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## Current concepts on burn wound conversion – a review of recent advances in understanding the secondary progressions of burns

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### Abstract

Burn wound conversion describes the process by which superficial partial thickness burns convert into deeper burns necessitating surgical intervention. Fully understanding and thus controlling this phenomenon continues to defy burn surgeons. However, potentially guiding burn wound progression so as to obviate the need for surgery while still bringing about healing with limited scarring is the major unmet challenge. Comprehending the pathophysiologic background contributing to deeper progression of these burns is an essential prerequisite to planning any intervention. In this study, a review of articles examining burn wound progression over the last five years was conducted to analyze trends in recent burn progression research, determine changes in understanding of the pathogenesis of burn conversion, and subsequently examine the direction for future research in developing therapies. The majority of recent research focuses on applying therapies from other disease processes to common underlying pathogenic mechanisms in burn conversion. While ischemia, inflammation, and free oxygen radicals continue to demonstrate a critical role in secondary necrosis, novel mechanisms such as autophagy have also been shown to contribute affect significantly burn progression significantly. Further research will have to determine whether multiple mechanisms should be targeted when developing clinical therapies.

### Keywords

Burn wound conversion; Secondary burn progression; Autophagy

## INTRODUCTION

Over the last ten years, more than 190,000 patients have been admitted for acute burns in the United States with 24,591 patients admitted in 2013 alone [1]. The majority of these cases

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involved a small total body surface area (TBSA). However, those patients with higher percent TBSA burns had significantly increased mortality in all age groups [1]. While the direct damage to the tissue area caused by the primary injury is often irreversible, the secondary insult caused by progression of the burn wound is amenable to therapeutic intervention.

Burn wound conversion can be defined as the progression of superficial partial-thickness burns to deep partial-thickness and full-thickness burns [2]. This concept originates in the traditional description of burn injury zones by Jackson, in which three different zones of tissue damage of varying degrees occurred after burn injury [2]. The central irreversibly damaged zone of coagulation, the surrounding damaged but threatened zone of stasis, and the outermost recoverable zone of hyperemia. The middle zone of stasis was noted to be viable by Jackson [3] and has since been deemed a therapeutically critical section of burn surface area to address as it loses perfusion in its natural course, dies, and regresses into the inner zone of coagulation that cannot be salvaged [2]. The issue of progression of injury in the zone of stasis is pivotal since burn wound conversion often contributes to both greater burn surface area and burn depth. This larger, deeper wound has multiple local and systemic consequences that increase complications and morbidity [4–6].

As halting the progression of damage in the zone of stasis is a logical point of medical intervention in burn treatment, much research has been devoted to better understanding the process of burn conversion, and halting it. A recent review found 29 studies in 2012 to 2013 investigating experimental burn conversion treatments [7]. While several advances have been made in elucidating new mechanisms of burn progression, some of the leading theories behind secondary burn damage have remained the same for many years. Microthrombosis was noted in burn wounds in 1949 [8] and shortly thereafter found to be reversible to prevent necrosis of otherwise viable burn tissue [9]. The importance of reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radicals, as mediators of tissue injury post-burn has also been known for some time [10]. On the other hand, more recent data has recognized the importance of other mechanisms, such as autophagy, in contribution to burn wound progression [11]. Though our understanding of burn wound progression pathogenesis is changing, the principles and applications of burn treatment have remained fairly stable.

Current therapies in medical burn wound treatment after stabilization and prior to reconstruction are aimed at treating the complications of burns, promoting healing, and preventing further complications. Fluid resuscitation serves as the mainstay of systemic treatment in moderate and severe burns to maintain organ and tissue perfusion. Local wound management includes topical antibiotics and various biologic and non-biologic dressing as means of protection from the environment, drainage absorption, pain control, and providing a moist environment for wound healing [5, 12]. Though such treatments may have secondary effects on wound conversion [13], as the pathogenesis of burn wound progression is still not well understood, few clinical therapies are designed to directly address the issue of wound progression.

The purpose of this study is to investigate the recent progress in understanding the pathophysiology of burn wound conversion. Elucidating the trends in burn wound

progression research may help provide a better understanding of whether therapeutic interventions for halting burn progression will continue to be targeted towards conventional therapies or whether new treatments might offer increased efficacy.

## MATERIALS AND METHODS

A review of the current literature was conducted to identify recent studies on the pathogenesis of burn wound conversion. A literature search was performed by querying the MEDLINE database for full-text articles over the last five years (2010 – 2014) using the keywords, “burn AND wound AND (conversion OR progression OR expansion)”. A preliminary review of article titles was used to include any basic or clinic science studies on burn progression. Review articles, articles in languages other than English, and abstracts were excluded from the study.

We identified abstracts focusing specifically on the pathophysiology of burn wound conversion, either proposing new theories or expanding on previously known mechanisms of burn progression. Full texts were then categorized according to the particular burn conversion pathophysiology investigated in each article. Studies describing new potential treatments for already defined mechanisms of progression were also addressed.

## RESULTS

The initial search criteria yielded a total of 254 potential articles for the time period of 2010 to 2014. After screening titles and abstracts, and applying the inclusion and exclusion criteria, a total of 24 articles were included for review in the study (Table 1). All studies utilized animal models for experiments and the majority (67 percent) used the comb burn model [14] to induce burn injuries and replicate the zone of stasis.

Articles were categorized according to the main theory of pathogenesis that was being investigated or treated in the study (Fig. 1). The majority of articles addressed better studied concepts such as ischemia (9 studies) and inflammation (6 studies), whereas more recently discovered mechanisms such as autophagy (3 articles) had less focus, as expected. Articles grouped under “other” included studies on stem cell therapies [15, 16], enzymatic wound debridement [17], burn excision [18] and hyperbaric oxygen treatment (HBOT) [19]. We found that most studies on burn wound progression over the last five years involved experimental treatments in animal models, and very few studies were solely focused on further elucidating the mechanisms behind burn wound conversion (Fig. 2). Studies focusing on pathophysiology addressed autophagy and ischemia while the remainder emphasized novel treatments for secondary ischemia and inflammation.

## DISCUSSION

The differentiation between salvageable and unsalvageable tissue in burns was a key breakthrough in burn research that has since led to better understanding the pathogenesis of burn wound conversion at the tissue, cellular, and molecular levels. As our understanding of this pathogenesis continues to evolve, most experimental therapies still focus on only a few of the current theories behind burn conversion. The goal of this study was to analyze recent

trends in burn progression research to develop a better understanding of new directions for this research, novel mechanisms of burn progression, and new potential therapies.

The majority of studies over the last five years have focused on assessing the efficacy of well-established therapies in other disease processes and applying these to burn wound conversion. The few articles dedicated to exploring pathophysiology were almost solely related to autophagy, or the process of cellular waste degradation, as this phenomenon is a much newer concept in burn progression research. However, the recent studies examining new treatments for well-known mechanisms of secondary burn progression also elucidate further details about the pathogenesis that contributes to burn wound progression. These findings, along with their implications for future trends in research and potential therapies, are reviewed below.

### Autophagy

Autophagy is the degradation pathway in which cellular waste is delivered to lysosomes to be broken down and recycled. These recycled substrates are then used by the cells for tissue regeneration [20]. This process appears to work effectively as an enhancement to tissue healing, provided that the system is not overwhelmed with excessive breakdown products from more severe cellular injury. Though not a new concept, autophagy is a new area of research in relation to burn wound progression. The studies over the last several years have focused on defining the role of autophagy in burn wound conversion and better understanding its relation to apoptosis in this process. Apoptosis was suggested to contribute to burn wound progression in 2006 when it was shown that more apoptotic cells were found in deep partial-thickness burns than in normal skin or superficial partial-thickness burns [21, 22]. Later work also demonstrated the contribution of apoptosis as well as oncosis, or ischemic cell death characterized by cell swelling, to tissue death in the zone of stasis [23]. Decreased apoptosis and improved healing in burns have also been reported after treatment with a c-Jun inhibitor, further supporting the importance of apoptosis in the process of secondary tissue loss in the burn wound [24]. The function of autophagy in the post-burn wound environment, however, was not well understood until recently.

In 2013, Tan *et al.* reported higher autophagy than apoptosis rates in hair follicle epithelium from two to 24 hours after burn injury using the comb burn model [25]. The authors concluded that both processes contribute to cell death in the zone of stasis but with a different time course, suggesting that different treatments may be necessary to target the two processes. The role of autophagy in cell death – specifically whether autophagy is a mediator or preventer of cell death – is controversial. Though autophagy has been referred to as “type II programmed cell death” [26] or “macroautophagy”, autophagy has been shown to protect against apoptosis [27] and is more widely believed to be a degradation pathway that supports cellular homeostasis [28].

Contrary to the results of Tan *et al.*, Xiao *et al.* reported that autophagy decreases early in the course of burn injury progression and increases later, though autophagy levels always remained below those in normal skin in full-thickness wound tissues [29]. The authors also observed, however, that in the deep dermal layer, which they state may correlate to zone of stasis, staining for autophagy marker LC3 was increased, similar to the observations of Tan

*et al.* Xiao *et al.* have also reported that augmenting autophagy with the antibiotic rapamycin lessened burn wound progression and improved wound healing, further suggesting that autophagy may have a beneficial role in preventing burn wound progression [30]. These studies are difficult to compare as different burn models are utilized, not all observed markers are similar, and different areas of tissue are analyzed. Autophagy may likely have both protective and detrimental effects on the cell, possibly depending on the degree of cell damage and the timing from initial injury. Further elucidating the role of autophagy in the zone of stasis will be crucial to determining whether potential treatments should be aimed at enhancing or inhibiting this process.

## Inflammation

The detrimental effects of the prolonged inflammatory response in burn wounds have been well established and can be attributed to a myriad of different factors including complement activation, cytokine production, delayed inflammatory cell apoptosis, and ROS production [31–37]. Current common methods of reducing inflammation involve wound debridement to remove surface eschar, bacteria, and inflammatory cells, as well as maintaining a favorable wound environment by using appropriate dressings [36]. More recent approaches have focused on targeting cytokines, signaling pathways, and inflammatory cells that contribute to the heightened inflammatory milieu of the burn wound microenvironment. Our laboratory is currently investigating the profile of these inflammatory mediators in the first 48 hours after partial thickness burns to guide the administration of local agents aimed at controlling and directing the initial immune response to reduce burn wound progression. In a similar approach, several specific mediators in the inflammatory signaling cascade have been targeted for potentially reducing excess inflammation.

Our review showed that recent studies have investigated novel uses for well-known anti-inflammatory agents or have focused on specific points in inflammatory signaling pathways that may be amenable to intervention. Singer *et al.* take a broader approach by investigating the efficacy of curcumin, a powerful anti-inflammatory and antioxidant agent [38–40]. The authors demonstrate that treatment of burn wounds with intravenous curcumin in a rat comb burn model decreased burn wound progression and that these effects were bimodal, suggesting more than one mechanism of action [41]. Eski *et al.* report that treatment with cerium nitrate baths immediately after thermal injury prevented progressive tissue necrosis in the zone of stasis in both short-term (3 day) and long-term (21 day) follow-up [42]. Cerium nitrate has been shown to reduce tumor necrosis factor alpha (TNF- $\alpha$ ) levels in burns [43], decrease transmigration of leukocytes [44], and denature the lipid protein complex (LPC) in burn eschars, which plays a role in phagocyte activation [45, 46]. As inflammation, particularly with regards to phagocyte activation, is inherently associated with the production of ROS [47] (discussed below), such treatment paradigms may reduce cell death in the zone of stasis through multiple different mechanisms.

Other studies have focused on the infiltration of inflammatory cells into the burn wound, particularly polymorphonuclear neutrophils (PMNs), which are known to occlude vasculature leading to ischemia [35, 36] as well as secrete a variety of deleterious proinflammatory mediators [48, 49]. Bohr *et al.* investigate the use of Resolvin D2 (RvD2),

a lipid mediator shown to block secretion of pro-inflammatory mediators such as TNF- $\alpha$  and interleukin-1 beta (IL-1 $\beta$ ) and reduce PMN adhesion and infiltration into tissues [50], to treat burn wound progression [51, 52]. The authors observed that treatment prevented secondary thrombosis of the deep dermal vascular network (DDVN) and subsequently decreased burn wound progression. RvD2, however, did not reduce the number of PMNs in the wounds, but instead decreased levels of TNF- $\alpha$  as well as the expression of inflammatory cell adhesion molecules, indicating that the activation and secretion of inflammatory mediators by PMNs may be more important in burn wound conversion than their physical obstruction of vasculature..

Efforts to reduce global inflammation in the burn wound have also utilized non-pharmacological means, such as hypothermia. Hypothermia has been shown to limit cellular injury after an insult [53] and downregulate inflammatory genes [54]. Rizzo *et al.* demonstrate that moderate systemic hypothermia after burn injury decreases burn depth progression [55]. Interestingly, some inflammatory genes including those for certain chemokines were upregulated after exposure to hypothermia. These genes were noted to be different than those upregulated by burns, suggesting that targeting of specific inflammatory mediators may be a more efficacious strategy than attempting to broadly limit inflammation.

One such target is TNF- $\alpha$ , a cell signaling protein that regulates inflammation and is observed at high levels in the burn wound microenvironment [56]. Sun *et al.* demonstrate that topical antibodies to TNF- $\alpha$  conjugated with hyaluronic acid (HA) reduce levels of downstream inflammatory mediators, decrease macrophage infiltration, and lessen secondary necrotic expansion of tissues [57]. In a later study, the authors demonstrate that anti-TNF- $\alpha$  conjugated to HA more effectively prevents necrosis of tissue and decreases inflammatory markers than anti-TNF- $\alpha$  alone or anti-TNF- $\alpha$  mixed with HA [58], further suggesting the need to better understand how to control the interactions of TNF- $\alpha$  in the post-burn environment.

Interleukin-6 (IL-6), another pro-inflammatory cytokine, is also a target in potential burn wound progression therapies. IL-6 is elevated in burn patients [59], and plays a role in cytotoxic T-cell differentiation and T-cell proliferation [56]. Antibodies to IL-6, however, did not reduce burn progression compared to controls [10, 36, 60], suggesting that TNF- $\alpha$ , a more upstream cytokine, may play a more significant role in mediating inflammation in the burn wound environment. Moreover, the success of a topical therapy suggests that immunomodulatory agents may be able to be utilized without the risk systemic administration.

The complement cascade has also served as a target for anti-inflammatory therapies since complement contributes to wound edema, vascular thrombosis, production of oxygen radicals, and reactive lysis of viable cells in the burn wound [37]. C1 inhibitors have previously been shown to reduce neutrophil adhesion and decrease the progression of wound depth in porcine deep partial-thickness burn models [61]. The only study focusing on complement in burn wound conversion over the last five years is a study by Begieneman *et al.* in 2012 that investigates the effects of long-term (14 day) C1 inhibitor treatment on burn progression [62]. Similar to prior studies, the authors demonstrate improved wound healing

and decreased local inflammation – particularly fewer infiltrating macrophages – in animals treated with the C1 inhibitor. As the critical intervention for burn wound progression must occur within the first 72 hours post burn injury, it is unclear whether the extended treatment seen in this study would be more clinically efficacious compared to treatment reserved to the critical window.

Taken together, these studies reinforce not only the multifactorial nature of burn wound conversion, but also the complex nature of the specific inflammatory interactions that contribute to burn progression. As multiple pathways converge on multiple different effector mechanisms, combined local therapies may potentially offer the most efficacious results while lessening the risks associated with immunomodulation. Furthermore, while most studies focus on reducing signaling cytokines in inflammatory cascades, certain factors, such as interleukin-4 (IL-4), are anti-inflammatory mediators and are beneficial in wound healing [36]. Therefore, therapy should be aimed at controlling the exaggerated inflammatory response in burns, while maintaining an environment of “balanced inflammation” in the burn wound.

### Ischemia

Ischemia is a well-known cause of cell and tissue death in a number of different disease processes and has been implicated in contributing to burn conversion as well [63]. The etiology of ischemia in the zone of stasis is multifactorial, and includes damage to the vascular endothelium from ROS and PMN aggregates as well as thrombosis, vasoconstriction, and edema [8, 64].

Prior studies have demonstrated the contribution of microthrombosis to postburn dermal ischemia [65] and have identified certain factors such as bradykinin, an inflammatory mediator, as potential therapeutic targets to prevent thrombosis [66]. Vasoconstriction [67] as well as local loss of fluid secondary to increased vascular permeability [68] cause impaired circulation and decreased tissue perfusion that can lead to tissue necrosis and progression of injury in the zone of stasis [13]. The systemic consequences of burns, such as sepsis and shock, can result in widespread lack of organ perfusion, including at the wound bed. The current mainstay of treatment for addressing these sequelae is aggressive fluid resuscitation, the extent of which has indeed been shown to influence burn wound progression [69]. Such treatments, however, do not address the cause of ischemia and are only methods of salvage. By better understanding the pathophysiology behind burn wound ischemia and targeting treatments toward causal factors for decreased perfusion, future therapies will help to prevent the unwanted effects of ischemia.

In 2013, Hirth *et al.* demonstrated that burn progression occurs within 24 hours of the initial burn, and that endothelial cell necrosis at one hour post-burn was predictive of the level of apoptosis at 24 hours post-burn as well as histologic tissue necrosis at 7 days post-burn [8, 64]. Traditionally, dermal ischemia has been implicated as a causal factor of burn wound progression [70], which is supported by these findings of initial endothelial cell necrosis prior to interstitial and adnexal cell damage in this study. The authors also suggest dividing Jackson’s zone of stasis into two subzones depending on the viability of endothelial cells at 1 hour: (1) the upper zone with necrotic endothelial cells but spared alternate cell types



which inevitably progresses to full necrosis secondary to ischemia and (2) the lower subzone with spared endothelial cells initially, which only sometimes progresses to necrosis. These findings reinforce the importance of ischemia and programmed cell death in burn wound progression while further elucidating the cellular mechanisms of cell death in a spatiotemporal-dependent manner in this critical area.

Fourman *et al.* attempted to predict necrosis in the zone of stasis by analyzing real-time perfusion using quantitative indocyanine green (ICG) angiography to delineate viable and non-viable areas up to one hour after the initial tissue insult [71–73]. However, this technique relies on how ICG dye angiography functions in the burn wound environment where perfusion patterns complicated by capillary leakage, microthrombosis, vasoconstriction, and vasodilation make it difficult to interpret ICG signals.

In our review, several studies over the last five years examined the role of erythropoietin (EPO), a hormone with known anti-inflammatory, angiogenic, and vasodilatory properties [74–76], in the prevention and treatment of burn wound progression [75]. Tobalem *et al.* demonstrate that treatment with EPO limits interspace necrosis and burn depth extension in a dose-dependent manner in a comb burn model [76]. Interestingly, while both high and low-dose EPO upregulated inducible nitric oxide synthase (iNOS) and decreased inflammation, only low-dose EPO treatment prevented burn progression. Additionally, neoangiogenesis was noted only after necrotic tissue had already demarcated. These results indicate that alterations in microperfusion during the early post-burn period may be more important than inflammatory effects or changes in angiogenesis in contributing to burn wound progression, and that factors other than iNOS may contribute to EPO's beneficial effects on the microcirculation. Tobalem *et al.* also show that the efficacy of EPO treatment is time-dependent, and though iNOS mediated vasodilation and anti-inflammatory effects occur independent of timing, only early (45 minutes post-burn) and not late (6 hours post-burn) treatment decreases burn progression [74].

The mechanisms behind the beneficial effects of EPO are further elucidated in a study by Bohr *et al.* who examined a secondary EPO signaling pathway not responsible for the traditional roles of hematopoiesis, platelet stimulation, and endothelial cell activation [63, 77, 78]. They demonstrate that activation of this alternative pathway using the peptide ARA290 lessens TNF- $\alpha$  secretion by immune cells, decreases microvascular thrombosis, and preserves the deep dermal vascular network in burn wounds, subsequently reducing burn wound conversion. A recent study by Yuhua *et al.* similarly showed reduced burn wound progression when secondary burns were treated with Poloxamer 188 (P188), a copolymer suggested to reduce microvascular stasis and decrease PMN ROS generation [79]. Both of these studies emphasize the effects of poor perfusion, microthrombosis, and pro-inflammatory mediators in burn wound conversion.

Other studies have taken different approaches to local tissue ischemia. Tobalem *et al.* demonstrated that topical application of warm water immediately after burn injury in an effort to induce vasodilation and decrease ischemia decreased burn wound conversion [80, 81]. This lies in contradiction to the mainstay of burn wound treatment which has been cold water exposure as a means to reduce heat as well as inflammation and edema [10]. While

cooling treatments may reduce initial injury, subsequent heat-induced vasodilation may improve perfusion, suggesting the need for possible temporally variable therapies.

### Reactive Oxygen Species

Free radicals have long been considered as mediators of progressive tissue damage after initial burn injury [82]. The occurrence of ROS in the burn wound can be attributed to direct production by the thermal energy of burns [83] as well as the increased activity of xanthine oxidase and NADPH oxidase [84, 85]. ROS such hydrogen peroxide, superoxide radical, and hydroxyl radicals contribute to cell death in the zone of stasis through lipid peroxidation and denaturation of protein [86]. In addition, decreases in antioxidants and free radical scavengers in the burn wound further oxidative stress and subsequent tissue damage [87].

Much recent research is dedicated to investigating the efficacy of different antioxidant agents in minimizing free radical damage and burn wound progression. Deniz *et al.* demonstrated that prevention of burn wound progression could be achieved by treatment with N-acetylcysteine (NAC) one hour after burns. NAC is a precursor to reduced glutathione, which has previously been shown to prevent necrosis in the zone of stasis [41]. Other antioxidant agents investigated included curcumin, which was shown to reduce burn wound progression in a rat comb burn model [88, 89]. Copper-zinc superoxide dismutase, (CuZnSOD), another free radical scavenger, has improved zone of stasis survival and wound healing in topical formulations [90]. Other studies, however, have failed to show any benefit when CuZnSOD was administered after burn injury or as a prophylactic intravenous treatment [17]. These discrepancies may be due to different routes of introduction of CuZnSOD as well different burn models, and suggest further investigation into optimal utilization of this agent is necessary.

These studies demonstrate that the recent free radical species research focus has been dedicated to refining current therapies and exploring alternative interventions rather than trying to better understand the production and interaction of ROS in the zone of stasis. While our understanding of the pathophysiology of these events has not changed significantly over the last several years, novel therapeutic interventions have shown promising results.

### Other Mechanisms

The majority of studies over the last five years have focused on applying well-known treatments for traditional mechanisms of burn wound progression pathogenesis. That being said, several studies in this review investigated alternative methods to prevent secondary necrosis. Two studies examined the adaption of conventional methods of local treatment of burn wounds, particularly wound debridement [18] and burn excision [17], in the context of burn conversion. Singer *et al.* evaluate the ability of a novel bromelin-based enzymatic preparation in salvaging tissue in the zone of stasis after immediately applying the compound to exposed dermis in burn wounds [18]. While animals with enzymatic debridement of eschar had significantly less interspace tissue necrosis in the burn comb model, two-thirds of the experimental group still exhibited partial-thickness necrosis of the zone of stasis and the remaining one-third had full-thickness necrosis. Similarly, a study by Macri *et al.* showed that excision of burn wounds immediately after thermal injury did not

limit burn progression of surrounding zone of stasis [42]. Though removal of eschar is critical for healing of deep burn wounds and studies have suggested that eschar may contribute to phagocyte activation [91], the inability to prevent necrosis in the zone of stasis in these studies indicates that the other pathophysiologic mechanisms described in this study may play a more important role in wound conversion.

Hyperbaric oxygen treatment has been another approach that has previously been used to treat burn wounds [92] and has been shown to improve wound healing in human burn wound models [19]. Turkaslan *et al.* investigated the effects of HBOT in the zone of stasis and reported increased cells in the synthesis stage by 24 hours post-burn, increased radioactive uptake by 5 days post-burn, and decreased necrosis overall in the treatment group [75]. Similar to the study by Tobalem *et al.* [93, 94], new vessel formation was not significantly different between the treatment and control group in the early critical stages of burn wound progression, suggesting a less important role for neoangiogenesis.

Cellular therapies have also emerged as potential treatments for burn wound progression. Mesenchymal stem cells (MSCs) are known for their ability to secrete numerous cytokines and growth factors that are important in wound healing [95, 96] and have been shown to improve wound healing in different environments [97, 98]. MSCs have also have an anti-inflammatory and immunosuppressive effect [16], which may be beneficial in the exaggerated inflammatory environment of the burn wound. With regards to burn wound progression, two studies by Singer *et al.* [15] and Oksuz *et al.* [16] have examined the effects of systemic and local administration of MSCs on the zone of stasis. Singer *et al.* report that systemic administration of MSCs 1 hour after burn injury reduced necrosis of the interspaces in a burn comb model by approximately 20 percent [69]. As necrosis of endothelial cells has been demonstrated to occur by 1 hour post-injury, earlier administration of systemic stem cell therapy [15], allowing time for localization to the wound bed, may improve results. Oksuz *et al.* similarly investigate the potential benefits of MSCs in the burn progression but utilize subcutaneous MSC injections 30 minutes post-burn, which may be a more favorable route of delivery for translational applications. In the treatment group, the authors reported lower apoptosis counts, increased perfusion, and higher percentage of vital tissue in the zone of stasis, reaffirming the multifactorial nature of progression pathophysiology and indicating possible mechanisms for the therapeutic effects of stem cell treatments. Again, earlier administration of therapy may possibly augment results based on the known timing of pathophysiologic events in the zone of stasis.

## CONCLUSIONS

Burn wound conversion continues to remain one of the least understood and poorly treated aspects of burn care. However, recent research has made strides in both better understanding of the contributing factors in the pathogenesis of wound conversion as well as applying novel treatments to well-known mechanisms of secondary progression. The majority of studies over the last five years have focused on adaption of therapies for other diseases to serve as potential interventions targeting the zone of stasis. Numerous experimental treatments aimed at reducing prolonged inflammation, decreasing free oxygen radicals, and improving perfusion have all shown promising results.

While ischemia and cell necrosis secondary to inflammation, ROS, and numerous other factors are important, the roles of apoptosis and autophagy have been each shown to be critical and must be included when considering potential therapies. The need for rapid treatment is also crucial, regardless of the mode of intervention, as cell necrosis has been shown to occur at the one-hour time point after initial insult. The wide variety of mechanisms studied and the varying degrees of success with targeted therapies for each mechanism further lend credence to the multifactorial and complex nature of burn wound progression. Moving forward, future research will have to determine the weight of each of these factors in burn wound progression in order to decide what combination of mechanisms need to be addressed to develop optimal clinical therapies.

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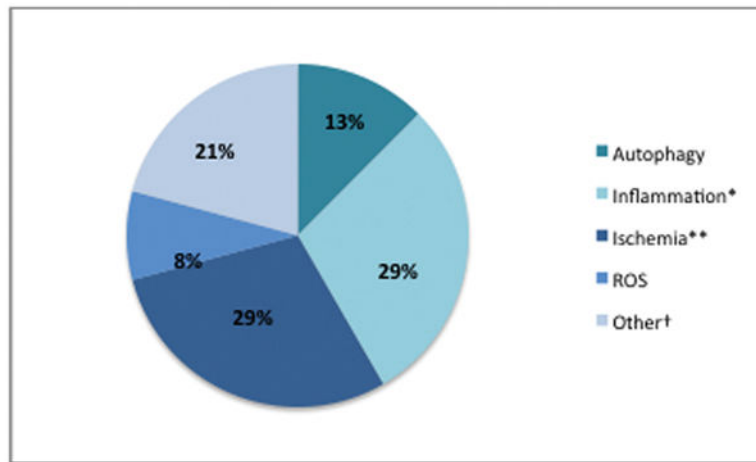
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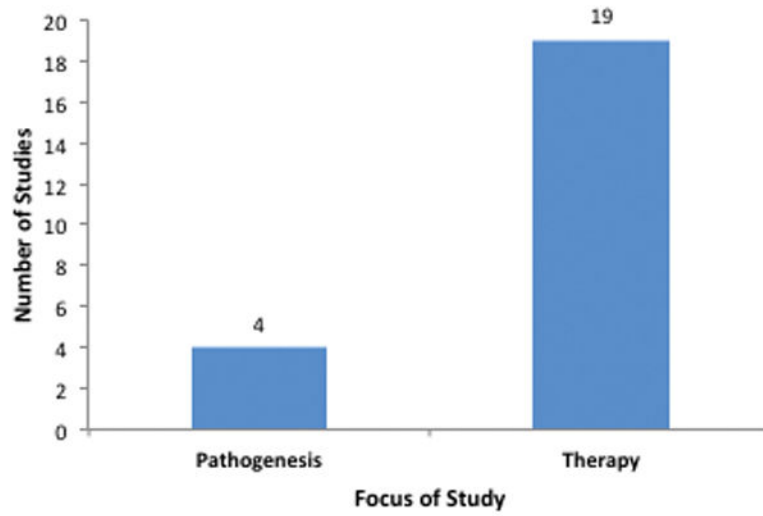
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### Highlights

- we review recent progress in the pathophysiology of burn wound conversion.
- halting burn progression will impact on the scar outcome and healing trajectory
- ischemia, inflammation, oxygen radicals are well accepted - autophagy is a new concept



**Fig. 1.** Categorization of studies on burn wound conversion from 2010 – 2014 according to particular pathogenic mechanism examined. ROS, Reactive oxygen species. \*One article also addressed ischemia, and another, ROS. \*\*Two articles also addressed inflammation. †Other category includes studies on wound debridement/excision, hyperbaric oxygen treatment, and stem cell therapies.



**Fig. 2.** Number of studies addressing pathogenesis (4) versus therapy (19). One study analyzed diagnostic methods for burn wound conversion.

Table 1

## Studies examining burn wound progression from 2010 to 2014

Mechanism	Author(s)	Year	Animal Model	Burn Model	Conclusions
<b>Autophagy</b>	Tan <i>et al.</i>	2013	Murine	Comb model	Autophagy contributes to cell death in zone of stasis; autophagy and apoptosis peak at different time intervals
	Xiao <i>et al.</i>	2013	Murine	Heated brass rod	Induction of autophagy (with rapamycin) decreases burn wound progression and improves wound healing
	Xiao <i>et al.</i>	2014	Murine	Heated brass rod	Autophagy is decreased and apoptosis is increased early in the course of burn progression in deep second-degree burns
<b>Inflammation</b>	Begienman <i>et al.</i>	2012	Murine	Heated copper stamp	Long term treatment with a C1 inhibitor post-burn improves wound healing and decreases local inflammation, particularly macrophage infiltration
	Bohr <i>et al.</i> *	2013	Murine	Comb model, scald burn	Resolvin D2 prevents thrombosis of the DDVN, TNF- $\alpha$ secretion and secondary burn progression; Resolvin D2 does not reduce PMN infiltration
	Eski <i>et al.</i>	2011	Murine	Comb model	Cerium nitrate baths immediately after burn injury prevent progressive tissue necrosis in the zone of stasis
	Friedrich <i>et al.</i>	2013	Murine	Heated brass disk	TNF- $\alpha$ conjugated to HA more effectively prevent burn progression and decrease inflammatory markers compared to TNF- $\alpha$ alone or mixed with HA
	Rizzo <i>et al.</i>	2013	Murine	Comb model	Systemic hypothermia after thermal injury decreases levels of tissue remodeling genes, increase expression of skin protective genes and reduce burn conversion
	Singer <i>et al.</i> **	2011	Murine	Comb model	Curcumin reduces burn wound progression when administered after burn injury
	Sun <i>et al.</i>	2012	Murine	Heated brass disk	Topical antibodies to TNF- $\alpha$ , but not IL-6, reduce burn wound progression
<b>Ischemia</b>	Bohr <i>et al.</i> †	2013	Murine	Scald burn	Alternative EPO-mediated signaling lessens TNF- $\alpha$ secretion, decreases microvascular thrombosis and reduces burn wound conversion
	Fourman <i>et al.</i>	2013	Porcine	Comb burn model	ICG angiography delineates viable versus nonviable tissue in the zone of injury up to 1 hour post-burn
	Hirth <i>et al.</i>	2013	Porcine	Heated aluminum bar	Endothelial cell necrosis at 1 hour post burn is predictive of apoptosis at 24 hours and tissue necrosis at 7 days; the zone of stasis is comprised of two vertically-divided subzones based on endothelial cell necrosis
	Tobalem <i>et al.</i>	2012	Murine	Comb model	Early (45 minute), but not late (6 hour) EPO treatment post-burn decreases burn progression
	Tobalem <i>et al.</i>	2013	Murine	Comb model	EPO decreases secondary burn progression in a dose-dependent manner, independent of iNOS expression, anti-inflammatory or angiogenic effects
	Tobalem <i>et al.</i>	2013	Murine	Comb model	Immediate application of warm water to burn wounds improves early perfusion and decreases zone of stasis necrosis
	Yuhua <i>et al.</i> †	2012	Murine	Heated brass rod	Systemic treatment with Poloxamer 188 reduces burn wound progression in deep second-degree burns
<b>ROS</b>	Deniz <i>et al.</i>	2013	Murine	Comb model	N-acetylcysteine prevents secondary tissue necrosis in the zone of stasis
<b>Other</b>	Shalom <i>et al.</i>	2011	Murine	Comb model	Prophylactic treatment with recombinant copper-zinc SOD does not improve zone of stasis survival
	Macri <i>et al.</i>	2013	Porcine	Comb model	Immediate burn excision does not prevent burn progression in surrounding zone of stasis
	Oksuz <i>et al.</i>	2013	Murine	Comb model	Subcutaneous MSC injections 30 minutes post-burn decrease apoptosis in the zone of stasis and necrosis in the zone of stasis
	Singer <i>et al.</i>	2010	Porcine	Comb model	Bromelain-based enzymatic debridement of burn eschar reduces tissue necrosis in the zone of stasis compared to controls

Mechanism	Author(s)	Year	Animal Model	Burn Model	Conclusions
	Singer <i>et al.</i>	2013	Murine	Comb model	Systemic MSC treatment 1 hour after burn injury reduces necrosis in the zone of stasis by around 20%
	Turkaslan <i>et al.</i>	2010	Murine	Comb model	HBOT increases cells in the proliferative phase at 24 hours post-burn, increases tissue perfusion at 5 days post-burn, and decreases secondary necrosis in the zone of stasis

PMN, Polymorphonuclear neutrophil; TNF- $\alpha$ , Tumor necrosis factor-alpha; HA, Hyaluronic acid; IL-6, Interleukin-6; ICG, Indocyanine green; EPO, Erythropoietin; iNOS, Inducible nitric oxide synthase; HBOT, Hyperbaric oxygen treatment; ROS, Reactive oxygen species; SOD, Superoxide dismutase; MSC, Mesenchymal stem cell.

Study also addressed \*ischemia, \*\*ROS, and <sup>†</sup>inflammation