–320.
5. Gallo, R.L.; Nizet, V. Innate barriers against skin infection and associated disorders. Drug Discov. Today Dis. Mech. 2008, 5, 145–152.
6. Sen, C.K.; Gordillo, G.M.; Roy, S.; Kirsner, R.; Lambert, L.; Hunt, T.K.; Gottrup, F.; Gurtner, G.C.; Longaker, M.T. Human skin wounds: A major and snowballing threat to public health and the economy. Wound Repair. Regen. 2009,

17, 763–771.

7. Gary Sibbald, R.; Woo, K.Y. The biology of chronic foot ulcers in persons with diabetes. Diabetes Metab. Res. Rev. 2008, 24 (Suppl. 1), 25–30.
8. Patel, S.; Srivastava, S.; Singh, M.R.; Singh, D. Mechanistic insight into diabetic wounds: Pathogenesis, molecular targets and treatment strategies to pace wound healing. Biomed. Pharmacother. Biomed. Pharmacother. 2019, 112, 108615.
9. May, K. Preventing foot ulcers. Aust. Prescr. 2008, 31, 94–96.
10. Rosenthal, S.P. Acceleration of primary wound healing by insulin. Arch. Surg. 1968, 96, 53–55.
11. Thalhimer, W. Insulin treatment of postoperative (nondiabetic) acidosis. J. Am. Med. Assoc. 1923, 81, 383–385.
12. Foster, N.B. Diabetic coma. J. Am. Med. Assoc. 1925, 84, 719–722.
13. Weringer, E.J.; Kelso, J.M.; Tamai, I.Y.; Arquilla, E.R. Effects of insulin on wound healing in diabetic mice. Acta Endocrinol. 1982, 99, 101–108.
14. Lima, M.H.; Caricilli, A.M.; de Abreu, L.L.; Araújo, E.P.; Pelegrinelli, F.F.; Thirone, A.C.; Tsukumo, D.M.; Pessoa, A.F.; dos Santos, M.F.; de Moraes, M.A.; et al. Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: A double-blind placebo-controlled clinical trial. PLoS ONE 2012, 7, e36974.
15. Li, X.; Liu, Y.; Zhang, J.; You, R.; Qu, J.; Li, M. Functionalized silk fibroin dressing with topical bioactive insulin release for accelerated chronic wound healing. Mater. Sci. Eng. C Mater. Biol. Appl. 2017, 72, 394–404.
16. Rohrmann, A.; Young, D.; Abbas, Z.; Kish, B.; Robinson, B. Profile of diabetic and non-diabetic patients treated for wounds or ulcers. Foot Care Can. J. Diabetes 2013, 1, 42–47.
17. Joseph, B. Insulin in the treatment of non-diabetic bed sores. Ann. Surg. 1930, 92, 318.
18. Udupa, K.N.; Chansouria, J.P. The role of protamine zinc insulin in accelerating wound healing in the rat. Br. J. Surg. 1971, 58, 673–675.
19. Wilson, J.M.; Baines, R.; Babu, E.D.; Kelley, C.J. A role for topical insulin in the management problematic surgical wounds. Ann. R. Coll. Surg. Engl. 2008, 90, 160.
20. Zhang, X.J.; Chinkes, D.L.; Sadagopa Ramanujam, V.M.; Wolfe, R.R. Local injection of insulin-zinc stimulates DNA synthesis in skin donor site wound. Wound Repair Regen. 2007, 15, 258–265.
21. Liu, Y.; Petreaca, M.; Yao, M.; Martins-Green, M. Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. BMC Cell Biol. 2009, 10, 1–15.
22. Rowan, M.P.; Cancio, L.C.; Elster, E.A.; Burmeister, D.M.; Rose, L.F.; Natesan, S.; Chan, R.K.; Christy, R.J.; Chung, K.K. Burn wound healing and treatment: Review and advancements. Crit. Care 2015, 19, 243.
23. Sakurai, Y.; Aarsland, A.; Herndon, D.N.; Chinkes, D.L.; Pierre, E.; Nguyen, T.T.; Patterson, B.W.; Wolfe, R.R. Stimulation of muscle protein synthesis by long-term insulin infusion in severely burned patients. Ann. Surg. 1995, 222, 283.
24. Zhang, X.J.; Chinkes, D.L.; Wolf, S.E.; Wolfe, R.R. Insulin but not growth hormone stimulates protein anabolism in skin wound and muscle. Am. J. Physiol. 1999, 276, E712–E720.
25. Zhang, X.J.; Chinkes, D.L.; Irtun, O.; Wolfe, R.R. Anabolic action of insulin on skin wound protein is augmented by exogenous amino acids. Am. J. Physiol. 2002, 282, 1308–1315.
26. Van den Berghe, G.; Wouters, P.; Weekers, F.; Verwaest, C.; Bruyninckx, F.; Schetz, M.; Vlasselaers, D.; Ferdinande, P.; Lauwers, P.; Bouillon, R. Intensive insulin therapy in critically Ill patients. N. Engl. J. Med. 2001, 345, 1359–1367.
27. Jeschke, M.G.; Boehning, D.F.; Finnerty, C.C.; Herndon, D.N. Effect of insulin on the inflammatory and acute phase response after burn injury. Crit. Care Med. 2007, 35, 519–523.
28. Jeschke, M.G.; Kulp, G.A.; Kraft, R.; Finnerty, C.C.; Mlcak, R.; Lee, J.O.; Herndon, D.N. Intensive insulin therapy in severely burned pediatric patients: A prospective randomized trial. Am. J. Respir. Crit. Care Med. 2010, 182, 351–359.
29. Jeschke, M.G.; Finnerty, C.C.; Herndon, D.N.; Song, J.; Boehning, D.; Tompkins, R.G.; Baker, H.V.; Gauglitz, G.G. Severe injury is associated with insulin resistance, endoplasmic reticulum stress response, and unfolded protein response. Ann. Surg. 2012, 255, 370–378.
30. Kabalak, A.A.; Ceylan, A.; Vural, Ç. Effect of insulin treatment to systemic inflammatory response in burn injury. N. Engl. J. Med. 2013, 30, 191–194.
31. Fram, R.Y.; Cree, M.G.; Wolfe, R.R.; Mlcak, R.P.; Qian, T.; Chinkes, D.L.; Herndon, D.N. Intensive insulin therapy improves insulin sensitivity and mitochondrial function in severely burned children. Crit. Care Med. 2010, 38, 1475–

1483.

32. Tuvdendorj, D.; Zhang, X.J.; Chinkes, D.L.; Aarsland, A.; Kulp, G.A.; Jeschke, M.G.; Herndon, D.N. Intensive insulin treatment increases donor site wound protein synthesis in burn patients. Surgery 2011, 149, 512–518.
33. Azevedo, F.; Pessoa, A.; Moreira, G.; Dos Santos, M.; Liberti, E.; Araujo, E.; Carvalho, C.; Saad, M.; Lima, M.H. Effect of topical insulin on second-degree burns in diabetic rats. Biol. Res. Nurs. 2016, 18, 181–192.
34. Dhall, S.; Silva, J.P.; Liu, Y.; Hrynyk, M.; Garcia, M.; Chan, A.; Lyubovitsky, J.; Neufeld, R.J.; Martins-Green, M. Release of insulin from PLGA-alginate dressing stimulates regenerative healing of burn wounds in rats. Clin. Sci. 2015, 129, 1115–1129.