OPTIMIZING WOUND HEALING AND MINIMIZING SCARRING WITH AMINO-PLEX®

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FOR DECADES, AMINO-PLEX® HAS BEEN USED WORLDWIDE TO PROMOTE HEALING IN DIFFICULT WOUNDS. THE BASIC SUBSTANCE IS A PROTEIN-FREE, ALL NATURAL SOLUTION CONSISTING OF LOW MOLECULAR WEIGHT ORGANIC AND INORGANIC COMPOUNDS. IT WAS ORIGINALY DEVELOPED AS A RESULT OF THE SEARCH FOR HUMORAL FACTORS AFFECTING GROWTH AND REPAIR. RESEARCH DEMONSTRATED THAT IT ACCELERATES TISSUE REPAIR, AND IT HAS BEEN WIDELY USED SINCE. THE BIOLOGICALLY ACTIVE PORTION HAS BEEN ANALYZED, AND FOUND TO CONTAIN A MIXTURE OF GLYCOLIPIDS (SPECIFICALLY HEXOSPHINGOLIPIDS). IN ADDITION, NUCLEOSIDES, NUCLEOTIDES, AMINO ACIDS, OLIGOPETIDES, ESSENTIAL TRACE ELEMENTS, ELECTROLYTES, AND INTERMEDIATE PRODUCTS OF CARBOHYDRATE AND FAT METABOLISM HAVE BEEN IDENTIFIED. THE COMBINATION OF THESE ELEMENTS HAS BEEN SHOWN CLINICALLY AND EXPERIMENTALLY TO

- INCREASE CELLULAR OXYGEN UPTAKE-
- STIMULATE ATP SYNTHESIS
- IMPROVE GLUCOSE TRANSPORT
- STIMULATE COLLAGEN FORMATION AND ANGIOGENESIS
- SIMULATE GROWTH FACTOR ACTIVITY
- INCREASE CYTOPROTECTIVE EFFECTS

AMINO-PLEX® IS THE BASIS OF THE OXY- MIST SYSTEM THAT HAS BEEN WIDELY USED FOR THE PAST FIVE YEARS TO ENHANCE THE RECOVERY AND SHORTEN THE HEALING TIME FOLLOWING CO2 LASER RESURFACING. THIS IMPROVEMENT WAS CLINICALLY STUDIED IN A CONTROLLED MANNER, TREATING ONE SIDE OF VOLUNTEERS' FACES WITH A STANDARD POST-LASER PROTOCOL (VASELINE, MASK) FOLLOWING A SIGNIFICANT CO2 LASER RESURFACING, AND THEN COMPARING THE HEALING WITH A SUBSEQUENT LASER RESURFACING ON THE OPPOSITE SIDE OF THE FACE TREATED WITH THE AMINO-OXY MIST PROTOCOL (DAILY APPLICATIONS FOR FIVE DAYS PLUS A HEALING BALM AND FREQUENT WASHING). ALL THE PATIENTS TREATED WITH THE AMINO-OXY MIST WERE HEALED WITHIN 7 DAYS, COMPARED TO 10-14 DAYS ON THE STANDARD SIDE. THE SUBSEQUENT ERYTHEMA WAS LESS INTENSE ON THE AMINO-OXY MIST SIDE, AND RESOLVED MORE RAPIDLY. BASED ON THIS CONTROLLED CLINICAL STUDY, THE AMINO-PLEX® HAS BEEN USED SUCCESSFULLY TO ENHANCE HEALING FOLLOWING MICRODERMABRASION WITH GLYCOLIC PEELS AND HAIR TRANSPLANTS, AS WELL AS IN SUTURED WOUNDS FOLLOWING FACE LIFTS, FLAPS, ABDOMINOPLASIES, AND BREAST SURGERIES. SINCE WOUND HEALING HAS THE SAME BASIC COMPONENTS WHETHER IN AN OPEN WOUND OR CLOSED (SUTURED) WOUND, IT IS REASONABLE TO EXPECT THAT AMINO-PLEX® WOULD ENHANCE AND IMPROVE HEALING OF ALL WOUNDS, OF WHATEVER ORIGIN, AND IT HAS EVEN SHOWN A DRAMATIC EFFECT IN VERY DIFFICULT WOUNDS, SUCH AS POST-RADIATION TISSUE NECROSIS, AND PRESSURE SORES (DECUBITUS ULCERS). AVAILABLE AS A SPRAY OR SOLUTION, IT CAN EASILY BE USED AT HOME, OR DELIVERED WITH AN OXYGEN SPRAY BY AESTHETICIANS OR MEDICAL PROFESSIONALS FOLLOWING LASER TREATMENTS, PEELS, DERMABRASIONS, OR ANY SURGICAL PROCEDURE. SINCE IT IS BIOLOGICALLY STANDARDIZED, PROTEIN-FREE, NON-ANTIGENIC AND NON-PYROGENIC, REACTIONS AND SIDE EFFECTS HAVE NOT BEEN SEEN. ENHANCED HEALING MEANS LESS INFLAMMATION, AND POTENTIALLY LESS SCARRING, LESS ERYTHEMA, LESS PAIN, AND A BETTER ULTIMATE RESULT, WHICH HAS BEEN CLINICALLY DEMONSTRATED.
Proposal: Effectiveness of Topical Nutrients for the Medical Management of Pediatric Thermal Burns

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Summary

Pediatric burns are a continuing problem and can have significant morbidity. Although the database is limited, clinical studies show that burn outcomes vary with the choice of dressing/management. A topical nutrient treatment increased the rate of re-epithelization in a neonatal pig model. Rapid wound healing is highly desirable to reduce scar formation, pain and to minimize the risk of infection. One of the limitations of current approaches to measuring wound healing rates is the lack of objective quantitative measurements of skin and scar features and lack of standardized procedures (i.e., variability due to observer differences). Therefore, we aim to examine the mechanism of wound healing in two animal models and on human pediatric subjects using objective skin imaging methods to quantify reepithelization and scar biomechanical properties.

Background and Significance
Approximately one million pediatric burn injuries occur each year in the United States alone 1 and three hundred pediatric scalds each day 2 . These children are at risk for permanent disfigurement and disability 3 . When skin grafting is required, the grafted skin is imperfect in that it cannot replace the normal form or function of the original, uninjured tissues. The donor site can become a source of further cosmetic deformity as well for select patients. Donor sites are areas of uninjured skin, from which a very thin shaving of skin is taken. These donor sites are functionally second-degree burn (created with deliberate mechanical trauma rather than thermally injury). The donor site wounds present additional morbidity for patients, including infection, pain, hyperpigmentation and scarring and strategies to accelerate healing are invaluable 4 . In the worst of cases, scarring at the donor site in pediatric patients can be more severe than in the original injury 1 . Fortunately, most burn injuries are not life threatening and do not require skin grafting. In one report, 75% of burns presenting to an urban pediatric emergency department were partial thickness (second degree) 5 . The upper extremities were the most common sites, particularly the digits (34%) and hands (27%). Deep hand and finger burns in very young children are susceptible to early complications, including necrosis and compartment syndrome 6 . However, as noted by a review endorsed by the American Burn Association, even more superficial burns of the hand can lead to impaired function 7 . Examining the factors that influence the outcome of partial thickness hand burns was identified as one of the priorities for research. It is unclear if protracted periods of pain, or impaired skin function with the development of hypertrophic scars has a greater impact on recovery, but both are exacerbated by the presence of an open wound for a longer period of time. Hypertrophic scar formation is positively related to wound healing time in burns of lesser severity, i.e., partial thickness (e.g., scalds), and are more likely to occur in wounds that require 21 or more days to heal 8 . Acceleration of wound healing holds the promise of decreasing morbidity of more-common lesser injuries, decreasing the morbidity of donor sites required for the current treatment of deeper burns, and decreasing the length of stay for the largest of burn injuries in which the care team may need to wait up to 2 weeks to successively re-harvest skin from limited donor sites. The limited research evidence on the effectiveness of treatments/dressings for pediatric wound care has resulted in reliance on clinician experience and/or anecdotal information to as the basis for selecting wound dressings 9 . For pediatric burns, treatments and dressings have been evaluated for general acceptability 10 and occurrence of complications 11 . Comparative studies for silver sulfadiazine versus Biobrane (pediatric patients) 12 and for silver sulfadiazine versus Acticoat (adults) 13 showed treatment differences for pain, wound healing time, infection, medication requirements and length of stay in pediatric patients. Treatment of donor sites with a carboxymethylcellulose hydrofiber dressing led to faster reepithelialization, reduced pain and improved scar quality relative to standard paraffin gauze (pediatrics) 14 . Pediatric partial thickness burns (superficial or mid dermal) were treated with one of two dressings, Biobrane ® (bicomposite, semipermeable silicone membrane plus knitted trifilament nylon) or Duoderm ® (occlusive, hydrocolloid) 15 . The dressings did not differ for healing time or pain, but Duoderm ® was more cost effective. Superficial scald burns were treated with one of two temporary cover dressings, Xe-Derma ® (acellular from pig dermis) or Askina THINSite ® (multilayer, hydrocellular, hydrogel). They were comparable for time to epithelialization and infection but Xe-Derma® required fewer dressing changes 16 .

**Rationale for Proposed Treatment**

The proposed wound treatment is Amino-Plex ® (APS, biO2 Cosmeceuticals International, Inc., Harbor City, CA, USA, Table I), a commercially available multi-component mixture containing of nutrients including amino acids, nucleotides, electrolytes, sugars, vitamins and electrolytes. Amino-Plex ® has been sold to clinicians since 1996 and used for a variety of wounds in an estimated 450,000 patients 18 . In post market monitoring by the company, no adverse effects, e.g., irritation, sensitization, wound exacerbation, have been reported. Amino-Plex ® was evaluated as an adjunct to laser debridement of partial thickness chemical vesicant burns and was associated with more rapid re-epithelialization than other adjuncts and the untreated control 19 . Collagen type VII was more normally immunolocalized to anchoring fibrils in the basement membrane suggestive of a more normal epidermal structure. Use of Amino-Plex ® for facial wound care after CO2 laser resurfacing resulted in less crusting compared to an occlusive dressing five days later 20 .
Rationale for Nutrients in Wound Healing

The Amino-Plex® Spray is a complex mixture of ingredients and specific nutrient effects cannot readily be isolated due to the complexity. However, a rationale for the investigation of a nutrient mixture is proposed as follows. Topical nutrients (calcium, insulin, hydrocortisone, and epidermal growth factor (EGF)) plus antimicrobials were compared with saline and EGF plus antimicrobials and dry dressings for the effects on engraftment and wound contracture in full thickness wounds grafted with cultured skin substitutes 21. Nutrient treated grafts were larger (less contracture) and percent human leukocyte antigens were higher than in both controls. Daily debridement followed by topical application of nutrients (salts, amino acids, ascorbic acid, D-glucose polysaccharide) increased repair and controlled infection in a variety of wounds, e.g., second and third degree burns, diabetic ulcers 22. Full thickness wounds treated with sericin, a mixture of silk proteins, peptides and amino acids, healed more quickly than controls (petrolatum-based vehicle only) in rats 23. Treatment of full thickness wounds with four amino acids (required for collagen synthesis: glycine, L-lysine, L-proline, L-leucine) in combination with hyaluronic acid (Vulnamin®, Professional Dietetics, Milano, IT) increased the rate of wound healing 24. Inflammatory cells and inducible nitric oxide synthase (iNOS) were decreased and endothelial nitric oxide synthase (eNOS), transforming growth factor-β1 (TGF-β1) and thin collagen fibers increased 24. Chronic ulcers in diabetic patients treated with Vulnamin had a higher healing rate at 3 months, reduced wound size and decreased severity compared to a placebo control 25. Since hyaluronic acid is associated with increased healing time, scar remodeling and improved scar quality 26, 27, the improvements found for Vulnamin may arise from both components. Extracellular nucleotides combined with epidermal growth factors increase cell proliferation in wound healing 28. Addition of adenosine triphosphate (ATP) to full thickness wounds in athymic mice increased wound healing and expression of wound tissue vascular endothelial growth factor (VEGF) 29. Adenosine exhibits anti-inflammatory effects in wound repair 30 and substantially increased the rate of wound closure 31. It also triggers the formation of collagen I and III by dermal fibroblasts in the tissue remodeling phase of wound healing 32. A uric acid derivative (6,8-dithio uric acid) enhanced full thickness wound healing in mice when topically applied 35. Additional studies are required to determine if the occurred via an anti-oxidant mechanism. Treatment with vitamin C extended cell viability and promoted engraftment of cultured skin substitutes used to treat full thickness wounds 36. Systemic administration of antioxidants, i.e., vitamins C, E and zinc, to pediatric burn patients reduced wound healing time and decreased lipid peroxidation relative to placebo (no supplementation), thereby mediating oxidative stress 37. To date, human studies of topical application have not been reported. Topical application of vitamins E and C (stabilized with ferulic acid) demonstrated protection from ultraviolet-induced oxidative stress in humans 38, 39.

Rationale for Objective Assessment with Imaging Methods

Visual skin assessment is the mainstay for clinical care but is limited by the lack of “universal standards” for normal skin (e.g., integrity, color), low reproducibility, low reliability, and observer variation 40, 41. The accuracy of clinical burn assessment is estimated at 50 – 65% 42, 43, in part because burn depth cannot easily be determined. Healing rates are difficult to assess clinically (by visual observation) in pediatric burns 8. A recent literature review indicated the need for reliable, objective instrumental techniques for the assessment of skin scars 44. Standardized digital photography, ultrasound, and three-dimensional reconstruction with non-visible light increase clinician efficiency and overcome the limitations posed by recall 45. Digital imaging techniques have been used to improve objectivity, quantify skin features (e.g., erythema, dispigmentation, abrasion) 46-48 and measure wounds, burns, lesions, photoaging, blanching, skin atrophy, and disease (e.g., psoriasis) 41, 48-52. The inherent skin pigmentation impacts the accuracy of color assessment and tissue damage may be underdetermined in dark skinned patients for whom pigmentation interferes with erythema detection 53, 54. Image analysis coupled with the wound electronic medical record provided the basis for effective wound treatment 55. Laser Doppler perfusion imaging (LDI) has been used to determine burn/wound healing potential in pediatric patients 56, 57 and allowed treatment decisions to be made more quickly in burn. Laser Doppler perfusion was related to re-epithelialization and predictive of grafting and scar management outcomes in
pediatric burns 59. Our laboratory has applied multiple imaging methods to quantify specific skin conditions and their response to treatment. They include irritant contact dermatitis 60-62, tissue injury in light and dark skinned subjects 62, gynoid lypodystrophy 63-65, facial rhytides 66-68, infant diaper skin 69, 70 and pediatric burn scars 71. The most recent research on pediatric burn scars used a within subject design to evaluate the effectiveness of Pulsed-Dye Laser (PDL) 71. One half of the graft seam was treated multiple times at 6-week intervals with the PDL plus compression therapy and the other with compression alone. Scars were assessed clinically with the Vancouver Scar Scale (VSS) by trained therapists who were blind to treatment. Both halves were evaluated with standardized imaging and analysis for color (erythema, blue color, lightness, excess erythema), 3D surface scanning for scar height, and biomechanical response for elasticity (BTC-2000, SRLI, Nashville, TN). Erythema and VSS vascularity were significantly lower for PDL + compression versus compression alone (p < 0.05). PDL treated skin was more elastic (via instrument and VSS) than with compression. Scar height (3D scans and VSS) was lower with PDL treatment. The results demonstrate the efficacy of the PDL as a burn scar treatment, providing the first comparative report 72 and support the feasibility of using multimodal skin imaging to quantify wound healing in the proposed research.

**Proposed Research**

We hypothesize that treatment of pediatric burn injuries with a nutrient mixture will result in more rapid epithelialization and reduced hypertrophic scarring than the current standard of care. We propose three specific aims to provide mechanistic and clinical outcomes in response to treatment. They are designed to address partial thickness wounds and engraftment of human skin substitutes (more extensive burns). The specific aims are as follows:

**Specific Aim 1:** Determine the effect of Amino-Plex® Spray (APS) on rate of re-epithelialization relative to the current standard of care (control) and no treatment in a C57/BL6 mouse wound model. Proposed Experimental Plan Standardized, partial thickness wounds will be created via 8 mm punch biopsies in C57/BL6 mice as previously described 73. Wounds will be treated daily for fourteen days with one of three treatments (12 animals per group): APS (test), control (current standard of care), no treatment (sterile gauze) following the procedures in Graham et al 19. The rate of re-epithelialization will be quantified as change in wound size, erythema and pigmentation via standardized high resolution digital image capture and analysis at baseline and on days 1, 3, 5, 10 and 14 71, 72, 74. At each time point (i.e., day 1, 3, 5, 10, 14), tissue samples will be obtained from two animals and analyzed for specific markers of wound healing using histology and immunohistochery. Histological sections will be stained with Sirius red and examined for inflammatory cells and distribution density of fibroblasts, collagen and fibrosis, e.g., percent of wound area with collagen fibers 24. Biomarkers of early inflammation, e.g., interleukin-1 alpha (IL1α), tumor necrosis factor-alpha (TNFα), will be evaluated. Nitric oxide production during inflammation will be evaluated as inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) to determine potential impact on cell proliferation, collagen deposition and contracture 24. Blood vessel density (vascularity with PECAM-1) and cell proliferation (Ki-67) in granulation tissue will be determined with immunohistochemistry as described by Scherer 75. To determine the effect on re-epithelialization rate, specifically the proliferative phase, expression of vascular endothelial growth factor (VEGF) 29, collagen I, fibronectin, and transforming growth factor-beta 1 (TGFβ1) will be determined from immunostains 76. APS, current standard (control) and no treatment samples will be compared for each outcome using appropriate statistical procedures (e.g., ANOVA, Chi-square analysis, logical decision-making methods). The relationships among biomarker expression and wound characteristics (size, color, pigmentation) will be determined over the wound healing period.

**Specific Aim 2:** Determine the effect of Amino-Plex® Spray (APS) on engraftment rate in wounds treated with cultured skin substitutes relative to the current standard of care (control) and no treatment in the athymic mouse model. Proposed Experimental Plan Cultured skin substitutes (CSS) provide an alternative to tissue harvesting in patients with extensive burns. However, CSS have deficient vasculature and varying engraftment rates relative to native skin 77. Addition of topical nutrients has been successful in increasing CSS engraftment in our...
Optimization of engraftment is an ongoing treatment goal overall and for this treatment modality. Standardized full thickness wounds will be created in athymic mice and grafted with cultured skin substitute (CSS) as previously described. The wounds will be treated daily for fourteen days with one of three treatments (6 animals per group): APS (test), control (current standard of care), no treatment (sterile gauze) following the procedures in Graham et al. The rate of re-epithelialization will be quantified as change in wound size, erythema and pigmentation via standardized high resolution digital image capture and analysis at baseline and on days 1, 3, 5, 10 and 14 as described in Aim 1. Animals will be sacrificed at the end of the wound healing period and tissue biopsies will be collected for standard histology and immunohistochemistry. Tissues will be embedded in paraffin, sectioned and treated with hematoxylin and eosin (H&E) and examined (scored) for reepithelialization, hyperplasia, elastic and collagen fibers, inflammatory cells and blood vessels. Tissues will also be frozen (-70 °C) and prepared for immunohistochemistry to determine granulation tissue formation, i.e., wound healing progression. Primary and cross-reacting antibodies will be use to evaluate collagens IV and VII, filaggrin, CD49f (alpha 6 integrin), laminin and laminin 5, vimentin and Von Willebrand factor. Image analysis of microscopy images will be used to obtain semi-quantitative information on amounts of each marker. APS, current standard (control) and no treatment samples will be compared for each outcome using appropriate statistical procedures (e.g., ANOVA, Chi-square analysis, logical decision-making methods). The relationships among biomarker expression and wound characteristics (size, color, pigmentation) will be determined over the wound healing period.

**Specific Aim 3:** Determine the effect of Amino-Plex® Spray (APS) on the rate of re-epithelialization, pain, scar size, and scar elasticity in pediatric burn patients. 1 – 18 years of age, with partial thickness donor site wounds (partial thickness) from tissue harvesting for burn grafts or scar restoration procedures or with thermal burns (e.g., scalds, fire) will be enrolled. Donor site wounds were selected for this aim because the wound depth and tissue thickness are known (e.g., dermatome settings of 0.008-0.012 in) thereby providing a more well characterized wound. Eligible subjects will be randomized to receive the treatment (APS) of the current standard of care (control) based on erythema prior to treatment and wound size. Patients will be recruited from the inpatient and outpatient clinics of Shriners Hospital Cincinnati. Based on sample size calculations from our previous work and that of others, we anticipate that a sample size of 60 subjects (30 per group) will be adequate to address the aim. Patients will be evaluated prior to treatment assignment at their first clinic visit or after tissue is harvested and on days 3, 5, 7, 10 and 14 for re-epithelialization rate and at subsequent clinic visits (every 3 months) for up to 18 months. The outcome measures are as follows: (1) rate of re-epithelialization measured quantitatively via digital imaging and analysis for wound size, erythema, color uniformity, blue coloration, excess erythema via high resolution digital photography, perfusion via laser Doppler speckle imaging, 3D surface characteristics using a three-dimensional surface scanner, and clinician evaluation (e.g., Vancouver Scar Scale). (2) Pain will be evaluated at the times of dressing changes for inpatients and at clinic visits for outpatients using the visual analog scale with faces and by pain medication requirements during the six hour period preceding the dressing change. (3) The effect of hypertrophic scar formation will be evaluated after re-epithelialization is complete and at normally scheduled follow-up visits at three month intervals. Scar color will be assessed using standardized digital imaging and analysis. Scar elasticity will be evaluated through measurement of biomechanical properties (BTC-2000™) including laxity (stiffness), elastic deformation, viscoelastic flow and as VSS pliability. Treatment effects for APS versus the current standard of care will be determined using linear mixed models repeated measures procedures with day as the repeat for re-epithelialization rate outcomes and visit (3-month intervals) for scar characteristics (F statistic, p ≤ 0.05). Covariates will include initial imaging data (e.g., erythema, perfusion) and scar characteristics after healing is complete. Marty O. Visscher, PhD December 21, 2010

**References**

