Addressing Full-Thickness Skin Defects: A Review of Clinically Available Autologous Skin Replacements

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ABSTRACT

Autologous keratinocyte culture, and combinations of scaffolds, different cell types, solutions of macromolecules, or growth factors have contributed to the resurfacing of full-thickness skin defects. Ideally, a treatment for full-thickness skin defects should not merely reestablish continuity of the surface of the skin but should restore its structure to allow skin to function as a dynamic biological factory that can participate in protein synthesis, metabolism, and cell signaling, and form an essential part of the body's immune, nervous, and endocrine systems. This paper provides a review of clinically available autologous skin replacements, highlighting the importance of regenerating an organ that will function physiologically.

INTRODUCTION

Skin is a complex organ providing a barrier to water loss and pathogens, and it protects against diverse forms of trauma, including chemical, thermal, and ultraviolet radiation. It allows us to communicate with our environment through a myriad of nerve endings, regulates body temperature, enhances metabolic functions, and plays a role in both innate and adaptive immunity.[1–5] To fulfil this purpose, skin requires a layered interface, epidermal appendages, and mechanical stability.[6]

The layered interface consists of an avascular cellular epithelium that spontaneously regenerates (the epidermis), a basement membrane zone (BMZ), and a fibrous neurovascular dermis that does not spontaneously regenerate and rests on a hypodermis or subcutaneous fat.[7]

The epidermis is mainly composed of sheets of keratinocytes but includes other cell populations, such as antigen presenting dendritic Langerhans cells as well as melanocytes and Merkel cells.[8–10] Among their functions, keratinocytes can proliferate to heal wounds, transport water and urea through aquaporins, receive melanin from melanocytes, and participate in innate and adaptive immunity through antimicrobial peptides.[11–15] The epidermis is nourished by diffusion of intercellular fluids from the dermal vasculature.

Reinforcing the epidermis is the dermis, which accommodates the vasculature, nervous system, lymphatics, and adnexa of the skin. This provides a durable base that can absorb mechanical forces. It is described as having a superficial papillary zone, comprising relatively thin collagen fibers, and a much thicker reticular dermis (a compact layer of thicker collagen fibers). The primary cell type is the fibroblast, which produces the extracellular structural proteins,

glycosaminoglycans, collagen, and elastin—the latter enhancing the deformability of the dermis. Between the cells and fibers is the extracellular matrix, largely composed of glycosaminoglycan/proteoglycan molecules, which hydrate the tissue due to the high-water binding capacity of hyaluronic acid.

Resistance to shear stress is supplied by the natural epidermal-dermal interface, which is composed of sinusoidal interdigitations of the rete ridges and the BMZ, a complex continuum of macromolecules that form a network providing stable association of the epidermis to the dermis.[16]

Left to its own devices, wounded skin heals by a process of repair in which continuity of tissues is achieved by contraction of wound edges and synthesis of scar tissue. Such scars are stiffer and more fragile than normal skin, have abnormal metabolism, and can give rise to itching, contracture, tenderness, and pain, as well as disfigurement.[17]

Skin wounds that are too extensive to heal by primary closure often require reconstruction with autologous tissue. For larger wounds, the standard of care is still the autograft, which contains the entire epidermis and part of the dermis. The procedure demands maintenance of contact between the apposed raw surfaces of the graft and a vascular bed, and it relies on a dynamic and intricate array of coordinated physiological events.[18] Initially bound to the wound bed by fibrin, the graft is nourished by osmotic diffusion in a process termed serum imbibition for 24–48 hours.[19,20] There follows an inosculatory phase, in which a new circulation is established within the graft, so that by the fifth or sixth day, the graft is pink and adherent to the defect, with further remodeling continuing in succeeding months. Disruption of any of these steps will prevent graft survival ("take").

Harvesting the split-thickness skin graft (STSG) fails to capture the deeper cellular entities, appendages, and tissue. STSG provides two incomplete defects: 1) the superficial skin graft within the recipient site which scars and contracts and is unable to secrete oil and sweat and grow hair, and 2) the donor site which results in a painful large trans/exudative wound in the short-term and a hyper/hypopigmented, scarred, dysesthetic region in the long term.[21] The situation at the recipient site is worsened by meshing since the interstices heal by secondary intention with pseudo epithelialization resulting in a "cobble stone" appearance. Skin harvesting can be repeated once the area has healed. Since dermis never regenerates, however, the number of times a site can be harvested will be limited by the thickness of the donor site and the depth at which the graft is harvested.

Full-thickness skin grafts (FTSG) contain all layers of the skin, but their size is restricted by the ability to achieve primary closure of the donor site. Thus, FTSGs are often reserved for smaller wounds and areas requiring full functioning skin, such as the eyelids or web spaces of the hands and feet.[22] Additionally, given increased tissue mass and metabolic demands, FTSGs require a robust vascular bed and are not suitable for all types of wounds, especially those on the lower extremity resulting from chronic diseases such as diabetes and venous stasis.[17] To address these limitations of traditional skin grafting, several modified approaches have been developed including but not limited to Meek grafting, Xpansion® micrografting (Applied Tissue Technologies, Hingham, Massachusetts), and pixel grafting.

MEEK GRAFTING

An alternative technique to skin expansion was developed by CP Meek who first patented a dermatome for producing small postage-stamp sized skin grafts.[23] His concept was based on the mathematical theory that the growing margin of the leading edges of the perimeter of a graft is proportional to the sum of its sides. Thus, grafting multiple small squares provides more active edges than one large square.[23,24] Furthermore, as graft size becomes smaller, these micrografts can survive on the wound bed exudate irrespective of their dermal orientation.[25,26] Although Meek grafting fell out of favor with the introduction of the Tanner skin graft mesher in 1964,[27] it was resurrected in 1993 with a modified version that was less cumbersome.[28] In this technique, harvested autograft is placed on a cork board and then run twice at 90-degree angles to each other through a compressed air dermatome, containing 13 blades spaced 3mm apart to cut the skin into squares. The processed autograft is then transferred onto a pre-folded polyamide gauze which is unfolded to achieve the desired expansion ratio (ranging from 3:1 to 9:1) before being placed on the wound bed. Unlike meshed grafts, these have been found to be 99.8% reliable in reaching the projected expansion ratio.[29] Although the Meek technique is more labor intensive than meshing, the literature describes excellent "take" rates clinically in major adult[30] and pediatric burn patients.[31] Menon has described the utility of the modified Meek technique combined with cultured epithelial autografts (CEA).[32] Reports indicate that micrografting results in shorter hospital stays.[33] This may be due to greater resistance to infection with faster re-epithelialization rates and wound closure.[34] The aesthetic outcome is also reported to be at least equal and possibly superior to grafts meshed to ratios greater than 3:1.[33]

XPANSION® MICROGRAFTING SYSTEM

The principle of micrograft systems is that the smaller the individual grafts, the greater the total epithelial border length from which epithelial cells can proliferate.[26] In a moist environment, orientation becomes irrelevant since the minute grafts can survive by diffusion from the wound bed alone.[35] Keratinocytes and fibroblasts migrate in the wound, form an epidermal layer with a dermal component,[36] and have demonstrated expression of factors including tumor necrosis factor-alpha, platelet-derived growth factor, and basic fibroblast growth factors, all capable of expediting the proliferative healing phase, neo-angiogenesis, and extra-cellular matrix deposition.[37] The skin micrografts are created using a mincing device, which consists of 24 parallel rotating cutting disks which are 0.8mm apart, permitting expansion ratios of 1:100 to be achieved, with wound healing quality comparable to split-thickness skin grafting. The system has been successfully used in the treatment of multiple chronic ulcers that were nonresponsive to conventional therapies.[38,39]

PIXEL GRAFTING

Studies were performed with pixel size grafts (0.3mm x 0.3mm) on full-thickness porcine wounds. A special mincing device was used to cut grafts 10 times (five in each perpendicular direction). The study demonstrated a faster rate of epithelialization compared to the micrograft technique, with equal degree of wound contraction, epidermal maturation, and rete ridge formation.[40]

OTHER TECHNIQUES

Given the foregoing limitations of skin grafts, numerous skin substitutes have been developed.[41,42] Most provide a scaffold for infiltration of fibroblasts and endothelial cells and still require subsequent skin grafting as a second stage. Skin substitutes, to date, have targeted the replacement of the epidermis or dermis, all of which have allogeneic, xenogeneic, or synthetic components, with no comprehensive approach to replicate or regenerate the hierarchical morphology of skin. Furthermore, the conceptual approach of recreating a simple two-layered, bilaminar structure fails to consider the functional appendages of the skin, as well as the resistance to shear stress supplied by the rete ridges. In this paper, we describe autologous skin replacement technologies that minimize donor-site morbidity and are available in clinical practice.

CULTURED EPITHELIAL AUTOGRAFTS

Keratinocytes can be grown in tissue cultures to produce sheets of autologous epithelial cells that can be transferred to the surface of a wound. Although the technology has been clinically available since the 1980s, drawbacks in its clinical application has caused enthusiasm for its use to wane.

The technique was developed by Rheinwald and Green in the mid-1970s and involves the isolation of keratinocytes from a full-thickness skin biopsy and their serial subcultivation in vitro onto a feeder layer of lethally irradiated mouse fibroblasts.[43,44] Added growth factors and serum provide optimal clonal expansion of proliferative epithelial cells and keratinocyte growth.[45,46] Although the 3T3 cells are washed away with successive media changes, the use of a non-human immunologically distinct cell line producing a host of foreign protein poses a theoretical concern. Other contributions stimulate colony formation including epidermal growth factor and cholera toxin, a known stimulant of cyclic AMP formation. Within three to four weeks, sheets of keratinocytes can be grown with expansion ratios of over 10,000. Each cultured epithelial sheet is detached from the vessel by enzymatic treatment with dispase and clipped to a backing of petroleum jelly-impregnated gauze before transfer to the wound bed.

An eight-year study by Compton performing serial biopsies on patients treated with CEA reported that at transplantation, the graft appears as unevenly stratified sheets of keratinocytes (three to nine cells thick) lacking both granular and cornified cell layers. By six days post grafting, CEA differentiate into all normal epidermal strata but lack rete ridges. All three types of epidermal dendritic cells (i.e., Langerhans cells, melanocytes, and Merkel cells) repopulate CEA during the first year.[47]

Since the first clinical application was reported in 1981, the use of CEA has had considerable impact in the treatment of patients with massive burns and other large wounds.[48–51] These excellent results, however, have not proved consistent in other burn units and their use has been questioned with regard as to its suitability for permanent skin coverage. Such limitations include poor engraftment and durability, sensitivity to colonization of bacteria, and high costs.[52–55]

The rate of successful engraftment (graft "take") depends on the nature of the recipient site, with chronic granulating wounds having a rate of take of 15% that rises to 28–47% in freshly excised or early granulating wounds.[56] The evidence suggests that in the absence of a dermal bed, application of CEA on a full-thickness skin wound does not lead to satisfactory bonding onto the underlying tissues (typically muscle), eventually leading to avulsion of the CEA.[6]

CEA is often friable and susceptible to shearing forces, especially within a year of grafting.[47] This can be explained by the absence of basement membrane, mature hemidesmosomes, and anchoring fibrils at the attachment face until three to four weeks post grafting. The anchoring fibrils remain immature, and there is an absence of rete ridges for a further six to 12 months.[47,57]

CEA is also highly susceptible to infection, explained by its vulnerability to bacterial proteases and cytotoxins during the first week of maturation.[58] Even after engraftment, its use is hampered by delays in obtaining grafts, high costs, and increased hospitalization compared with STSGs.[59] Quantitative sensory testing three years after grafting in an 11-year-old boy showed fine fiber sensory function (temperature) remaining subnormal compared to normal skin.[47]

The use of CEA is still recommended mainly for use in patients with large full-thickness burns where donor sites are limited and is approved for use by the FDA in patients with greater than 30% total body surface area (TBSA) injuries under a Humanitarian Device Exemption (HDE). To help mitigate the lack of a dermal component, higher take rates require alternative techniques, such as that described by Cuono et al., in which CEA is grafted to deepithelialized allograft.[60] Detractors of this method cite the problem of timing related to a two-stage procedure, premature loss of engrafted allograft, and infection.[61] For this reason, CEA can be placed over widely meshed autograft to provide a stronger base and accelerate the key events in wound repair and skin regeneration (Fig. 1).[60]

Best results are achieved when CEA is applied on the anterior surface of the body to decrease shear.[62] For circumferential use on the extremities, the limbs can be elevated by use of external fixators. Immobilization of the grafts for weeks to prevent shearing, coupled with the extreme sterile precautions necessary for dressing changes, places an enormous burden on the nursing staff.

AUTOLOGOUS SKIN CELL SUSPENSION

In an attempt to overcome some of the limitations of CEA, several different strategies have been explored.[63] Autologous keratinocytes have been harvested, digested, and made into cellular suspensions, which are sprayed onto wound sites, with or without fibrin, or delivered on various carrier dressings ranging from bovine collagen to a chemically defined polymer carrier.[64,65]

Autologous skin cell suspension (ASCS) is a technology in which a small, thin split-thickness skin biopsy is enzymatically digested to produce a suspension containing keratinocytes, melanocytes, Langerhans cells, and fibroblasts that can be sprayed directly onto a wound.[66] In this way, skin

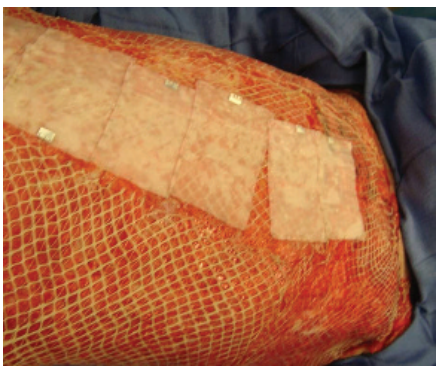

Figure 1. Excised burn of the chest resurfaced with widely meshed STSG overlaid with CEA.

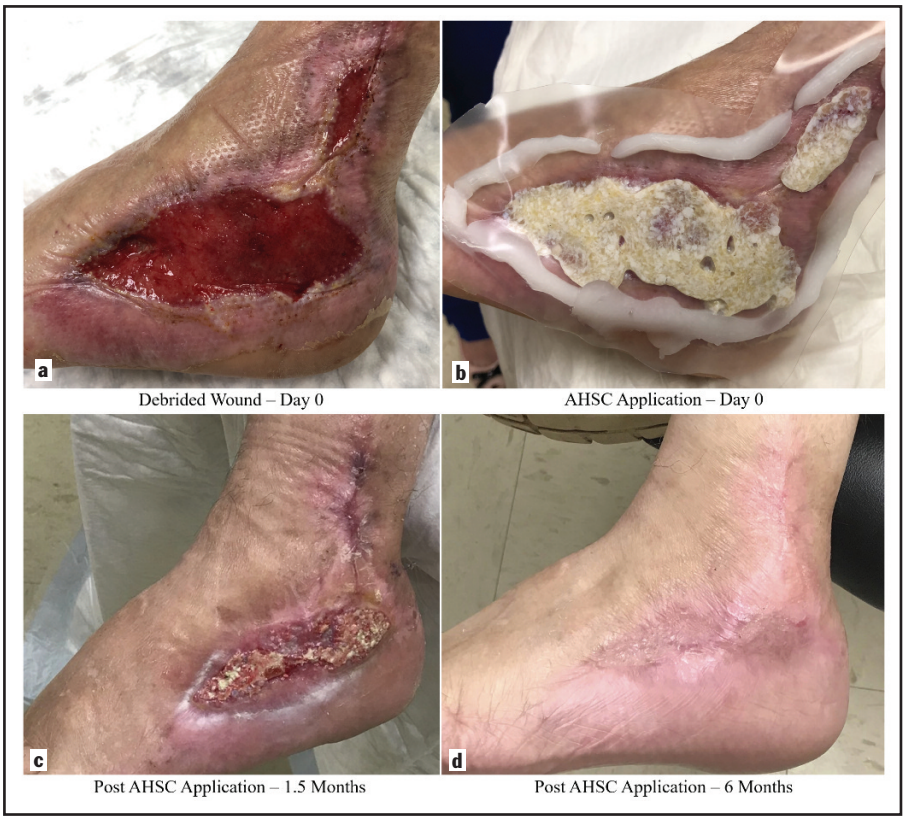

Figure 2a–d. Seventy-year-old male suffered a traumatic crush injury two months prior to AHSC therapy with multiple lower extremity fractures and an open wound on his left foot with exposed bone. The patient underwent debridement and application of urinary bladder matrix but was still left with an open wound with exposed bone. AHSC closed the remaining wound bed, including the previously exposed bone.

expansion to a ratio of 80:1 can be attained for partial-thickness wounds. Harkin reported cell viability is unaffected when delivered at pressures below 20psi. Pressures above this value, however, are associated with reduction in cellular metabolism. Bursts of a 0.2ml cell suspension ($1.5x10^6$/ml) delivered from a height of 10cm, delivering 5000–10,000 cells/cm^2, was enough to epithelialize an area of 10–15cm^2 within seven days in vitro.[67] Application with fibrin spray on a swine model was found to optimize the cellular response.[68]

A randomized study was conducted by Gravante et al., followed by two recent multicenter, prospective, randomized clinical trials conducted by Holmes et al.[69–71] The first of these recent multicenter trials compared the use of ASCS applied over widely meshed STSGs to meshed STSG (control). The second examined the clinical performance of ASCS against 2:1 meshed STSG. In all three studies, wound healing, pain, patient satisfaction with appearance, and scarring at the treatment sites were similar to the control groups. The advantage of ASCS was the reduced donor harvest site, reduced donor morbidity, and the utility of the therapy when donor sites are limited.

ASCS has been reported in a small series for the treatment of vitiligo and full-thickness burns in combination with a dermal regeneration template, in trauma, in a large melanocytic nevus and in an experimental pig model of sulfur mustard cutaneous injury.[66,68,72–75]

MICROCOLUMN GRAFTING

Microcolumn grafting is being pursued as an alternative technique in which narrow skin columns (microcolumns) of full-thickness skin, representing a fraction of the recipient surface, are harvested and transferred onto the target wound. Compared to traditional harvesting techniques, there is said to be minimal donor-site morbidity. The scientific principle was established following the development of fractional photothermolysis, whereby laser microbeams were used to create thousands of thin columns of microscopic thermal burns per cm^2. In contrast to full-thickness burns, these laser wounds remodeled and epithelialized without scarring.[76]

The use of microcolumns to reconstitute skin wounds was demonstrated in a mouse model by Tam et al. where fresh human skin microcolumns were applied to full-thickness wounds on immunodeficient mice.[77] Many features of normal human skin were present in the restored skin including epidermis, diverse skin cell populations, adnexal structures, and sweat production in response to cholinergic stimulation. These promising preclinical results suggested that harvesting and grafting of microcolumns may be useful for reconstituting fully functional skin in human wounds, without donor-site morbidity. Full-thickness skin tissue columns have also been evaluated in porcine studies.[78,79]

A recently developed device (Autologous Regeneration of Tissue [ART]; MedlinePlus, Northfield, Illinois) harvests 316 full-thickness columns with a 500mM diameter in a 28.7mm circular array.[80] The columns are then placed into the wound, and multiple harvests can be taken. Jaller et al. assessed donor-site pain in nine patients with non-healing lower extremity wounds. Although wound outcomes were not the focus of the study, nor reported, patients were able to tolerate the harvest procedure. Donor sites were still apparent but demonstrated qualitative visual improvement within the 60-day follow-up period. The use of microcolumn grafting for chronic wounds is being further evaluated in a single arm, open-label clinical trial (https://clinicaltrials.gov/ct2/ show/NCT03368534), which is designed to assess donor-site morbidity and wound closure.

AUTOLOGOUS HOMOLOGOUS SKIN CONSTRUCT

In an effort to incorporate the endogenous regenerative populations found within the dermis, Autologous Heterogeneous Skin Construct (AHSC, SkinTE™, PolarityTE MD, Inc., Salt Lake City, Utah—also referred to as Autologous Homologous Skin Construct in prior publications) was derived from a small full-thickness harvest of healthy skin. In addition to the basal keratinocytes of the epidermis, full-thickness skin includes the endogenous cellular niches within the follicular bulge and glandular appendages that are involved in native skin repair.[81–83] The skin is harvested as an ellipse sufficiently small to allow the donor site to be closed primarily.[84] The AHSC manufacturing

optimizes the construct for graft take in austere wound environments, such as chronic wounds, and primes the contained cellular populations for wound closure.[85] The AHSC is returned to the patient expeditiously without any exogenous enzymes, supplementation, or culturing. It is evenly spread across the wound bed, which provides the necessary sustenance. The construct engrafts and expands within the wound facilitating closure from the inside out (Fig. 2a–d). This is in contrast with other advanced wound care therapeutics that often still rely on the residual skin cells surrounding the wound that are potentially injured and or inadequate to close critical-sized defects.[86–88] Additionally, data suggest that resulting skin has mature epidermis and dermis including dermal appendages such as hair follicles and sweat glands, which is related to the return of skin function such as sweating and proprioception.[89]

In the first reported case, the AHSC generated from a 12cm^2 harvest was applied to a 200cm^2 two-year-old wound that had failed multiple STSGs.[89] Complete epithelial coverage was achieved in eight weeks, and complete wound coverage with functional skin occurred in 12 weeks. At six-month follow up, the wound was healed with durable, pliable skin, which was qualitatively and quantitatively similar to surrounding normal skin across multiple functions and characteristics, including sensation, hair follicle morphology, bio-impedance and composition, pigment regeneration, and gland production.[89]

Patterson et al. reported on the resurfacing of a wound during scar revision in a pediatric burn patient using a 17.5cm^2 harvest to treat a 200cm^2 full-thickness scar excision defect. The AHSC demonstrated graft take and initial epithelialization with repigmentation by one week postoperatively, progressing to complete epithelial coverage at eight weeks with minimal contracture. At five months, imaging and biopsy of the reconstructed site revealed regeneration of full-thickness skin, including hair, rete pegs, and subdermal fat.[86] A multi-institutional case series of early clinical use evaluated AHSC for complex refractory wounds of different etiologies including acute burn, complex traumatic wounds, burn reconstruction, and chronic wounds. The entire cohort of 15 patients had complete AHSC take and wound closure. When performed, two-point discrimination, bioimpedance, Raman spectroscopy, and histomorphologic analyses showed that AHSC-regenerated skin was analogous to native skin complete with hair follicles.[90]

Two pilot studies evaluating the use of AHSC in chronic wounds have shown promising results.[85,90] Eleven patients with diabetic foot ulcers (DFUs) were treated with a single application of AHSC created from a 1.5cm^2 piece of healthy skin. Ten DFUs were closed within eight weeks, with a median time to complete closure of 25 days. Mean percent reduction at four weeks was 83%, with no adverse events related to the treatment site. One patient developed an infection of previously placed hardware for a Charcot foot reconstruction that required extensive debridement.[85] In the second pilot study, 10 patients with venous leg ulcers (VLUs) were treated with a single application of AHSC created from a 1.5cm^2 piece of healthy skin. Nine VLUs healed in a mean time of 34 days. One patient took over 13 weeks to achieve full closure. There were no adverse events related to the treatment site.[90] These results are being evaluated in a larger randomized controlled trial comparing AHSC to standard of care (DFU: NCT03881254 and VLU: NCT03881267).

FUTURE PERSPECTIVE

Wound care treatments have undergone significant progress in the last two decades with the emergence of sophisticated tissue engineering approaches including decellularized tissue matrices, engineered scaffolds, and seeded allogenic constructs. These therapies often require the response of the patient's tissue, surrounding or within the wound, which may be insufficient to close critical-sized defects and refractory chronic wounds. The potential for human skin to regenerate itself has been appreciated for centuries and was first harnessed in the form of skin grafting and then in the last century as cultured epithelial autografts. More recently, skin's endogenous regenerative potential has been harnessed in novel autologous therapies that allow for the use of the patient's own skin while minimizing donor-site morbidity.

Despite these advancements, treatment of full-thickness defects has remained challenging. The importance of generating not just the epithelium but all the functional components of skin including durable, elastic dermis, hair-follicles, and glands has become more apparent with the appropriate and increasing attention to patient quality of life.[21] Skin is not only a barrier but the body's largest organ and its critical functions rely on the presence of its native complex architecture. Novel therapies that leverage not only keratinocytes but the potent regenerative populations important for native wound healing, which are found within the dermis and dermal appendages, show promise for the treatment of full-thickness defects. Furthermore, they present an exciting opportunity to advance wound healing beyond just epithelial closure to achieve the complex array of skin functions and ultimately a better quality of life for the patient. STI

AUTHORS' DISCLOSURES

Dr Milner, Dr. Swanson, and Dr. Sopko are paid employees of PolarityTE, Inc. Dr. Granick is a clinical consultant of PolarityTE, Inc.

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