The Impact of Diabetes on Myofibroblast Activity and Its Potential Therapeutic Treatments

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INTRODUCTION

- According to the International Diabetes Federation, ~463 million adults (age 20-79 years) were living with diabetes in 2019, which is predicted to rise to 700 million by 2045.
- Those with diabetes are 10-20x more likely to undergo an amputation due to chronic ulcers.
- In normal wound healing there is a reliable progression of inflammation, proliferation, and tissue remodeling.
- Chronic hyperglycemic states can dysregulate fibroblast to myofibroblast differentiation and myofibroblast activity which leads to a lack of cellular proliferation and an increase in proteolytic activity that prevents sufficient deposition of the ECM.

Influence of Diabetes on Wound Healing Phases

Inflammatory phase	Proliferative phase	Remodeling phase
• M2↓ • Growth factors ↓ (IGF-1, VEGF, TGF-β1)	• Angiogenesis ↓ • Epithelialization ↓ • Fibroblast differentiation ↓	• MMPs ↑ • TIMPs ↓ • Collagen deposition ↓ • Wound contraction ↓

SIGNALING PATHWAYS THAT AFFECT MYOFIBROBLASTS IN DIABETES

TGF-β/Smad Signaling Pathway

- TGF-β promotes myofibroblast formation directly through the canonical pathway.
  - The interplay between TGF-β isoforms and specific transcription factors, including Smad proteins, leads to transcription and expression of α-SMA, a marker for myofibroblast differentiation.
- In non-obese Goto-Kakizaki diabetic rats, levels of TGF-β1 mRNA and protein were found to be decreased in nearby excisional wounds which correlated with a downregulation of α-SMA and myofibroblast differentiation as well as disturbed myofibroblast proliferation.[1]
- Cells at the wound margins on genetically diabetic db/db mice also found TGF-β1 to be reduced as well as consistently lower α-SMA levels, which presented with delayed wound closure and impaired contraction.[2,3]

IGF-1 Signaling Pathway

- IGF-1 is a cytokine that is primarily found in the epithelium at the later stages of granulation that is responsible for the mitogenic action of myofibroblast proliferation and fibroblast differentiation
  - It also plays a key role in ECM collagen synthesis and stimulation of fibroblasts and keratinocytes.
- In streptozotocin diabetic rats, IGF-1 levels were found to be decreased during wound healing.[4]
- Fibroblasts were found to be resistant to growth factors such as IGF-I and EGF after creating a hyperglycemic environment in fibroblast cultures.[5]
- An absence of IGF-1 proteins was noted at the edge of the diabetic foot wounds in humans[55], which led to lower levels of myofibroblasts and overall wound healing and repair.[6]

HIF-1 Signaling Pathway

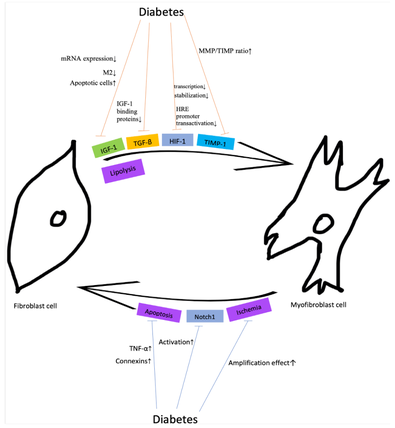
- HIF-1 triggers fibroblast differentiation into a myofibroblast by increasing lactic acid and decreasing intracellular pH.
  - Similarly, inhibition of HIF-1 expression inactivates TGF-β induced myofibroblast differentiation
- Tissues from a diabetic ulcer biopsy displayed reduced HIF-1 expression compared to non-diabetic ulcers.[7]
- Hyperglycemia inhibited HIF-1 mRNA expression directly by lowering the HRE promoter transactivation which is thought decreased amount of myofibroblasts.[8]
- Hyperglycemia interfered with stabilization of HIF-1 by impairing its protective ability against degradation.[9]
- HIF-1 defects that took place in pre-clinical models showed a failure of VEGF and SDF-1 formation response in hypoxic environments and led to impaired myofibroblast function and chronic hypoxia in wounds.[10]

Notch1 Signaling Pathway

- The Notch1 signaling pathway was recently identified as a key regulator of the plasticity and function of fibroblasts in wound healing and angiogenesis.[11]
- An experimental study found that the Notch1 signaling pathway was only activated in the diabetic mouse but not in normal skin or non-diabetic wounds.[12]

MYOFIBROBLAST APOPTOSIS

- When functioning normally, myofibroblasts are highly specialized cells that are differentiated early in the wound healing process and are removed after the granulation tissue phase.
- In streptozotcin diabetic mice, there was a delay in myofibroblast recruitment and differentiation in the early healing phase as well as a long-term persistence after the granulation phase.[13]
- Many in vitro models have shown that hyperglycemia alters fibroblast physiology leading to granulation refractoriness, senescence, and apoptosis.[14]
- An increase in apoptosis of fibroblasts and reduced cell proliferation resulted in delayed epithelial and connective tissue repair.[15]
- Diabetic wounds in db/db mice were found to have significantly higher levels of TNF-α and fibroblast apoptosis and by manipulating TNF-α, fibroblast apoptosis was reduced and myofibroblast density increased.[16]
- Connexins in diabetic wounds were found to be up-regulated, which allowed for increased signaling of cell apoptosis and removal of fibroblasts.[17]



Abbreviations

ECM	Extracellular matrix
M2	Macrophage phenotype type 2
α-SMA	alpha smooth muscle actin
TGF-β	Transforming growth factor beta 1
Smad	mothers-against-decapentaplegic-homolog
IGF-1	insulin-like growth factor 1
HIF-1	hypoxia inducible factor-alpha 1
HRE	hypoxia response element
VEGF	vascular endothelial growth factor
SDF-1	stromal cell derived factor 1
MMP	matrix metalloproteinase
TIMP	tissue inhibitors of metalloproteinases
TNF-α	tumor necrosis factor alpha

IMPACT OF MYOFIBROBLASTS ON ECM IN DIABETIC WOUNDS

MMPs and TIMPs

- Myofibroblasts produce MMPs and TIMPs during the remodeling and maturation phase of wound healing to balance the building up and degradation of the ECM to ensure complete remodeling.
- Hyperglycemic conditions found in diabetes are reported to induce higher levels of MMPs and reduced TIMPs, resulting in abnormal ECM modification and chronic wound healing.[17,18]
- A study on urethral scars found TIMP-1 significantly raised levels of α-SMA, TGF-β and subsequently induced transformation of fibroblasts to myofibroblasts.[19]
- Research on db/db mice has shown severe impairment in VEGF production in combination with the pro-degradative activity of fibroblasts due to the increased amount of MMP-9.[20]

Quality of ECM

- By using 3D biomimetic in vitro models of diabetic foot ulcers, the analyzed fibroblast cell strains responded abnormally to TGF-β1 and produced significantly thinner ECM, which was covered in fibronectin for an increased duration of time.[21]
- Isolated primary fibroblasts in diabetic foot ulcers were also found to have impaired angiogenesis, enhanced proliferation of keratinocytes, and diminished re-epithelialization of the ECM.[22,23]
- In type 1 diabetic humans, subjects were found to have impaired hydroxyproline, a major form of protein in collagen produced by myofibroblasts, which resulted in improperly twisted and stabilized ECM.[24]

Potential Therapeutic Agents for Diabetic Wound Healing

Product/Technique	Author	Year	Title	Type of Study	Conclusion
Photobiomodulation	Mokoena et al.	2020	Photobiomodulation at 660 nm Stimulates Fibroblast Differentiation	Irradiated cell model comparing diabetic to control wound cells	Increased α-SMA (myofibroblast marker), Thy-1 (fibroblast cell marker) decreased in diabetic wounds.
Jamun Honey	Chaudhary et al.	2020	Wound healing efficacy of Jamun honey in diabetic mice model through reepithelialization, collagen deposition and angiogenesis.	Rat model (diabetic vs. control)	Diabetic mice treated with Jamun honey showed enhanced wound closure, collagen deposition, and re-epithelization.
Topical Insulin	Wang et al.	2020	Effects of topical insulin on wound healing: A review of animal and human evidence.	Comprehensive Review of available animal and human studies	Current animal and clinical studies bolster efficacy of topical insulin to treat diabetic wounds.
Keratinocyte Growth Factor 1 (KGF-1)	Peng et al.	2019	KGF-1 accelerates wound contraction through the TGF-β1/Smad signaling pathway in a double- paracrine manner.	Rat model (treated with KGF-1 vs. control)	Secretion of functioning TGF-β1 was induced by KGF-1and wound contraction was accelerated in a double- paracrine manner.
Vitamin D	Yuan et al.	2018	Vitamin D Ameliorates Impaired Wound Healing in Streptozotocin- Induced Diabetic	Diabetic mice vs control	Vitamin D decreased the rate of apoptosis in wound healing. Also, cell viability was significantly
Insulin Growth Factor (IGF-1)	Achar RAN et al.	2014	Use of insulin-like growth factor in the healing of open wounds in diabetic and non-diabetic rats.	Rat model (diabetic vs. control)	Increase in myofibroblast activity (per α-SMA activity), more rapid re-epithelization in diabetic wounds with IGF.
Heparin	Hehenberger et al.	1998	Fibroblasts derived from human chronic diabetic wounds have a decreased proliferation rate, which is recovered by the addition of heparin	Fibroblast cells from the chronic wounds of diabatic and nondiabetic patients	Fibroblast mitogenic activity was stimulated and it is dose-dependent.

CONCLUSION

- Myofibroblasts play an essential role in all phases of wound healing.
- Hyperglycemic conditions in diabetes can alter fibroblast to myofibroblast differentiation and function.
- This interference of myofibroblast activity leads to inappropriate ECM modulation and wound contraction, which is necessary for wound regeneration.
- Understanding the role of myofibroblasts in diabetic wound healing can further benefit management for non-healing diabetic wounds.

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