A NEW TREATMENT FOR LONG-TERM DIABETIC WOUNDS

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INTRODUCTION

In medicine, chronic nonhealing wounds are debilitating with high morbidity and mortality in a highly vulnerable patient population. Despite thousands of wound dressings developed in the past century, none has shown good results in enhancing the healing process of diabetic wounds, including the only FDA-approved prescription growth factor, Regranex.

Multiple factors contribute to nonhealing diabetic wounds, and many of the pathways involved are not entirely clear. Surgeons have long recognized that ischemic tissues heal poorly and are easily infected. One direct effect of ischemia is a reduction of tissue high energy phosphate reserve, mainly adenosine triphosphate (ATP). However, a direct application of ATP does not work because it cannot pass through the cell membrane. Our group has developed a new technique for wound care by using intracellular ATP delivery technique (ATP-vesicles). This study was designed to test this treatment in animals with long-term diabetic ulcers and explore possible mechanisms in this rapid tissue regeneration.

HYPOTHESIS

Our hypothesis is, intracellular ATP delivery can generate new tissue within a short period of time. It keeps growing and covers the wound cavity within a few days. This extremely rapid tissue regeneration is the result of a combination of very early and rapid macrophage accumulation, in situ proliferation, M2 polarization, and direct collagen production to form extracellular matrix long before traditional fibroblasts come into play. The provided energy is the key to cell survival and function before neovascularization is established.

METHODS

Twenty-five diabetic rabbits were used in this study and another 25 non-diabetic rabbits were used for comparison. Diabetes was induced by alloxan (100 mg/kg, IV) injection. After stable diabetes was established, the rabbits were kept for 3-12 months before the wound study. In each rabbit, the left ear was made ischemic using vascular disruption or adding a silicone ring buried in the ear base to prevent vessel and nerve regeneration. The right ear vessel and nerve supply was not disturbed as normal control. Four wounds (5 mm in diameter) were created on the ventral side of each ear, resulting in 100 ischemic wounds and 100 non-ischemic wounds. On each ear, the two wounds on one side were treated with ATP-vesicles while the other two wounds were treated with Regranex (50 for ATP-vesicles and 50 for Regranex). Animals were sacrificed after the wounds were healed or sacrificed at an earlier time post-surgery for comparison.

RESULTS

1. The use of ATP-vesicles causes extremely rapid tissue regeneration—granulation starts at 12 hours after surgery. It keeps growing and fills the cavity quickly. The control wounds treated by Regranex, did not have any growth before day 5 (Fig. 1, 2).
2. The early growth by ATP-vesicles is full of cells, mainly by macrophages (Fig. 1).
3. The wound healing comparisons among three groups (non-diabetic, 2-month diabetic, and 12-month diabetic) show a significant healing difference between ATP-vesicles and controls (Fig. 3).
4. Wound collagen production by macrophages has shown much higher by ATP-vesicles than all other ingredients (Fig. 4).
5. More lab tests have shown other differences between ATP-vesicles and Regranex. These include platelet, neutrophil elastase, CD44 stem cells, CD106 stem cell marker, macrophage M2 marker, IL-13 cytokine marker, CD34 hematopoietic marker, CD31 neovascular marker, and CD105 vascular endothelial marker (Fig. 5).
6. Because this is the first time a new intracellular ATP delivery is used for wound care, the mechanisms are complex. So far, we have explored multiple related mechanisms (Fig. 6).

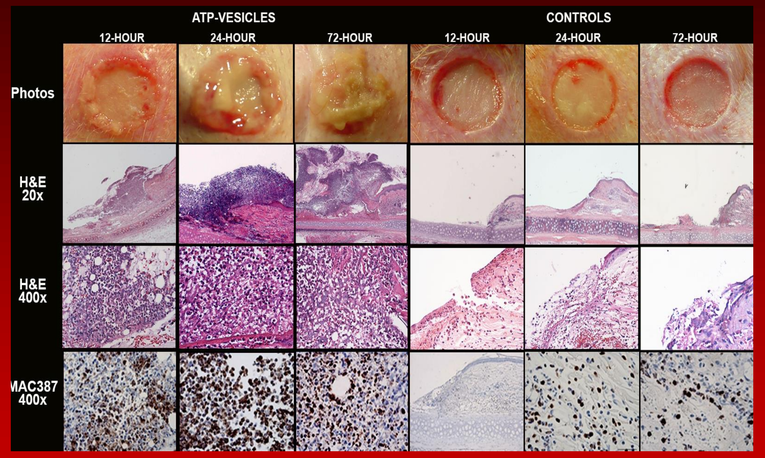

Fig. 1. A comparison of early wound healing time. In wounds treated by ATP-vesicles, granulation starts to appear within 12 hours. It keeps growing and fills the wound cavity in less than 72 hours. The main component is the macrophages. When the wounds are treated by controls such as Regranex, no new tissue growth is seen within 72 hours.

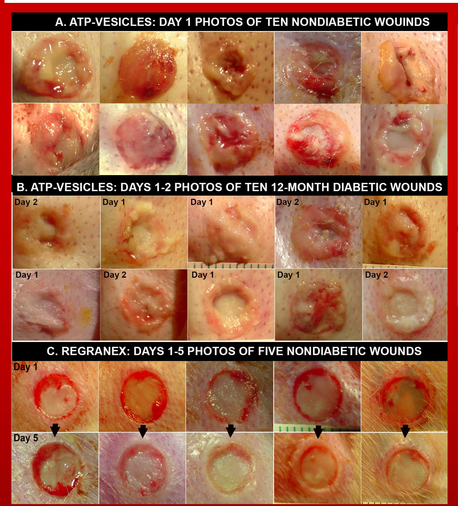

Fig. 2. A. Examples of wound healing. A. When treated by ATP-vesicles, granulation starts to appear during day 1. B. When diabetic wounds are treated by ATP-vesicles, healing starts at days 1 to day 2. C. When wounds treated by Regranex, no new tissue growth is seen from day 1 to day 5.

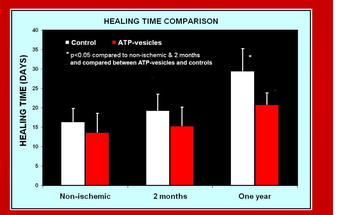

Fig. 3. Wound healing comparison in non-diabetic, short (2 months) diabetic, and long (12 months) diabetic. ATP-vesicles heal the wounds faster in all three groups.

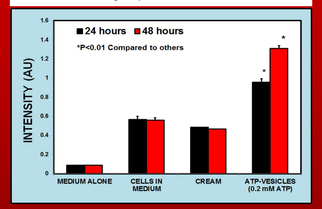

Fig. 4. Comparison of the collagen production. Wounds treated by ATP-vesicles show a much higher volume of collagen at 24 and 48 hours than all other controls dressings.

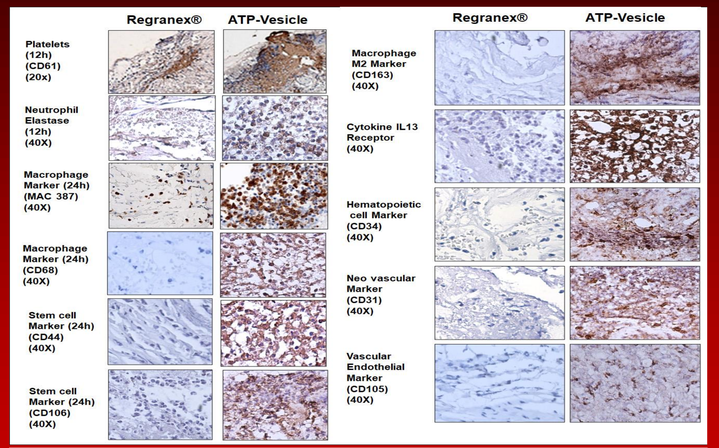

Fig. 5. In the ATP-vesicle treated wounds, multiple cells and cell markers show much high accumulation than Regranex. This includes platelets, neutrophil elastase, anti Mac 387, CD68m CD44, CD106, CD163, IL13R, CD34, CD31, and CD105.

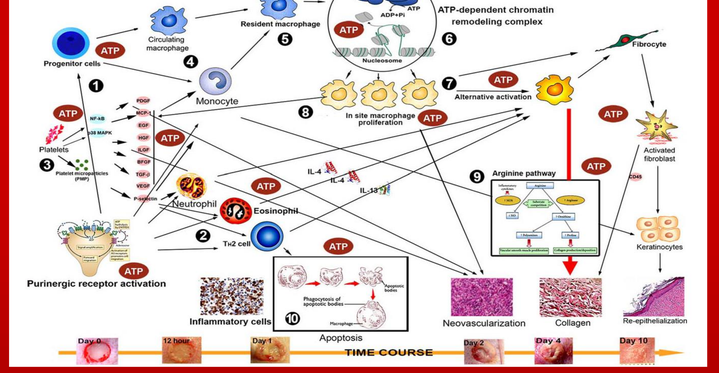

Fig. 6. Schematic illustration of the complex cascades when ATP-vesicles are used. (1) Stem/progenitor cell attraction mostly via purinergic receptor activation. (2) Leukocyte chemotaxis caused by ATP. (3) Enhanced platelet accumulation by ATP. (4) Monocyte accumulation and activation from the above processes. (5) Monocyte transformation to macrophages via platelet and platelet microparticles. (6) Massive cell accumulation caused by in situ macrophage proliferation. (7) Energy provided by ATP-vesicles nourishes massive cell accumulation and enhances their function. (8) Activated M1 macrophages exert their phagocytic function. (9) Activated M2 macrophages produce collagen directly via alternative pathway. (10) Up-regulated apoptosis keeps the growth in check and maintains the balance between proliferation and regression.

CONCLUSIONS

1. Using intracellular ATP delivery can enhance wound healing in short or long-term diabetic wounds significantly.
2. The extremely rapid wound healing is the result of very early and rapid macrophage accumulation, in situ proliferation, M2 polarization, and direct collagen production to form extracellular matrix quickly.
3. The extremely rapid tissue regeneration is accompanied by rapid cell accumulation and proliferation such as platelets, neutrophiles, macrophages, stem cells, vascular endothelial cells, and multiple proliferation cells.
4. If we can duplicate the results in humans, it will overturn the current paradigm in wound healing to save billions of dollars in healthcare cost and has the potential to open up a new avenue for tissue regeneration.

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