Keratin Hydrogel Film Accelerates Wound Closure in a Diabetic Mouse Model of Delayed Wound Healing

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Introduction

Diabetes is a pandemic that affects one third of the United States population[1]. One of the common complications of this disease is a chronic wound termed a diabetic foot ulcer (DFU), which affects 15% of diabetics and can lead to osteomyelitis, amputation and death[2-4] at an annual cost of $5 billion in the United States alone[5]. Chronic wounds are first debrided (cleaned) by removal of dying or dead (necrotic) tissue and any infection present. They are then treated for the infection and followed up to make sure infection is absent prior to further treatment. Chronic wounds are typically stalled at the inflammation phase and/or fail to re-epithelialize. When this occurs, often more skin tissue dies off and becomes infected such that the unhealed wound becomes larger with each debridement[6]. DFUs therefore often become chronic wounds that grow larger, fail to heal, or upon healing have a recurrence rate of over 60%. The demand for alternative treatment is therefore substantial.

We reasoned that a wound covering should therefore contain keratin, a major component of the epidermis. Keratin is a complex mixture of subtypes and associated proteins that varies throughout the body. Human hair is readily available and well known to contain keratins that are compatible with *in vitro* cell culture of human fibroblasts and keratinocytes. Thus we developed a proprietary process for selectively extracting a fraction of keratins from human hair and transforming it into a clear, strong, flexible hydrogel film (ProgenaFilm™) as well as a sponge structure featuring highly interconnected porosity (ProgenaFoam™).

Our Mouse Model

Although the leptin receptor-deficient (*db/db*) mouse has been used previously to study delayed wound healing, we developed this model further to quantify a critically important human clinical endpoint: *the time it takes for an open wound to become closed*.

All mice received four equal 6mm diameter full thickness excisional wounds consisting of a control wound and three wounds treated with either keratin film, keratin foam or a commercially obtained advanced wound care product: Oasis Wound Matrix® (Smith & Nephew) or Revitalon™ (Medline). Dressings were placed over the test materials and from bottom to top were: Iodosorb◊ (Smith & Nephew), Skintegrity® (Medline), Band-Aid® Gauze (J & J) and covered with Tegaderm™ transparent film dressing (3M). Dressings and test materials were changed weekly (as is done clinically) at which time the extent of wound healing was measured as the current unhealed wound size. The week the wounds healed was recorded as wound closure.

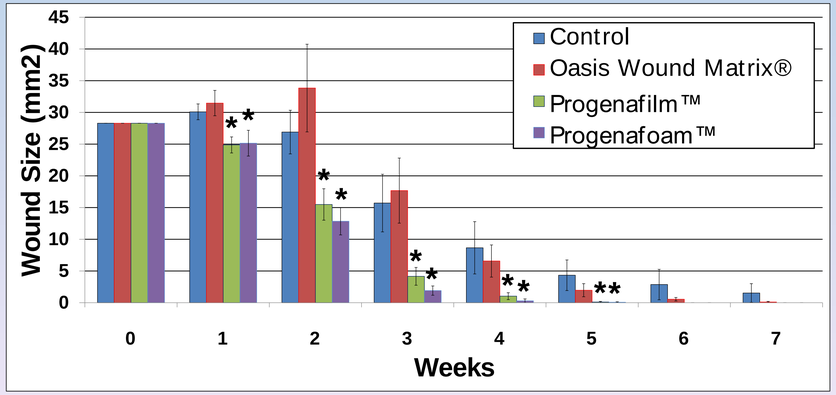
Results

Table 1.

Mouse Type	Time To Wound Closure	# Wounds
Nude Mouse (*nu/nu*)	19.8 ± 1.2 days	6
Black-6 Mouse (*C57BL/6*)	22.4 ± 0.9 days	10
Diabetic *C57BL/6* (db/db)	38.6 ± 2.1 days	39

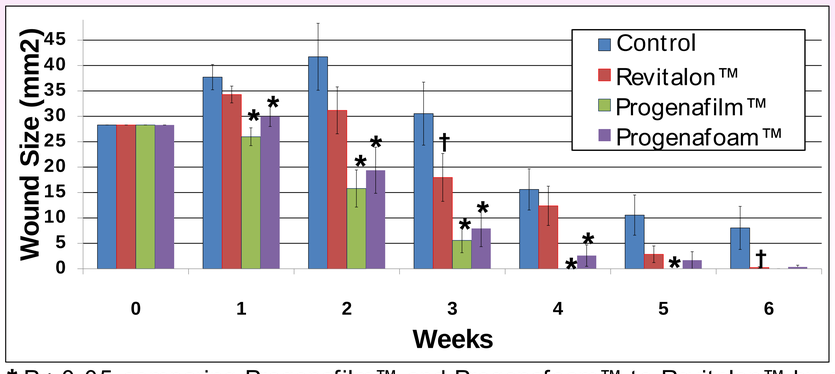
The data in this table indicates that the *db/db (000642, Jackson Labs)* mice have impaired healing as compared to the other mice.

Figure 1. Progenafilm™ and Progenafoam™ Heal Wounds Faster Than Oasis Wound Matrix®.



* P< 0.05 by a paired T-test comparing Progenafilm™ and Progenafoam™ to the control or Oasis Wound Matrix®. (n = 17 mice)

Figure 2. Progenafilm™ and Progenafoam™ Heal Wounds Faster Than Revitalon™.



* P< 0.05 comparing Progenafilm™ and Progenafoam™ to Revitalon™ by a paired T-test. † P< 0.05 comparing Revitalon™ to control by paired T-tests. (n = 13 mice)

Figure 3. Progenafilm™ and Progenafoam™ Close Wounds Faster Than Oasis Wound Matrix®.

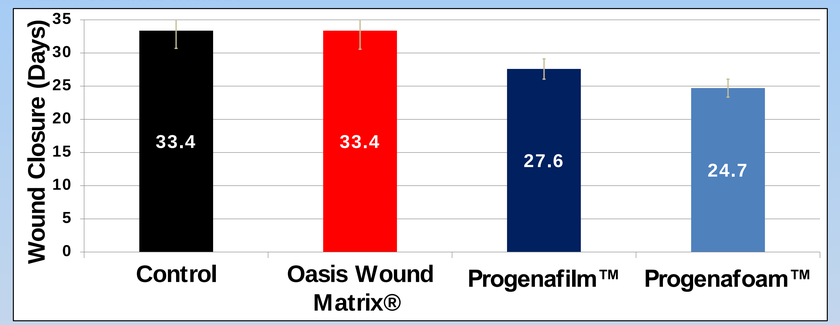
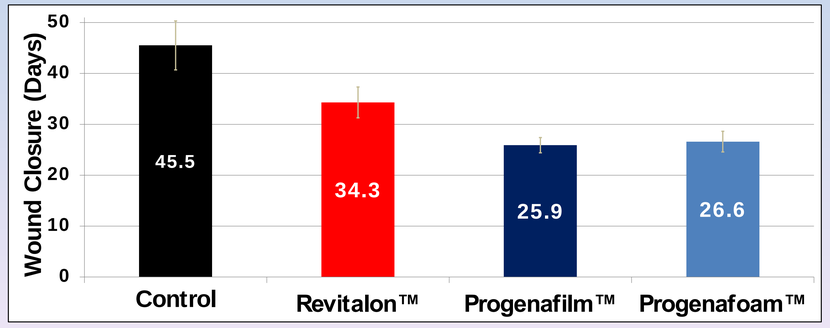


Figure 4. Progenafilm™ and Progenafoam™ Close Wounds Faster Than Revitalon™.



Conclusion

Our diabetic mouse model of chronic wounds is able to show differences among materials applied to the wound bed and is useful as a predictor of advanced wound dressing efficacy. This model suggests that Progenafilm™ and Progenafoam™ dressings are likely to be equally or more effective than the two products tested in treating human chronic wounds. Future wound care products are therefore likely to benefit through the use of this model prior to human clinical trials.

References

1. CDC. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta, GA: US Department of Health and Human Services.
2. Brem H, Tomic-Canic M. Cellular and Molecular basis of wound healing in diabetes. J Clin Invest. 2007 May;117(5):1219-22. PMC1857239
3. Snyder RJ, Hanft JR. Diabetic Foot Ulcers- Effects on Quality of Life, Costs, and Mortality and the Role of Standard Wound Care and Advanced-Care Therapies in Healing: A Review. Ostomy Wound Management. 2009. Nov 1;55(11):28-38. PMID: 19934461
4. Tsourdi E, Barthel A, Rietzsch H, Reichel A, Bornstein SR. Current Aspects in the Pathophysiology and Treatment of Chronic Wounds in Diabetes Mellitus. BioMed Research International. 2013, Article ID 385641, 6 pages. PMC3638655

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