Enhanced wound-healing performance of a phyto-polysaccharide-enriched dressing – a preclinical small and large animal study

Chih-Hsin Wang¹, Shu-Jen Chang², Yuan-Sheng Tzeng¹, Yu-Jen Shih¹, Chang Adrienne³, Shyi-Gen Chen¹, Tim-Mo Chen¹, Niann-Tzyy Dai¹ & Juin-Hong Cherng⁴,⁵,⁶

¹ Department of Plastic and Reconstructive Surgery, Tri-Service General Hospital, Taipei, Taiwan (R.O.C)
² Department of Dentistry, National Yang-Ming University, National Defense Medical Center, Taipei, Taiwan (R.O.C)
³ Department of Chemistry, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates
⁴ Department and Graduate Institute of Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan (R.O.C)
⁵ General Clinical Research Center, Tri-Service General Hospital, Taipei, Taiwan (R.O.C)
⁶ Department of Gerontological Health Care, National Taipei University of Nursing and Health Sciences, Taipei, Taiwan (R.O.C)

Key words
Burn; Calcium alginate; Carboxymethyl cellulose; Cytokines; Swine

Abstract
Alginate is a natural rich anionic polysaccharide (APS), commonly available as calcium alginate (CAPS). It can maintain a physiologically moist microenvironment, which minimises bacterial infection and facilitates wound healing at a wound site. Patients with burn injuries suffer from pain and an inflammatory response. In this study, we evaluated the CAPS dressing and traditional dressing containing carboxymethyl cellulose (CMC) for wound healing and scar tissue formation in a burn model of rat and swine. In our pilot study of a burn rat model to evaluate inflammatory response and wound healing, we found that the monocyte chemoattractant protein (MCP)-1 and transforming growth factor (TGF)-β were up-regulated in the CAPS treatment group. Next, the burn swine models tested positive for MCP-1 in a Gram-positive bacterial infection, and there was overproduction of TGF-β during the burn wound healing process. Rats were monitored daily for 1 week for cytokine assay and sacrificed on day 28 post-burn injury. The swine were monitored over 6 weeks. We further examined the pain and related factors and inflammatory cytokine expression in a rodent burns model monitored everyday for 7 days post-burn. Our results revealed that the efficacy of the dressing containing CAPS for wound repair post-burn was better than the CMC dressing with respect to natural wound healing and scar formation. The polysaccharide-enriched dressing exerted an antimicrobial effect on burn wounds, regulated the inflammatory response and stimulated anti-inflammatory cytokine release. However, one pain assessment method showed no significant difference in the reduction in levels of adenosine triphosphate in serum of rats after wound dressing in either the CAPS or CMC group. In conclusion, a polysaccharide-enriched dressing outperformed a traditional dressing in reducing wound size, minimising hypertrophic scar formation, regulating cytokines and maximising antimicrobial effects.
Introduction

A good dressing should be easy to handle, prevent infection and inflammation, alleviate pain, have no toxicity, permit easy and early mobilisation, cause no allergic reactions, be inexpensive and facilitate quick and solid healing with an acceptable scar (1,2). Alginate, a rich, natural anionic, phylot polysaccharide (APS) commonly derived from seaweed, comprises mainly differing ratios of d-Mannuronic and l-Guluronic acid, which are covalently bound through 1,4-glycosidic linkages. Alginate is a biocompatible, hydrophilic and biodegradable material, which benefits wound healing because it provides a moist microenvironment (3,4). Calcium alginate polysaccharide (CAPS) has been found suitable for use in pharmaceutical drugs, as a bioactive food ingredient and for cell encapsulation or tissue regeneration (5). Carboxymethyl cellulose (CMC) is a derivative of cellulose that can be obtained from natural cellulose by chemical modification. It has been widely used in wound dressing for many years (6,7).

In the clinic, most patients with burn injuries suffer pain during burn wound debridement, which they describe as severe to excruciating despite the use of powerful opioid analgesics (8). During the last decade, important advances have been made in lowering the mortality rate in burns; however, treatment methods are still not satisfactory. Evidence has shown that the underlying mechanism of pain is associated with adenosine triphosphate (ATP) because ATP is released from damaged cells, and its extracellular concentration during tissue injury is sufficient to activate purinergic P2X3 and P2X2/3 receptors, expressed by nociceptors. Laycock et al. (9) indicated that ATP is a principal algogenic component of the early burn injury tissue fluid. In addition, ATP is involved in maintaining pain in the later stages of burn injuries because P2X3 receptor expression is up-regulated following burn injury (10). Monocyte chemoattractant protein (MCP)-1 is also part of the ATP-related pain pathway (11).

In addition, the pathogenesis of pain is associated with the modulation of transforming growth factor (TGF)-β, which is an important inflammatory cytokine and anti-inflammatory factor (12–14). Moreover, TGF-β can enhance cell proliferation, wound healing and scar formation, which are important in the process of burn injury recovery (15–17). An inflammatory response occurs in patients with burn injuries. The systemic inflammatory response includes the release of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, interferon (INF)-γ and tumour necrosis factor (TNF)-α after thermal injury (18,19). Abnormal levels of anti-inflammatory cytokines, such as IL-2, IL-4 or IL-10, are then released to mitigate the effects of the pro-inflammatory cytokines through a feedback mechanism. Such abnormal levels of anti-inflammatory cytokines have been reported both systemically and locally in patients with burns. In rodents, escharotomy was found to inhibit the overexpression of both early and later inflammatory mediators and maintain the balance of pro-/anti-inflammatory responses, thus improving multiple organ functions following severe burns (20–23).

Furthermore, a dominant activity of type 2T-cells is generally noticed in animals and patients with severe burn injuries (20,24). Moreover, alterations in the cytokine/chemokine profile, especially MCP-1 levels, modulated innate responses to favour microbial clearance, thus controlling infection (25). Hence, treatment with CAPS resulted in positive effects in clinical studies of patients with burns or multiple traumas. However, the underlying mechanism remains unclear.

One of the leading limitations of research into practical treatments for burn patients is the lack of an appropriate animal model that captures all the prominent factors associated with burn trauma. However, animal models continue to be required to uncover the molecular and cellular (26) aspects that characterise human burn trauma. Burns have a heterogeneous nature; therefore, a variety of animal burn models have been developed as valuable tools to review the pathophysiology of burns. Rats, as a research model, provide researchers with key insights into the molecular signalling pathways active in the recovery process, mainly because of the number of rat-specific reagents and the practicality of performing transgenics in rats. In addition, as a result of their substantially reduced healing time and superior defence mechanisms (27), the morbidity of rats in scientific studies are very reasonable. Even though a rat model has its own specific advantages, its major drawback is its failure to totally mimic the human wound recovery process. Rat wound healing occurs mainly through wound contraction (28), which occurs quite rapidly. In comparison, reepithelialisation is the main healing approach observed in humans (29). Another potential hindrance in utilising rats to review wound healing is that unlike humans, rats are not susceptible to hypertrophic or keloid scar formation (28). Wound repair in pigs has recently become a research focus because the pig’s skin architecture is similar to human skin; the recovery process of swine and human occurs through physiologically similar phases (inflammation, proliferation, reepithelialisation and remodelling). In most cases, however, burns in pigs heal by 21 days, with reepithelialisation occurring between 7 and 14 days post-wound infliction (30,31), which are similar to the timelines observed in humans. Pigs also show greater morbidity in comparison with rats because their body size makes them more prone to wound infection, putting them in danger of sepsis. Considering that no one animal is the best model for all the biological aspects of burn wound healing, a good approach is to integrate the data produced from multiple model systems. Wound healing in each type of animal has its own pros and cons, and the integration of molecular and cellular data from more than one animal type will
benefit research into burn wound healing. A rat model, using its well-characterised immune system mechanisms (32), revealed the suppression of cell-mediated immune responses after burn injury and the increased risk of subsequent septic complications and mortality (33). By contrast, insufficient scar formation in rat wound-healing models (28) has prompted researchers to use the swine model to determine the mechanisms underlying hypertrophic and keloid scar formation in patients with burns. Thus, the rat and swine have each contributed significantly to revealing the biological process and the diseases affecting the human skin. In this study, we found that, in rats, wound contraction was rapid, resulting in reduced healing time, and rats displayed a superior immune response, unlike reepithelialisation, which involves the development of new skin tissue. We created a swine model because of its primary benefit of producing burn wounds compared with the rat model, facilitating research into the mechanisms underlying scar formation and bacterial infection in patients with burns.

In the present study, we first confirmed the release of inflammatory cytokines and the wound-healing rate in the rat model treated with dressings enriched with CAPS or CMC for a third-degree burn injury for 28 days. Subsequently, we examined the pain-related factors and inflammatory cytokines in third-degree burn injury rats treated with CAPS or CMC dressings. Next, we observed the wound repair and scar formation in a third-degree burn injury swine model treated with CAPS or CMC dressings and confirmed the anti-bacterial activity of CAPS and CMC treatment. Data from the present study provide new insights into how a CAPS-containing dressing affects pain and inflammatory cytokines in burn injuries.

Material and methods

Animal selection and pre-procedure monitoring

This study was conducted in the Animal Center Laboratory at the National Defense Medical Center. Three domestic pigs were used for this study. Animals were fed a standard diet ad libitum several days before the investigation and were fasted overnight before any procedure. The animals were housed in individual pens upon their arrival and allowed to acclimatise for at least 7 days. The pigs were pre-treated with a transdermal fentanyl patch (50 μg/hour) for pain management. On the day of burn creation, animals were sedated intravenously with Zoletil 50 (Virbac, Carros cedex, France; 25 mg/kg). The swine were then intubated endotracheally and maintained under a surgical plane of anaesthesia with isoflurane 0.5–2.5%. Their blood pressure, heart rate and body temperature were monitored during the surgery to check for complications. The flanks and back hair was shaved with hair clippers, and the skin was scrubbed with a povidone iodine solution.

Burn procedure of rats and swine

Burns were produced in 10 Sprague–Dawley rats by means of a brass block measuring 2.5 cm in diameter. The blocks were heated in boiling oil until the temperature reached 190°C. The brass block was applied onto the rats’ backs parallel to the midline for 20 seconds resulting in third-degree burns (30). Using the brass block device, two burn wounds were created on each animal under anaesthesia by an intraperitoneal injection of Zoletil 50 (Verbac; 5 mg/100 g). Two replicate burns were created for each contact time, and treatment and control wounds were randomised as to their location, with equal distribution of wounds on either side of the dorsal midline and with respect to rostral or caudal positioning. Sixty minutes after burning, the wounds were covered with saline-soaked non-adhesive gauze. The burns were covered with gauze, CAPS (CoreLeader Biotech Co., Ltd, Taipei, Taiwan) or CMC (Aquacel) dressing for 28 days after the burn. All wounds were protected with a non-stick cotton and acrylic fibre pad, fixed with adhesive dressing and covered with a special garment to prevent tearing of the dressings.

Moreover, three swine weighing 20–25 kg were anaesthetised by an intramuscular injection of ketamine (5 mg/kg), sternal (cazaporonum, 20 mg/kg) and atropine (5 mg/kg). Six uniform burn wounds (190°C, 30 seconds) were then made symmetrically on the back of each swine using a modified soldering iron with a flat contact area of approximately 20 cm² (31). The burn injury was equivalent to a full-thickness burn in humans and caused uniform coagulation and necrosis of the dermis. Dressings were changed every 2 days for the first 10 days and then twice a week for 6 weeks. All wounds were cleaned and measured before each dressing reaplication.

Analysis of wound healing

Wound healing was assessed by evaluating the rates of wound reepithelialisation and contraction. The open wound area and the surrounding area of normal skin was measured using macrophotography. The healing rate was monitored every 2 days for the first 10 days and then twice a week for 6 weeks. Wound reepithelialisation or contraction was calculated as the percentage of the original wound size according to a previously described method. The analysis of wound closure was conducted in a blinded manner.

Vancouver scar scale

Based on the human burn scar scales (34), a porcine burn scar scale was established. The Vancouver scar scale (VSS) consists of four variables: vascularity, height (thickness), pliability and pigmentation. Each variable has four to six possible scores. The total score ranges from 0 to 14, whereby a score of 0 reflects normal skin.

Haematoxylin and eosin staining

Following euthanasia, burn wound tissue was excised en bloc to include the underlying musculature and surrounding unwounded tissue. Tissues were fixed in 10% neutral buffered formalin, and paraffin-embedded sections were cut, stained with haematoxylin and eosin (H&E; Sigma Chemical Co., USA) and visualised under 100× power using a light microscope.

Bacterial growth experiments

Forty-eight isolated burn wound areas were included in the study. The samples were collected on post-burn days 0 and 3.
Polysaccharide-enriched dressing for better burn treatment

C.-H. Wang et al.

and weeks 1, 2, 3, 4, 5 and 6 placed in sterile tubes. One hundred microlitres of each dilution ($10^{-1} - 10^{-5}$) was plated on selective and non-selective media, and the plates were incubated under aerobic conditions for 24–72 hours. The media employed contained 5% sheep blood agar to isolate aerobic Gram-positive organisms. To determine the number of colony-forming units (CFU), a sample was prepared and spread or poured uniformly on the surface of an agar plate and then incubated at 37°C overnight. An inverted sheep blood agar dish, with the bottom scored into four equal quadrants using a sharpie pen and small ruler, was used. We then placed the dish onto the stage of a dissection microscope and counted the colonies on each plate. By definition, a colony must have a minimum of 300 CFU to be enumerated.

Serum cytokine assay

The serum samples were collected, and the production of cytokine/chemokines was quantified using a MILLIPLEX MAP Rat Cytokine/Chemokine Kit (RACYTMAG-65K; Millipore Corp., Billerica, MA). Serum specimens for testing were collected from the ventral tail artery of the rats after burn injury on days 0, 1, 3 and 7. All samples were acquired on an xMAP instrument (Millipore, Merck KGaA, Darmstadt, Germany). Analytes tested included IL-4, IL-6, MCP-1, IFN-γ, TNF-α, TGF-β1, TGF-β2 and TGF-β3.

ATP assay

ATP in a 20-μl specimen of ventral tail artery blood serum collected from rats with burns was determined using an ATP Assay Kit (Cat. No. 119107; Calbiocem, Taipei, Taiwan) and a Biotek Synergy H4 luminometer. ATP (μg/μl) in each specimen was calculated against an ATP standard curve.

Statistical analysis

Statistical analysis between the two treatment groups was performed using the two-tailed paired t-test and Student t-test for continuous data and the chi-square test for non-continuous data. Differences between groups were declared statistically significant when the P value was less than 0.05.

Ethical considerations

Rats were purchased from the Animal Laboratory Center for National Defense Medical Center, Taipei, Taiwan, and the in vivo animal experiments were performed with the approval of the National Defense Medical Center of Animal Care and Use Committee.

Results

In the present study, we created 2 burns on each of six rats, giving a total of 12 burns, with three replicates dressed with CAPS and CMC. A panel of eight cyto-/chemokines was analysed in the serum of rats with burns of anti-inflammation or pro-inflammation. The cyto-/chemokines included IL-4, IL-6, MCP-1, IFN-γ, TNF-α, TGF-β1, TGF-β2 and TGF-β3.

The levels of cyto-/chemokines of serum in rats with dressings were measured at 0, 1 and 7 days (Figure 1). There was no significant difference in the levels of IL-4, IL-6, MCP-1, IFN-γ, TNF-α, TGF-β1, TGF-β2 and TGF-β3.

The levels of cyto-/chemokines in serum of rats with dressings were measured at 0, 1 and 7 days (Figure 1). There was no significant difference in the levels of IL-4, IL-6, MCP-1, IFN-γ, TNF-α, TGF-β1, TGF-β2 and TGF-β3.

The CAPS group had a significantly higher level of TGF-β3 compared with those in the CMC group on day 1 post-burn injury ($t = 3.354, P = 0.01$).

Levels of serum ATP were used to investigate pain activation. On burn day 1, the CMC group ATP concentration
The results showed wound area closure between each group. There was no statistically significant difference in the healing rate of the CMC and the CAPS treatment groups in the rat model. Although the wounds contracted rapidly, the levels of serum MCP-1 and TGF-β increased, which suggested that the wounds suffered from continuous internal infection. According to the results of the pilot study in rats, we designed the burned swine model to observe large-scale burn wounds and long-term wound repair.

We created six burns on each of the three pigs for a total of 18 burns, with three replicates dressed with CAPS and CMC. The wound size was measured periodically during the healing process and photographed. The efficacy of CAPS and CMC are shown in Figure 4. Wounds dressed with CAPS exhibited rapid reepithelialisation and less scar formation (e.g. at week 2; Figure 4A). Reepithelialisation, which progressed from the wound margins inwards, appeared smooth in wounds dressed with CAPS (Figure 4A). These animals were also used to assess the secondary outcomes of the depth of scar formation at post-burn days 0, 3 and weeks 1, 2, 3, 4, 5 and 6, as well as reepithelialisation, as determined by gross inspection. The healing rate was defined as the greatest average wound margin distance from the wound centre divided by the time to complete wound closure. The CMC and CAPS groups showed that wound closure was accelerated by nearly 50%; with 65.57 ± 6.49% and 73.43 ± 6.33% wound closure on post-burn week 6, respectively. At this time point, the cutaneous burns dressed with CMC and CAPS exhibited 3.22 ± 0.55 and 2.59 ± 0.63 cm² wound areas, respectively (Figure 5). Wounds dressed with CAPS showed the greatest decrease in wound area by post-burn week 6 (P = 0.001). At 6 weeks after injury, all burns were reepithelialised based on gross inspection.
The VSS scores with respect to scar vascularity, pliability, pigmentation and height on post-burn days 0 and 3 and weeks 1, 2, 3, 4, 5 and 6 are shown in Figure 6. There was less scar formation in the wounds dressed with CAPS. In post-burn week 3, wounds dressed with CAPS and CMC were scored as 7.4 ± 0.5 and 7.8 ± 0.5, respectively. This reduction was maintained until 6 weeks in each study group, at which time the score in the CAPS group was less than that of the CMC group (3.3 ± 0.58 versus 4.67 ± 0.58, respectively). However, there was no significant difference between the CAPS and CMC groups.

Photomicrographs of representative wounds at post-burn week 6 are shown in Figure 4. H&E staining of swine skin collected 6 weeks after burn injury confirmed the full-thickness burns. The burns resulted in necrosis of the epidermis, dermis and dermal components without apparently affecting the underlying muscle (Figure 7A, B). The morphology of the full-thickness wounds was smooth and continuous, and the papillary layer resembled the features of hypertrophic scarring, whereas wounds in the CAPS group (A) appeared completely healed. In contrast, the CMC group (B) showed...
Burns are a serious injury that can be further complicated by infection, leading to increased mortality and morbidity. There is considerable variation in the treatment of burns, such as skin grafts and donor site wounds, between different medical institutions. Despite some controversy, a consensus was reached that the optimal dressing material should promote healing, cause minimal pain to the patient, prevent infection, produce minimal scarring and be inexpensive and easy to use (1,2). In this study, we evaluated the efficacy of a wound dressing with a rich source of phytol polysaccharides (i.e. CAPS) and CMC on a third-degree burn injury model in swine and rats, particularly with respect to pain, inflammatory cytokines and scar formation.

The management of burn scarring is one of the major complications encountered during the wound-healing process, which occurs through the biological processes of haemostasis, inflammation, proliferation and maturation. The healing process can go awry, resulting in fibroblastic proliferation that produces a hypertrophic scar, which by definition is confined to the wound site (35). Reepithelialisation and reducing scar formation are the most important stages of burn repair, and their success requires a supportive microenvironment, which may be provided by a suitable bio-matrix. Our data showed that CAPS has better healing efficacy in terms of faster wound closure and reduction of wound area than CMC treatment.

Moreover, in the present study, the VSS was used in the swine model, which is a validated subjective scale score (34). It is of interest to document what percentage of the originally treated wound surface has become hypertrophic, particularly with respect to pain, inflammatory cytokines and scar formation.

The CAPS wound dressings keep the wounds moist and promote the growth of granulation tissue, and the resultant epidermis decreases pain and reduces scar formation. Because CAPS consists of calcium and the polysaccharide alginate, alginate promotes wound healing by providing a moist microenvironment, which promotes granulation of intermittent and ragged skin, and the thickness of the features of hypertrophic scarring presented more deposition than in the CAPS group. Note the presence of neo-epidermis covering the wound surface in the CMC group. The thickness of the dermis dressed with CAPS was less than that in the CMC group (5-4 mm versus 7-2 mm, respectively). In addition, the burn resulted in sloughing of the dermis and significant lymphocytic infiltrate, with the red arrow indicating the dermal connective tissue-infiltrated inflammatory cells in the burn wound eschar above from the viable dermis below (Figure 7C, D).

Bacterial culture and counting showed that CAPS significantly reduced the amount of bacteria growth at post-burn week 3 (Figure 8 and Table 1). The wound was swabbed on post-burn days 0 and 3 and weeks 1, 2, 3, 4, 5 and 6. The anti-bacterial activity was evaluated using a CFU assay, and the resulting average value was divided by 1000. We then compared the value of the CAPS group with that of the CMC group for the dilution ratio. The results of 1000 continuous dilutions showed that CAPS demonstrated markedly better inhibition of bacterial growth than CMC. At week 2 after burn injury, the ratio of CFU for the CAPS group was 0.91 times less than that of the CMC group. This reduction was maintained until 6 weeks in each group, at which time the ratio of the CFU assay in the CAPS group was 0.53 times less than that of the CMC group. The CFU assay was performed in triplicate, and data were presented as ratios compared with the CMC group.

**Discussion**

Burns are a serious injury that can be further complicated by infection, leading to increased mortality and morbidity. There is considerable variation in the treatment of burns, such as skin transplants and donor site wounds, between different medical institutions. Despite some controversy, a consensus was reached that the optimal dressing material should promote healing, cause minimal pain to the patient, prevent infection, produce minimal scarring and be inexpensive and easy to use (1,2). In this study, we evaluated the efficacy of a wound dressing with a rich source of phytol polysaccharides (i.e. CAPS) and CMC on a third-degree burn injury model in swine and rats, particularly with respect to pain, inflammatory cytokines and scar formation.

The management of burn scarring is one of the major complications encountered during the wound-healing process, which occurs through the biological processes of haemostasis, inflammation, proliferation and maturation. The healing process can go awry, resulting in fibroblastic proliferation that produces a hypertrophic scar, which by definition is confined to the wound site (35). Reepithelialisation and reducing scar formation are the most important stages of burn repair, and their success requires a supportive microenvironment, which may be provided by a suitable bio-matrix. Our data showed that CAPS has better healing efficacy in terms of faster wound closure and reduction of wound area than CMC treatment.

Moreover, in the present study, the VSS was used in the swine model, which is a validated subjective scale score (34). It is of interest to document what percentage of the originally treated wound surface has become hypertrophic, particularly in clinical trials (36). CAPS treatment had consistently lower scores than CMC treatment up to post-burn week 6. A lower score indicates proximally normal skin colour pigmentation, vascularity, pliability and height. CAPS showed consistently lower scores compared with CMC (P = 0.003).
Polysaccharide-enriched dressing for better burn treatment
C.-H. Wang et al.

Figure 7 Histological analysis of wounds dressed with calcium covalently linked with calcium alginate polysaccharide (CAPS) or carboxymethyl cellulose (CMC) of the basal layer of epidermis by haematoxylin and eosin staining at post-burn week 6 in the swine model. (A–D) The black dotted boxes show photomicrographs of the repaired burn tissue. The morphology of the full-thickness wounds was smooth and continuous, and the papillary layer resembled features of hypertrophic scarring, whereas wounds in the CAPS group (A) appeared to be completely healed. In contrast, the CMC group (B) appeared to be intermittent and ragged, and the thickness of the features of hypertrophic scarring displayed more deposition than those in the CAPS group. Note the presence of neo-epidermis covering the CAPS group wound surface. The red arrow indicates the dermal connective tissue-infiltrated inflammatory cells in the burn wound eschar above the viable dermis below (C, D). Original magnification, ×10.

Figure 8 Anti-bacterial tests of calcium alginate polysaccharide (CAPS) and carboxymethyl cellulose (CMC) of the extravasate from the burn wounds in vivo at post-burn days 0 and 3 and weeks 1, 2, 3, 4, 5 and 6. One hundred microlitres of each dilution (10^{-1}–10^{-5}) was plated on sheep blood agar dish, and the plates were incubated under aerobic conditions for 24–72 hours.

In the present study, CAPS treatment demonstrated a markedly better inhibition of bacterial growth than CMC treatment, suggesting that CAPS reduced the bacteria growth through the release of Ca^{2+}, which has been recommended as an antimicrobial agent (36,40–44), resulting in superior bactericidal and bacteriolytic effects compared with other antimicrobial agents (41,43,44). Thus, wound dressings that are enriched with polysaccharides, including alginate, are efficacious because they maintain a physiologically moist microenvironment, minimise bacterial infection at the wound site, facilitate wound healing and are effective in reducing wound size.

In a burn injury, pro-/anti-inflammatory cytokines act as important modulators of immune cell proliferation, differentiation and clonal growth of lymphocyte sub-populations, and also attract immune cells to the site of injuries (22). In the present study, we found that IL-4, IL-6, TNF-α, IFN-γ, MCP-1 and TGF-β were present in the serum of burned rats and that IL-4, IL-6, TNF-α and MCP-1 are involved in the early stages of the
rat’s response to burning. Our cytokine data are consistent with previous studies (18,20,21). These cytokines attract immune cells to the site of injuries to initiate an immune response after burning. IL-4-secreting T cells were produced when normal T cells were cultured with rMCP-1, without any cells derived from mice, early after thermal injury, which showed that MCP-1 initiates the generation of type 2T-cells and is launched from macrophages, fibroblasts and neutrophils, which appear early after a thermal injury (20). The control of bacterial infection is a key issue in the management of patients with burns. In addition, MCP-1 is essential for optimal microbial elimination through a mechanism that requires the discharge of nitric oxide supplement, which is an antimicrobial agent (41). The role of MCP-1 in Gram-positive bacterial infections has been demonstrated in the control of Listeria monocytogenes infections (42). This implied that the presence of MCP-1 is an indicator of bacterial infection. The level of MCP-1 in the CMC group was significantly higher than that in the CAPS group. Although the wound had already contracted, we hypothesised that bacteria were present inside the wound, resulting in a sustained inflammatory response that delayed reepithelialisation. In support of this hypothesis, in the burned swine model, the bacterial growth assay showed that the number of CFUs in the CMC group was obviously higher than that in the CAPS group.

IL-6 production is an alarm signal and triggers wound healing in patients with burns. The amount of IL-6 after injury is an indicator of the severity of the burn (24). In addition, elevation of TNF-α was reported at time points less than 72 hours after burning (19). The level of IFN-γ was elevated in untreated thermally injured rats (11), implying that the CAPS might regulate IFN-γ release to reduce the inflammatory response.

TGF-β is involved in many processes in wound healing, including inflammation, stimulation of angiogenesis, fibroblast proliferation, collagen synthesis and depositing and remodelling from the new extracellular matrix, demonstrating its important role in the burn injury recovery process (15–17). TGF-β functions as a wound healing-promoting factor; therefore, if produced in excessive amounts, it may produce over-healing outcomes, such as keloid and hypertrophic scarring. Activation of latent TGF-β occurs at two time points during wound healing: immediately after wounding and during reepithelialisation. The activated TGF-β then functions like a potent chemoattractant for inflammatory cells, which attack the wound microenvironment, resulting in further activation of latent TGF-β during the reepithelialisation phase (45–47). However, the rat burn wound was too small and the wounds contracted rapidly, and the statistical healing rate (Figure 3) showed no difference in efficacy between the CAPS and CMC groups. Therefore, we used the burned swine model to observe large-scale burn wounds and long-term wound repair to compare CAPS and CMC treatments. In the swine study, we observed that although the CMC treatment group was initially observed to have superior wound contraction and less scar characteristics, the CAPS treatment group showed significantly better wound-healing ability (Figures 4, 5) and lower VSS (Figure 6) scores at 6 weeks post-burn injury. In addition, H&E staining (Figure 7) showed that the lesion site of the epithelium and stratum corneum layer was continuous in the CAPS treatment group but was disturbed in the CMC treatment group.

Our results showed the CAPS group had higher levels of TGF-β1, TGF-β2 and TGF-β3, which demonstrated that CAPS might help to reduce scarring after serve burn injury in the acute stage; moreover, it is consistent with the VSS results. TGF-β1 is responsible for the fibrotic scarring response, whereas the scar-less wound healing observed in foetal wounds is the result of elevated amounts of TGF-β2 and TGF-β3 (12). Not only do hypertrophic scar-derived fibroblasts produce TGF-β1, but they also display prolonged expression of TGF-β receptors in comparison with normal skin. TGF-β receptors have overlapping functions and predominantly mediate their effects through the intracellular SMAD pathway (12,17).

TGF-β is a modulator of the pathogenesis of pain in chronic inflammation. TGF-β participation in the mechanism of pain signals includes peripheral and central processing (13). Increased TGF-β1 can particularly result in peripheral sensitisation and contributes to the enhanced nociception that accompanies chronic inflammation (13). It is effective in the treatment of neuropathy by targeting both neurons and glial cells, contributing to the attenuation of neuropathic pain (12). Our serum data indicated that TGF-β1 was elevated in the CAPS treatment group, suggesting that it might contribute to reduced pain perception.

We attempted to measure the ATP level as an indicator of burn pain before and after dressing. Nociceptors, which express the P2X3 receptor, can be subdivided into two subsets based on their reliance upon neurotrophins and the existence of certain immunohistochemical markers (9,10). The nocifensive response elicited by ATP in burned rats was associated with potentiation in the hyperalgesic skin and dependency on capsaicin-sensitive nociceptors (10). It is believed that burn pain caused by the activation of nociceptors by ATP analogs is mediated, at least in part, by a receptor encompassing the P2X3 protein in rats (9). Burn injury-released endogenous ATP might contribute to ongoing pain (9,10). According to our data, both the CAPS and CMC groups showed reduced ATP concentration on dressing days 1 and 7; however, the differences between the CAPS and CMC groups were small and did not reach statistical significance. Moreover, the MCP-1-related pain signalling pathway acts through the c-Jun N terminal kinase/MCP-1 pathway, which is also regulated by ATP-sensitive potassium

The anti-bacterial activity of calcium covalently linked with anionic polysaccharides (CAPS) and carboxymethyl cellulose (CMC). The anti-bacterial activity was evaluated by a colony-forming units (CFU) assay, and the resulting average value was divided by 1000 for the taken ratio. The CFU assay was performed in triplicate, and data are represented as a ratio compared with the CMC group.

<table>
<thead>
<tr>
<th>Day</th>
<th>CAPS</th>
<th>CMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3 days</td>
<td>1.00</td>
<td>0.08</td>
</tr>
<tr>
<td>1 week</td>
<td>1.00</td>
<td>0.14</td>
</tr>
<tr>
<td>2 weeks</td>
<td>1.00</td>
<td>0.91</td>
</tr>
<tr>
<td>3 weeks</td>
<td>1.00</td>
<td>0.73</td>
</tr>
<tr>
<td>6 weeks</td>
<td>1.00</td>
<td>0.53</td>
</tr>
</tbody>
</table>

The CFU assay was performed in triplicate, and data are represented as a ratio compared with the CMC group.
channels (11). To clarify the mechanism of pain assessment in different dressing treatments, the P2X3 receptor and its downstream signalling should be investigated in a future study.

Animal burn experiments increase our knowledge of the physiological and pathophysiological mechanisms associated with this particularly devastating trauma. One of the leading barriers to extrapolating this information to humans is the fact that, because of ethical and financial restrictions, researchers rarely utilise large animal models that are clinically relevant. In the present study, we chose rats and pigs, which could be viewed as complementary, to help uncover the pathology behind burn trauma. This study presented preliminary results of a pilot experiment that will be further analysed, expanded and replicated in a larger animal study.

Conclusions

Our study demonstrated that the use of a wound dressing with a rich source of polysaccharides (i.e. CAPS) has advantages over a traditional CMC-containing dressing for third-degree burn injuries in an animal model by promoting natural wound healing, less scarring formation, minimum bacterial infection, appropriate inflammatory response and pain regulation. Our results suggest that phytol polysaccharide-enriched wound dressings may be more advantageous than traditional wound dressings.

Acknowledgements

This study was supported by a grant from the Tri-Service General Hospital, National Defense Medical Center, Taiwan (TSGH-C101-174); Cardinal Tien Hospital, Taiwan (CTH-103-1-2C13); and the Ministry of Science and Technology, Taiwan (MOST 103-2314-B-016-038; MOST105-2314-B-016-054).

References


学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具