# Application of Topical Exogenous Nutrient Supplementation to Improve Graft Take on a Poorly Vascularized Wound Bed

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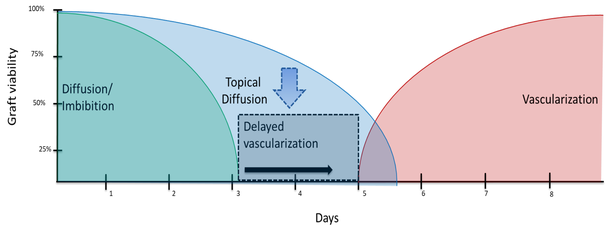
## Introduction

Skin graft survival relies on imbibition, inosculation, and revascularization from the wound bed [1,2]. When a wound bed is poorly vascularized, as in the case of exposed fascia, tendon or bone, skin grafting may be delayed until the wound bed improves [3]. We propose that topical nutrient supplementation may be able to increase take of skin grafts applied over a poorly vascularized wound bed. With topical nutrient supplementation providing the environment to maintain graft survival until the wound bed itself is able to support the skin graft, single-stage skin grafting procedures may become feasible.




## Objectives

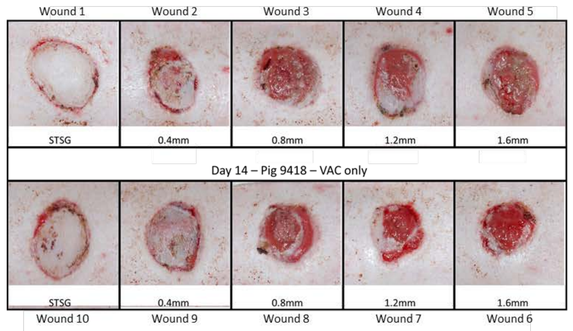
- Develop a poorly vascularized porcine wound bed model
- Determine the optimal nutrient supplementation
- Compare graft take with wound vacuum-assisted closure (VAC) alone to wound VAC with instill treatments

## Methods

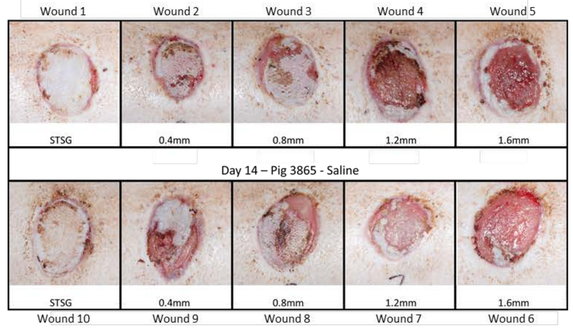
To create the porcine model, twenty excisional full-thickness 5cm-diameter wounds were created on the dorsum of anesthetized swine (*Sus scrofa domestica)* and a dermal substitute (0.4mm, 0.8mm, 1.2mm, or 1.6mm thick) was placed on each wound. Negative pressure therapy with and without intermittent saline instillation was applied (Figure 1C). Instillation was performed 3 times per day with a soak time of 15 minutes. Reepithelialization was measured at day 7 and day 14 using a non-contact 3D camera (Figure 1A and 1B) and measured as percent graft take (Figure 2).

Optimal nutrient supplementation was determined by obtaining 12mm biopsy punches from skin grafts harvested from the dorsum of female Yorkshire and red Duroc swine within 1 hour of euthanasia. Under sterile conditions, biopsies were submerged in multi-well culture plates containing one of the media listed in Table 1 supplemented by an antibiotic-antimycotic solution. Plates were stored at 37°C and subsequently tested for markers of high metabolism: lactic acid and enzymatic carbonate (Figure 3A and 3B). The amount of cell death was also measured by determining the amount of lactate dehydrogenase (LDH) activity (Figure 3C). The amount of glucose consumption was quantified for each media as well (Figure 3D).

## Results




**A.**




**B.**

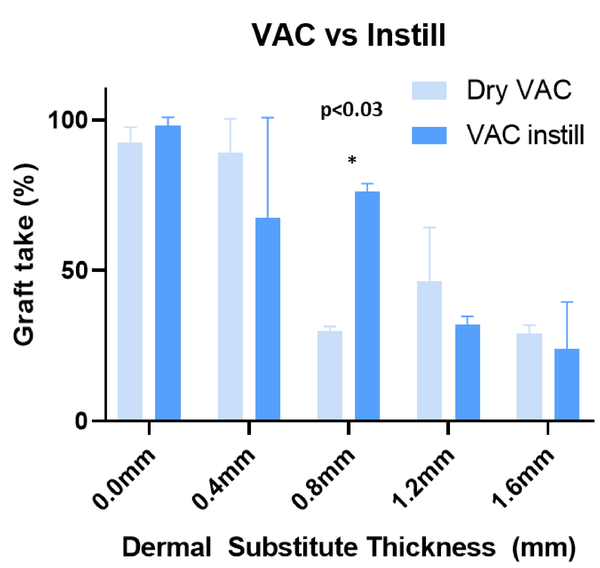



**Figure 2.** Reepithelialization results as measured by percent re-epithelialized of total wound area on day 14. Dermal substitutes of 0.8mm, 1.2mm, and 1.6mm thickness inhibited graft take significantly for all wounds treated with wound VAC alone. Addition of normal saline instill showed a significant improvement in graft take (p = 0.03) over wound VAC alone for the wounds treated with the 0.8mm dermal substitute. Wounds covered with 1.2 and 1.6mm dermal substitute continued to show significantly decreased graft take.

**Table 1. Media Tested**

| Media | Cell Type | Components |
|---|---|---|
| Phosphate-buffered Saline | N/A | Sodium Chloride, Potassium Chloride, Disodium Phosphate, Monopotassium phosphate |
| Dulbecco's Modified Eagle's Medium (DMEM) + 1g glucose | Fibroblast | Amino Acids, Glutamate, Glucose, Vitamins |
| Dulbecco's Modified Eagle's Medium (DMEM) + 4.5g glucose | Fibroblast | Amino Acids, Glutamate, Glucose, Vitamins |
| Tyrode's Salts | N/A | Sodium Chloride, Potassium Chloride, Calcium Chloride, Magnesium Chloride, Monosodium Phosphate, Sodium Bicarbonate, Glucose |
| EpiLife w/ 60M calcium | Keratinocyte | Amino Acids, Vitamins, Trace Minerals, Inorganic Salts |
| William's E | N/A | Amino Acids, Vitamins, Inorganic Salts, Glucose, Glutathione, Methyl Linoleate |

**Table 1.** List of media tested with respective cell types and components.

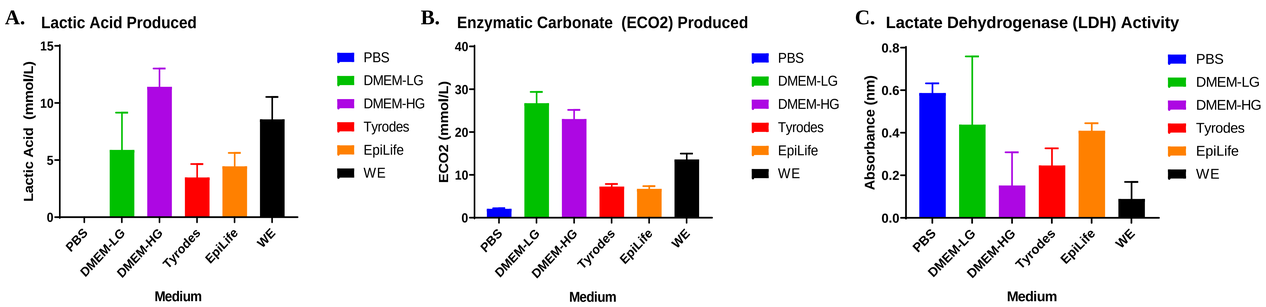



**Figure 3.** Ex vivo data. **B.** Lactic acid produced by grafts in respective media. The Dulbecco's Modified Eagle's Medium (DMEM) with low (1g) glucose (LG) and high (4.5g) glucose (HG) and William's E had the highest amount of lactic acid produced. **C.** Enzymatic Carbonate (ECO2) produced by grafts in respective media. Again, DMEM and WE showed the highest amount of metabolic activity. **D.** Lactate dehydrogenase (LDH) activity as measured by absorbance in nm. LDH activity was lowest for WE indicating the least number of dying cells found within this media. **E.** Glucose consumption by grafts in respective media. While DMEM-HG had the highest amount of glucose consumption, there was also a large amount of the starting glucose that went unconsumed, indicating that this media had too much glucose (not shown). As William's E had a more favorable ratio of starting to final glucose, indicating the majority of glucose available was consumed, this was determined to be the optimal media to utilize for the remainder of the experiments.

**C.**

**Figure 1.** Wound images captured on day 14 and set-up. **A.** Excisional wounds treated with wound VAC alone. Split-thickness skin grafts (STSGs) were placed on Wounds 1 and 10 without underlying dermal substitute to serve as a control. Overall, as dermal substitute thickness increases, graft take decreases. **B.** Excisional wounds treated with wound VAC with instill treatments. Wounds 1 and 10 serve as controls with no underlying dermal substitute. Graft take is significantly improved for Wound 3 and Wound 8 for which 0.8mm dermal substitute thickness was placed. **C.** Digital photographs of wound VAC placement and attachments.

## Conclusions

- Acellular dermal substitute with a thickness of 0.8mm produces a successful model of a poorly vascularized wound bed.
- William's E serves as a source of topical nutrients ex vivo, producing a high amount of dividing cells and the lowest amount of dying cells
- Wound VAC with instill treatments significantly improves graft take for skin grafts placed over ≤0.8mm dermal substitutes when compared to wound VAC alone.

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## Statements

The views expressed are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DOD, or the U.S. Government.

Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC.