Combination Platelet Leukocyte Rich Plasma (PLRP/PRP) and Hyperbaric Systemic Oxygenation (HBO) is Safe and Effective in the Treatment of Refractory Wounds

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Rationale & Protocols

INTRODUCTION

It is becoming increasingly clear that the cost of wound care continues to rise as patients are living longer only to face different medical challenges in their later years. In particular, the lower extremity becomes more vulnerable to wound formation as patients survive cardiac, pulmonary and malignant diseases. Aging limbs tend to succumb to poor end point perfusion in the 7th, 8th and 9th decades as arteriolar, capillary and venular integrity diminishes. *Patients are also healthier in their later years and are now more active enjoying a higher quality life.* Seemingly small wounds, which develop on the lower extremities often lead to life changing and sometimes catastrophic events such as hospitalization for wound infections, sepsis, amputation and or death. The cost of wound care in the United States is staggering now approaching 3 billion dollars annually (1). In addition to this cost is the associated disability and loss of productivity as a result of amputation, which is difficult to quantify. Approximately 50% of non-traumatic amputations in the US are associated with complications of diabetic lower extremity wounds (2). To worsen matters is the fact that the 3-year survival rate after a major lower extremity amputation is only 50% and the survival rate after 5 years is only 40%. Approximately 80 % of these cases began with a seemingly small wound on the lower extremity necessitating the need to consider aggressive and more effective treatment strategies earlier in the course of disease.

There is a growing trend in the development of wound management centers in the U.S. that utilize multi-disciplinary team approaches leading to the successful treatment in approximately 80 percent of their cases (3). However, there remains the subset which are refractory despite appropriate treatment strategies necessitating the need to consider more aggressive intervention by utilizing tissue engineering concepts including growth factor therapy, hyperbaric medicine, human skin equivalents (HSE) and now autologous living tissue free graft techniques (PRP). These new technologies represent a safe and non-surgical alternative to full thickness skin grafts and in some cases can prevent a significant number of limb amputations. Further discussed is the role of these technologies and how “combination” therapy pushes the envelope in the successful treatment of these refractory wounds.

Given the magnitude and its associated cost of the problem, an explosion of new treatment strategies has emerged over the past decade for wound care. These include the recent development of growth factor therapy, bio-tissue engineering, hyperbaric medicine in addition to the myriad of new dressings that are now available. Indeed, wound care has come a long way however in some regions of the U.S. it is still thought to basically consist of a simple wet to dry saline dressings in the hope that the wound will heal. When these wounds fail to heal patients are often referred for surgical consultation for traditional full thickness skin grafts requiring

hospitalization. Wounds appear to be static but are quite dynamic requiring an understanding of the complex "physiological" changes in the different stages of the wound-healing cascade. The new technologies for wound care address these physiological issues requiring additional training making it challenging for primary care physicians to manage complex wounds.

These second line therapies are reserved for wounds which are refractory to appropriate first line basic treatment strategies which include (4): bio-mechanical off-loading, frequent debridement, treatment of infection, appropriate wound dressings, nutritional supplementation, better control of serum glucose (5) and correcting other underlying metabolic factors which contribute to an immunocompromized state. All of these strategies, whether first or second line, were developed to optimize or specifically enhance individual steps within the wound healing cascade of which there has been a significant growth in our of understanding at a cellular and molecular level. The summation of individual steps in the treatment plan attempts to "tilt" the wound-healing cascade in favor of forward progression of soft tissue regeneration. When wounds fail to demonstrate adequate granulation after 30 to 60 days of first line therapies, more aggressive second line non-surgical treatment strategies can be employed including combination therapy. These techniques are now available to be employed by wound management personnel in an outpatient setting allowing easier patient access to advancing technologies.

Not all wounds are the same. The diversity of wound types and their treatments are rather extensive including anatomical location, pathophysiology, age of the patient, various comorbidities, size and depth of the wound and socioeconomic factors including staff and patient levels of education (6). For example, a surgical knee dehiscence will respond differently when compared to a lateral leg or Achilles wound. A rheumatoid associated vasculitic cutaneous wound will respond differently than a diabetic foot ulcer. Wounds will respond differently depending upon the age of patients whether they are in their 4th, 5th, and subsequent decades. Identical wounds will respond differently when patients have different co-morbidities such as CHF, history of malignancy, diabetes or renal disease, etc. The various sizes of wounds also lead to different healing rates depending upon its initial severity. Patients demonstrate various levels of education resulting in varying degrees of compliance.

When considering these many variables it becomes increasingly clear that the responsibility for appropriate wound care falls upon the patient and wound care multi-disciplinary team. For these same reasons it is also difficult to conduct evidence based randomized double-blinded controlled studies. The challenge in wound care is addressing the multiple risk factors, which are known to inhibit the wound-healing cascade and intervene with safe and effective treatment strategies, which promote the forward progression of granulation tissue formation or secondary closure. Despite the diversity of wounds and their varying response rates to treatment, there remains the fundamental principle of successive stages of the wound healing cascade beginning with the inflammatory phase followed by fibroblast proliferation and completed in the remodeling phase with epidermis formation (7).

Another important consideration in wound healing is determining the "capacity" for healing. The capacity for a wound to heal is dependent upon cellular ATP energy stores, which is only available at the wound margins. Essentially, wound models are energy dependent equations with different "slopes" of response. These slopes are dependent upon the available energy

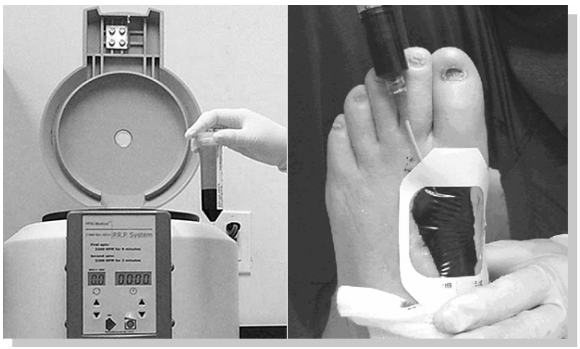
measured as a function of ATP stores with in the cells involved in wound healing; platelets, leukocytes, monocytes-soft tissue macrophages, fibroblasts and keratinocytes, etc. Mitogenic activity, cellular differentiation, protein transcription and chemotaxis are active cellular metabolic processes, which are energy dependent. For example, the metabolic requirement of an activated leukocyte engulfing bacteria is approximately 15 fold when compared to the resting basal state. Chronic nonhealing wounds often are shown to have end point perfusion deficits resulting in cellular hypoxia and hypoglycemia making the histological "milieu" energy poor for tissue regeneration. It is reasonable to assume that the complex growth factor driven processes of the inflammatory, proliferative and remodeling phases are energy dependent upon the availability of peri-wound capillary end point perfusion.

The objective of wound healing is to "optimize" the wound-healing cascade, which are temporal, and spatial, requiring signal transduction mechanisms, paracrine activity and adequate energy stores sufficient for soft tissue regeneration. The cascade also requires an understanding of the importance of end point perfusion including adequate oxygenation and glucose for mitogenic and cell differentiation metabolic requirements. Principles of treatment target the various steps in the hope of driving soft tissue regeneration to secondary closure. It then becomes clear that a single treatment is not likely to be effective in a complex wound healing model. Chronic refractory wounds that have failed appropriate first line therapies should require "combination" treatment strategies addressing many individual step requirements simultaneously. Bio-tissue engineering techniques in combination with hyperbaric medicine represent an effective multi-faceted targeted approach to refractory wounds.

PLRP

The concept of growth factor therapy for the treatment of wounds is now well accepted and utilized. Platelet derived growth factor (PDGF) was one of the first growth factors to be identified in the wound healing cascade and now is available for topical application in the form of Regranex® (8). The autologous source of PDGF was initially identified in the alpha granules contained within platelets. Subsequently, many additional growth factors have been identified including transforming growth factor (TGF), fibroblast growth factor (FGF), and many others known to play an important role within the wound-healing cascade. Studies have shown that multiple growth factors tend to be more effective than the use of a single growth factor. The concept of harvesting and concentrating autologous platelets with subsequent transfer and fixation to the wound site within the medium of a autologous soft tissue graft allows access to higher concentrations of multiple growth factors placed directly into the wound site (9,10,11). This process is known as platelet gel or platelet rich plasma.

Platelet rich plasma has been used extensively within the surgical setting primarily with autologous or synthetic bone osseo-integration techniques in cranial facial surgery (12). It is now extensively used in orthopedics, neurosurgery, ENT, plastics and other surgical fields. The role of autologous derived growth factors promoting bone and softer tissue growth has been well studied (13, 14, 15, 16, 17, 18, 19, 20, 21, 22). This procedure initially required cell saver technology within the OR and large volumes of blood (500ml). Further refinement of the technique has led to the use of a small tabletop centrifuge allowing the procedure to be performed with low volume whole (50ml) blood in an out patient setting. From 50 cc of whole blood can be derived approximately 5-10 cc of platelet rich plasma, which is activated with



thrombin and calcium resulting in a thick, gelatinous soft tissue mass, which can be cut and placed immediately into the wound site where it remains for 5 to 7days. The autologous graft fills the wound void thereby serving as an occlusive dressing in additional to its grafting properties. The procedure can be performed in an out patient setting in one hour without the storage of any blood products eliminating infectious disease concerns.

Platelets play an important role in wound healing providing initial hemostasis and early growth factor provision to the wound site. Activated platelets lead to secretory expression of the alpha granules known to contain multiple growth factors including PDGF, TGF, PDAF, PDEGF and many others (23). These growth factors possess paracrine related properties, which are stimulatory for mitogenic activity, cell differentiation, protein transcription and chemotaxis (24). These same growth factors also possess inhibitory properties that stop the inflammatory phase and allow transition to the proliferative phase. Normal serum concentrations of platelets in whole is blood is 150,000 to 400,000 which is concentrated approximately 10 fold by the PRP process often resulting in platelet counts in excess of 1.5 million. There is evidence that the target concentration level should be approximately a minimum of 750,000 and further evidence that higher concentrations appear to be more effective in granulation tissue induction. Autologous sources of platelets allow access to " multiple" growth factors without concerns for tissue rejection, infectious disease related issues and eliminates the need for out of facility tissue manipulation.

Most, if not all of the attention regarding platelet rich plasma has been focused on platelets. However, the density of a leukocyte is approximately the same as a platelet and as a result the procedure also concentrates leukocytes. A recent study has shown that leukocytes are also concentrated to higher levels representing almost a 10-fold increase similar to that seen in platelets (25) and may represent an additional source for growth factors, in particular, insulin like growth factor (IL-1). In addition to its anti-bacterial role, leukocytes have also been shown to produce combinations of proteases important for soft tissue remodeling. Excessive protease production leads to self-destruction of soft tissue and inhibits transition to the proliferative phase of wound healing. Monocytes transform to soft tissue macrophages which are known to produce secretory leukocyte protease inhibitor (SLPI), which have been shown to inhibit excessive protease activity. The increase in leukocytes and monocytes along with platelets in the formation of the autologous living soft tissue graft suggests renaming the process to platelet-leukocyte rich plasma (PLRP). This increase in leukocyte concentration probably explains the absence of infection noted with the PLRP procedure.

PLRP represents a safe, autologous source of connective tissue, which can easily be prepared in the out patient setting without exposing the patient to the risk of contamination. The principles of treatment are multi-factorial addressing deficiencies within the wound healing cascade including autologous growth factor therapy, soft tissue matrix grafting and protease inhibition. PLRP preparations are versatile as it can be formed into soft tissue solid grafts,

sprayed into thin layers over large surface area wounds, or injected as a viscous liquid form into fistulas and deep surgical wounds which are hard to reach. Given its versatility, PLRP can be used in a variety of different wound models not restricted to lower extremity wounds.

HBO

Hyperbaric oxygen therapy (HBO) is the systemic provision of elevated partial pressures of oxygen for the purpose of delivering molecular oxygen to hypoxic tissues. This is achieved by utilizing hyperbaric chambers which can pressurize patients to controlled depths whereby the patient breathes 100% O2 for set periods of time as prescribed by the physician. At 2.4 ATA (atmospheres)of pressure which is the equivalent to diving to 45 FSW (feet sea water) a patient breathing 100% O2 will achieve a serum partial of O2 of 1800 mmHg, the majority of which is free and unbound dissolved as a gas in serum plasma (26). The ambient partial pressure of O2 in the lower extremity at a cutaneous level is approximately 40 mmHg. During a standard HBO treatment this level can rise to 500-1000 mmHG (10 to 15 fold increase). Chronic wounds, which are demonstrated to be hypoxic as measured by transcutaneous oximetry, qualify for a course of HBO the premise of delivering sufficient molecular O2 to the wound margins supporting mitogenic activity and other metabolic processes that are required for soft tissue regeneration (27,28). Physicians trained in hyperbaric medicine are essentially gas physiologists who take advantage of gas laws and diving chamber technology to deliver systemic oxygen at high partial pressures addressing various hypoxic pathophysiological models.

Increased molecular oxygen availability to the wound margin has been demonstrated to have multiple supportive processes. These include promotion of collagen formation (the molecule itself requiring oxygen within its structure), increased leukocyte activity, potentiation of certain antibiotics, neutralization of certain bacterial endotoxins as well as serving as a metabolic substrate for cellular metabolism (29). Recent studies have also demonstrated growth factor receptor upregulation reflecting the cells ability to engage in protein transcription in a hypoxic pathophysiological model (30,31). Recent studies have also shown that oxygen may serve as a "signal messenger molecule". When considering the chronic wound model, it becomes clear that oxygen plays an important supportive role in multiple cellular processes important for soft tissue regeneration.

Standard courses of HBO treatments consist of 20 to 30 treatments over a 4 to 6 week period with each treatment lasting approximately 2 hours. Clinical evidence of wound improvement is generally noted towards the end of the 2nd week of treatment although cellular changes can be demonstrated as early as the first treatment. The effect of treatments tends to be "accumulative" requiring consistent delivery to achieve maximum therapeutic effect. During this daily treatment phase the wound care team is able to deliver high-level acuity management of the wound.

COMBINATION PLRP/HBO

When considering the multiple treatment benefits of each modality it stands to reason that combination therapy is likely to be more effective. Further evidence suggests that combination therapy is not only more effective in an additive fashion but also is synergistic. PLRP induced

protease inhibition with autologous growth factor provision induces mitogenic and cellular protein transcription that require increased oxygen and glucose delivery for cellular metabolism within the wound-healing cascade. It appears that the timing of delivery of these two modalities is essential in optimizing wound healing. For example, one can argue that PLRP could be applied 24 hours after the 1st HBO treatment, which has been demonstrated to up regulate growth factor receptors. While the graft remains in the wound for 5 days, HBO treatments would support signal transduction pathways as autologous growth factors begin to see multiple growth factor receptor up-regulation within the wound field. For further consideration is the combination of metalloproteinase inhibitor (MMPI) cellulose matrix dressings with PLRP and HBO. MMP's are a class of proteases that have been shown to inhibit wound healing due to excessive self-destruction of healthy tissues within the wound (32). MMPI's inhibit these proteases by utilizing oxidized regenerated cellulose that increase the uptake of MMP's out of the wound site and provide a scaffold matrix promoting cell migration. The combination of MMPI's with SLPI's maximizes the inhibition of multiple classes of proteases. The cellulose matrix maximizes cellular migration that is essential in wound healing.

PLRP FACILITY AND SAFETY EXPERIENCE

Given its safety profile in surgical applications over the past 10 years it was determined that the established safety profile would also be seen in the out patient setting. Since many surgical centers still use large volume (500ml) techniques it was felt that the low volume (50ml) sequestration utilized at the Brevard Regional Hyperbaric Center (BRHC) would be even safer. At the BRHC, over the past 3 years we have applied 1000 PLRP autologous living tissue grafts without any adverse events to the graft site itself. The only difficulties noted were with the dressing itself, particularly Tegaderm® that occasionally causes a topical dermatitis to the surrounding epidermis. The use of multiple-layered Vaseline® dressings eliminates the potential for contact dermatitis. At no point was it noted, standard 3 to 5 days of graft application, any occurrence of an adverse reaction to the wound region. In addition, there has been no noted increase for wound infection. As a matter in fact, PLRP has been applied to cultured proven polymicrobial colonized wounds (mostly MRSA and pseudomonas) with no adverse reaction. Patients are followed closely and instructed to call the BRHC when any difficulties arise before their next scheduled visit. Careful follow up is provided to all patients who receive PLRP and often return to the BRHC 3 to 5 days later for graft removal and reassessment. For patients who do not return to the BRHC, further coordination of care is extended to in-home nursing who are knowledgeable about the procedure. The safety profile experienced, in part, reflects the careful screening and facility protocols established and maintained at the BRHC.

PRLP CONTRAINDICATIONS

Contraindications for PLRP include patients with significant anemia (Hg < 9.5), thrombocytopenia (PLT < 100,000), and history of blood dyscrasias. These contraindications are on the basis of lack of efficacy of PLRP preparations. Patients who are critically ill, hypotensive, febrile and or are terminally ill are excluded on a clinical basis. Certain religions have restrictions pertaining to autologous blood that may not be compatible with their religious beliefs.

PLRP INDICATIONS AND PROTOCOLS

PLRP is reserved as 2nd line therapy similar to HBO when wounds fail to demonstrate adequate clinical change for at least 30 to 60 days despite appropriate primary preventative strategies as described above. PLRP than becomes adjunctive while preventative measures are followed in the center. The PLRP remains in place for 5 to 7 days (at the discretion of the wound care team) and formal reassessment of the wound is performed 2 weeks after application. In severe wound cases, PLRP can be applied weekly for 2 to 4 weeks at the discretion of the treating physician. Most applications are twice monthly for 4 to 8 weeks. The average number of applications per patient is approximately 5 PLRP applications in the course of wound care treatments. Once a wound is demonstrating an adequate clinical response 2nd line therapy is no longer needed unless the wound reaches a "plateau" for a 2 to 4 week period.

EFFICACY

Recent studies have shown bio-tissue engineering transplanted living human dermal tissue (human skin equivalents- HSE) to be effective in wound healing (33). The primary mechanisms are attributed to the graft acting as a substrate for cellular migration and the growth factors contained within the transplanted tissue. PLRP autologous living tissue transplants share many of the same characteristics as in HSE's (34). A recent controlled study demonstrated similar effectiveness for soft tissue regeneration treated with PRP (35). As a result, patients were treated with PLRP when wounds were greater than 30 days in duration (average being 90 to 120 days) having demonstrated no clinical evidence despite appropriate preventative wound management strategies. Autologous transplanted PLRP was applied weekly, twice monthly and or monthly depending upon the severity and location of the wound. End points of treatment was determined when the wound demonstrated sufficient granulation tissue formation and or when the remodeling phase was achieved. As long as there was clinical evidence of continued improvement seen in the wound no further treatment was given unless the wound arrested in a plateau for more than 4 weeks.

Based upon these protocols, 100 patients were treated with varying combinations of PRP with and without HBO at the Brevard Regional Hyperbaric Center. The patients represented failures from appropriate standard wound care treatment protocols. Many were referred for 2nd opinions from remote locations having been treated in other wound care centers. The types of wounds were variable as some were surgical dehiscence cases and others were Grade 3 and 4 Wagner wounds. The average duration of the wound on presentation to the facility was 5 ½ months with the longest wound duration being 24 months.

Based upon this initial protocol, multiple variations in treatment strategies were developed as determined by treatment response, socioeconomic factors and severity of the wound. For example, some patients responded to a single PLRP treatment that was sufficient to achieve secondary wound closure. Other patients only required 2 PLRP treatments separated by 2 to 4 week intervals. Others required extended treatments over longer periods of time, 3 to 6 months, due to the initial severity of the wound. Nursing home patients were found to be excellent candidates for PLRP due to the ease of treatment provision (36). Patients with dementia who have significant wounds, which do not respond appropriately to facility wound care

protocols were considered for PLRP at the request of the family and care providers. Patients who were referred for HBO therapy but were found to have contraindications for HBO were evaluated for PLRP. Some patients were provided both PLRP and HBO in various combinations.

COMBINATION PLRP/HBO SAFETY AND REVIEW

Patients chosen for combination therapy were those who were most severe and refractory to other treatments in the past. These patients were at the highest risk for limb amputation and served as controls as they had failed aggressive and extensive wound care treatment strategies. Patients who were treated with both HBO and PLRP were found not to have any adverse reactions to either treatment. As a matter of fact the wounds appeared to respond faster with significant changes were noted weekly. Various combinations in the timing of both treatments were developed. Some patients were provided PLRP at the beginning of the week followed by 4 HBO treatments weekly for 4 to 6 weeks periods. Others were provided PLRP after a full course of 20 to 30 HBO treatments or were provided PLRP with 2 to 3 HBO treatments in succession twice monthly or monthly. It became clear that the total number of both PLRP and HBO treatments could be reduced and shaped to fit the patient's socioeconomic restrictions. For example, patients receiving renal dialysis are typically very difficult to treat, as their wounds tend to be refractory. Also, they find it difficult to meet the rigorous time commitments to HBO which typically is daily therapy Monday thru Friday for 4 to 6 weeks when they are already committed to 5 hour dialysis treatments 3xs a week. Renal dialysis patients could receive PLRP and HBO on off dialysis days, which was easier for them to tolerate with good wound healing.

At the Brevard Regional Hyperbaric Center, 100 patients have been treated with PLRP and or combination PLRP/HBO of which 79 patients received HBO/PLRP and 21 received PLRP alone. Of those treated with PRP alone, 4 patients failed to respond while 17 achieved secondary closure (81% closure rate). Of the 79 patients treated with HBO/PLRP, 5 patients failed to respond while 74 achieved secondary closure (94 % closure rate). Treatment failures were defined as adequate treatment despite appropriate patient compliance while failures due to non-compliance were not included. Patients treated with PLRP alone were found to have contraindications to HBO. Patients who underwent combination therapy were treated in various combinations based upon socioeconomic constraints. . The majority of the patients received bi-monthly PLRP grafting after a full course of 20-30 HBO treatments. 9 patients received concomitant therapy where PLRP grafting was applied on Monday followed by daily HBO for the remainder of the week for 4 to 6 weeks. Although subjective and not controlled, the clinical response determined by weekly assessments appeared to be significant when compared to the other treatment groups. Patients with end stage renal disease on dialysis were treated with 3 HBO treatments per week on alternating dialysis days and received PLRP grafting bi-monthly.

DISCUSSION

The high success rate of this difficult and refractory subgroup seen at the Brevard Regional Hyperbaric Center (BRHC), in part, can be explained by strict patient criteria selection protocols. Careful review of fundamental treatment principles were ensured followed by clinical observation at the BRHC before combination therapy was recommended and provided. Very

close observation during the treatment phase yielded higher compliance as patients are seen daily while receiving combination therapy. Many patients were excluded from combination therapy due to non-compliance and or to the identification of disease processes where it was predicted that the patient would not respond.

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The efficacy of PLRP appears to be related to the concentration levels of both platelets and leukocytes. Platelet concentration in excess of 1.5 million tend to be more effective than 750,000. Further refinement of the procedure should also include platelet count elevations as well as qualitative analysis of alpha granule integrity. It would be helpful to know that the sequestered alpha granules contain adequate numbers of growth factors by quantitative analysis with the application of each graft. This would ensure maximum efficacy with each treatment and result in the need for fewer applications thereby reducing the cost of treatment.

The role of sequestered, autologous and concentrated leukocytes within the graft also requires further study. The known benefits of bactericidal activity in combination with protease inhibition can be further extended with additional growth factor studies. Studies of leukocyte-derived growth factors may shed additional information pertaining to wound healing.

A significant benefit of combination PLRP/HBO is the ability to access difficult to reach wounds by constructing the graft to fit the physical shape of the wound. Surgical wounds in particular have responded very well where the aperture of the wound is small while the volumetric surgical cavity is quite large. Examples include lumbar, cervical and abdominal surgical dehiscence with small apertures. Whether it is injected as a thick viscous liquid which then converts to a solid graft inside a deeply penetrating fistula, sprayed as a thin layer over a large surface area within the wound, or converted into a solid graft and fixed to smaller wound, allows versatility in the application of difficult and refractory wounds. This in combination with systemic oxygenation allows better treatment “access” to difficult to reach wounds.

Another significant benefit of PLRP is in the treatment of nursing home patients who are not appropriate for hyperbaric medicine due to dementia. Unfortunately, these patients often develop significant wounds, which increase the burden of care that is already high. These patients are often hospitalized due to wound infections and sepsis increasing the cost of medical care. Family members are quite sensitive to these wounds and expect wounds to heal quickly. When these wounds become refractory despite appropriate wound management, family frustration increases and in some cases lead to litigation that is already becoming a significant problem for nursing homes. Given the ease in provision, safety and efficacy of PLRP, 2nd line therapy can be coordinated and extended into the nursing home environment by a collaborative effort with the regional wound management program.

Much of the attention in wound healing has been focused on cellular mechanisms that stimulate cell growth. Additional areas for study should also include inhibitory processes, which are also important for wound healing. Tissue regeneration involves a balance of stimulatory growth in balance with proteolytic soft tissue remodeling. Also important is the role of certain growth factors that inhibit the inflammatory phase allowing transition to the proliferative phase, i.e. TGF. This is becoming increasingly important especially in cutaneous wounds associated with autoimmune disease i.e. rheumatoid arthritis, where the wound is in a perpetual

inflammatory state. The principle of treatment in these types of wounds should include inhibition of cytokines and the simultaneous provision of growth factors that induce fibroblastic activity. Vasculitic wounds treated at our center with PLRP and PLRP/HBO have responded very well with noted decrease in inflammation and pain followed by sequential granulation tissue formation and secondary closure.

Combination therapy does not need to be limited to 2 entities. As long as each entity is safe and there is no evidence of cross entity adverse reaction, multiple combinations can be further considered. One example includes metalloprotease inhibitors with PLRP/HBO. As discussed above, this triple combination is synergistic due to inhibition of the MMP class of proteases as well as serving as a cellulose matrix, which promotes improved cellular migration. The oxidized regenerated cellulose is applied to the wound as a singular layer followed by application of the PLRP graft. The patient then proceeds with a course of HBO while the combination graft remains in the wound for 3 to 5 days.

When considering the new emerging treatment options for refractory wounds it is becoming clear that more patients are achieving secondary closure of refractory wounds. It is no longer a case of "*can we heal the wound?*" but rather "*how long will it take?*". The rate of healing is important since wounds that remain open are portals for microorganisms that can lead to infection and sepsis. The sooner a wound closes the sooner the risk for wound complications decreases.

SUMMARY

Combination PLRP/HBO offers a safe and effective treatment option for refractory wounds that are at the highest risk for amputation. Its varying combinations can be shaped to the socioeconomic needs of the patient, care providers and family. Given it safety profile, ease of provision and efficacy, PLRP/HBO represents a treatment option alternative to patients who are at high risk for amputation. Combination therapy is also cost effective as high-risk refractory wounds lead to more frequent hospitalizations for sepsis, gangrene, full thickness skin grafting, amputation and death. Further combinations of new emerging technologies will mostly likely yield improved results.

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