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AWC ADVANCED WOUND CARE

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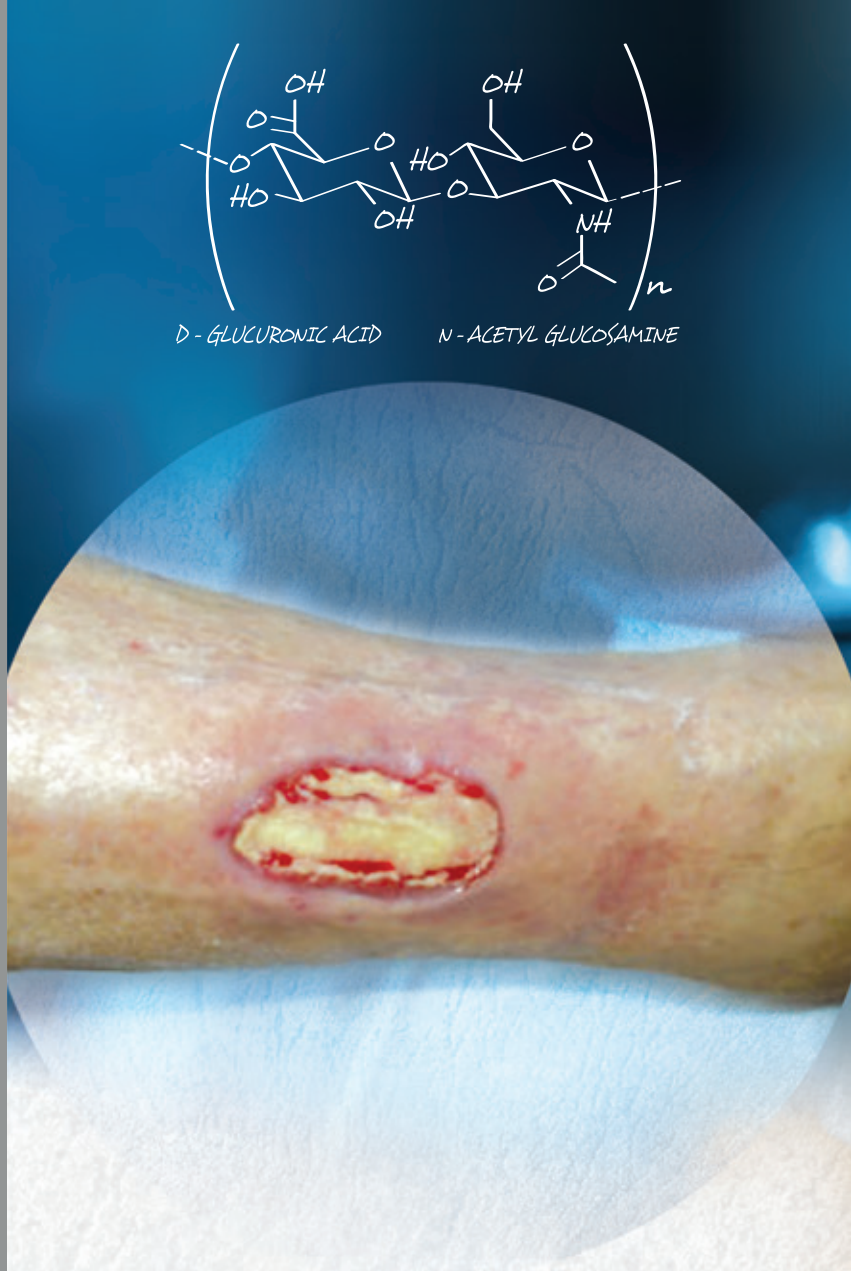

REMOVAL OF NECROTIC AND NON-VIABLE TISSUE WHILE SUPPORTING WOUND HEALING

The need for WOUND BED PREPARATION

Routine care of non-healing acute and chronic wounds often comprises removal of adherent, dead or contaminated tissue and must be clearly distinguished from cleansing, which is defined as the removal of dirt[1]. Consequently, wound bed preparation is a basic necessity to induce the functional process of tissue repair, and is thus a central intervention in the management of acute and chronic, non-healing wounds.

Wound bed preparation in non-healing wound involves the act of removing necrotic material, eschar, devitalised tissue, serocrusts, infected tissue, hyperkeratosis, slough, pus, haematomas, foreign bodies, debris, bone fragments or any other type of bioburden from a wound, with the objective to promote wound healing[1].

Methods used to prepare the wound bed of non-healing wounds.

There are a wide variety of methods to prepare the wound bed. Each is described briefly below[1].

MECHANICAL METHODS

This type of preparation involves the use of dry gauze dressings, wet-to-dry gauze dressings, impregnated gauze/tulle dressings or a monofilament fibre pad, to remove non-viable tissue from the wound bed.

← Wet-to-dry

In this method, a moist gauze pad is applied to the wound. As the devitalised tissue dries, it re-hardens and becomes attached to the gauze; when the dressing is removed, the adhered material is pulled free. The disadvantages of this method are described as injury to normal tissue and pain, along with the necessity for frequent dressing changes.

← Gauze

Gauze has been used as the basic dressing in wound management and is frequently used as a comparator in wound studies. The primary limitation for the use of gauzes is that it is associated with significantly more pain for patients than with use of other dressings.

AUTOLYTIC DRESSINGS

The term 'autolytic' describes a natural process in all kinds of wounds, which can be supported by a moist wound management strategy. Autolytic debridement products can be found in many different varieties. They can be defined in the following groups:

- **Hydrogels**, or hydrogel-based dressings, which are three-dimensional, cross-linked homopolymers saturated with water.
- **Hydrocolloids** are composed of carbomethyl cellulose, gelatin, pectin, elastomers and adhesives that turn in to a gel when exudate is absorbed.
- **Highly absorptive dressings** with autolytic and occlusive properties.
- **Multi-component dressings**; some combine autolytic, absorptive and antimicrobial features.

Autolytic dressings are indicated for different kinds of acute and chronic wounds with necrotic tissue or fibrin coatings, to rehydrate, soften and liquefy hard eschar and slough. Products for autolytic debridement are fairly well documented as easy to use and causing little-to-no pain. They do not damage healthy tissues (selective debridement) and are claimed to promote the formation of granulation tissue and epithelialisation. Most of these products are likely to require few dressing changes.

ENZYMATIC DRESSINGS

Enzymatic dressings are specific methods that uses proteolytic enzymes contained within in gels or ointments, which should work synergistically with endogenous enzymes. Enzymatic debridement can be useful in patients with wounds where mechanical debridement options are not available or are contraindicated; for example, in patients with bleeding problems.

During debridement, proteolytic enzymes are used to hydrolyse peptide bonds, in order to facilitate the removal of non-viable tissue from a wound.

Matrix metalloproteases (MMPs) are zinc-dependent endopeptidases, with a subgroup of metalloenzymes called **collagenases**. Humans generate endogenous collagenases to facilitate the physiological balance between the assembly and degradation of collagen. Collagenases are the only endoproteases that can degrade human triple helical collagen, but do not attack keratin, fat, fibrin or haemoglobin. The most commonly used wound products contain the collagenase Clostridiopeptidase A from *Clostridium histolyticum*.

The main advantages of the use of proteolytic enzymes in the debridement of patients with chronic wounds are their easy and safe handling. Therapies are bloodless and generally considered rather painless.

LARVAE

Larval therapy is a form of mechanical preparation wherein live maggots, raised in sterile conditions, usually *Lucilia sericata* (common green bottle fly), are placed on necrotic/sloughy wounds. Maggot secretions contain antibacterial substances that reduce bacterial load by exerting a bacteriostatic effect, and proteolytic enzymes cause eschar degradation by disrupting the tissue collagen matrix. These actions promote wound healing and amplify human fibroblast and chondrocyte growth. Thus, larval therapy works through mechanical preparation, antimicrobial effects and stimulation of healing.

Larval therapy is a cost-effective debriding treatment, which can reduce pain, bacteria and malodour, while promoting wound healing with little or no side effects. One of the major advantages of larval therapy is that the maggots separate the necrotic tissue from live tissue, allowing for an easier surgical debridement. The therapy can be easily applied in any environment (inpatient/outpatient) and can be left in place for 48–72 hours.

JET LAVAGE/HYDROSURGERY

The principle of jet lavage debridement (hydrosurgery) is basically an evolution of the lavage of wounds, used since the ancient times in acute wounds and, more recently, in chronic wounds. Its mechanism of action is related to water irrigation, which can physically remove foreign bodies, debris and any other kind of loose material from the wound. The more intense and fast the irrigation, the more intense are the energies transferred to tissues and consequently the more extensive preparation.

The principal limitation of this technology is that it may be painful for some patients and for this reason it should only be used when adequate pain control can be achieved, such as by use of local anesthesia. Another issue is that jet lavage has been suspected to disseminate bacteria in the environment because of the formation of an aerosol. This may contribute to the contamination of the setting in which the procedure is carried out.

ULTRASOUND

Ultrasound can, depending on the frequency and intensity of the mechanical energy transmitted, interfere with many different structures, from inert protein material to cellular bodies, exerting a range of effects that may vary from destruction to dislocation and physical modification. This characteristic makes this technology suitable for applications in different conditions and in different clinical settings; primarily for debriding purposes, but eventually also as an adjunct to the reparative phase.

An aspect that is relevant for some ultrasound devices, as well as hydrosurgery, is the nebulisation of material from the wound bed. If not properly managed, this can be substantial and, due to this aspect, the setting in which the procedures are carried out is of crucial importance for the safety of patients and clinicians.

A focus on HYALO4 Start

HYALO4 Start

HYALO4 Start is a bioactive ointment containing hyaluronic acid and collagenase, and is ideal for wounds with necrotic/devitalised tissue.

It is used in the local management of chronic ulcers (i.e. pressure sores, vascular ulcers of the legs, diabetic ulcers). It is intended to provide an optimal moist environment and wound bed preparation that supports the natural healing process.

A POWERFUL AND SYNERGIC COMBINATION:
Hyaluronic acid promotes natural healing, while collagenase assists in preparing the wound bed.

Hyaluronic Acid
(200 kDa Molecular Weight)

- Favors a moist environment, where the process of wound healing takes place more rapidly[2]
- Due to its hydrophilic properties, it provides hydration around and between cells, thus facilitating cell migration[2]
- Protects new, viable tissue[2]

Collagenase from *Vibrio alginolyticus* strain

- The collagenase in HYALO4 START is >98% pure and shows no contaminants bands compared to *C. Histolitycum* electrophoretic profile[3]
- The absence of non-specific proteases ensures a specificity of action leaving peri-lesional skin intact[3]
- Allows selective removal of necrotic tissue, does not degrade other minor, but structurally important components of the dermal ECM[3]

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Biochemical characterization of *Vibrio alginolyticus* collagenase versus a commercial preparation from *Clostridium histolyticum* strain on various dermal extracellular matrix (ECM) substrates

Di Pasquale R, Vaccaro S, Caputo M, et al. Collagenase-assisted wound bed preparation: An in vitro comparison between *Vibrio alginolyticus* and *Clostridium histolyticum* collagenases on substrate specificity. *Int Wound J. 2019;16(4):1013-23.*

This paper describes the preparation of the collagenase used in Hyalo4 Start. *V. alginolyticus* collagenase is obtained through a fermentation process and is purified chromatographically, **resulting in a highly purified 82 kDa protein that does not contain non-specific proteases or other microbial impurities.**

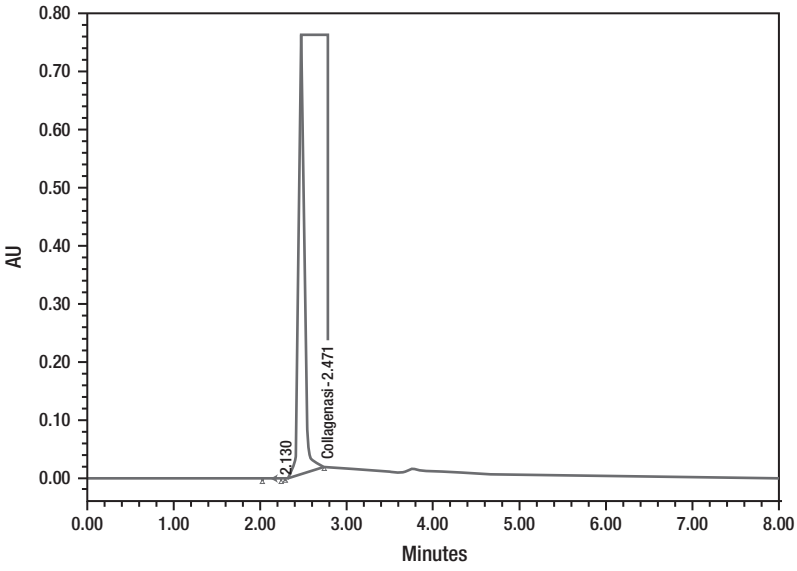


Figure 1. UPLC-SEC chromatogram of the *V. alginolyticus* collagenase product after purification.
(Di Pasquale et al., 2019).

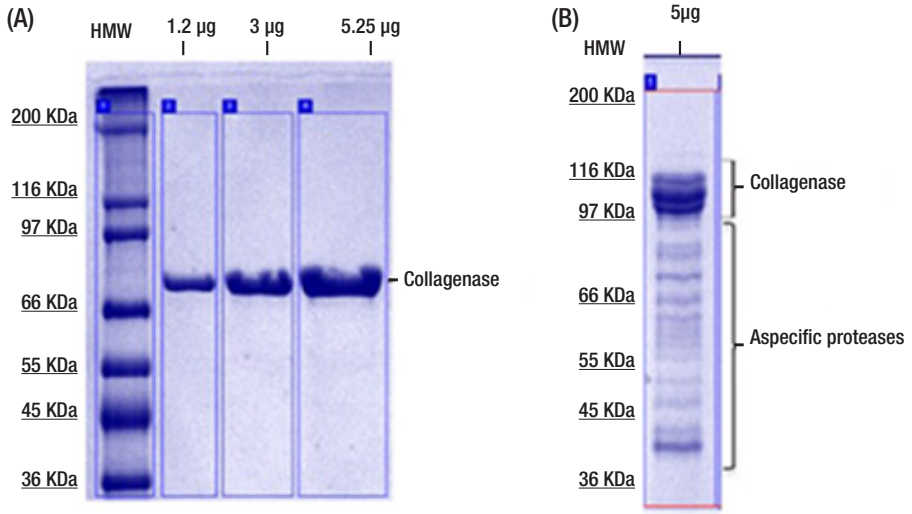


Figure 2. Purity by 12,5% SDS-PAGE and detection in Coomassic Gel Blue stain. A, Electrophoretic profile of *Vibrio alginolyticus* collagenase; B. Electrophoretic profile of *Clostridium histolyticum* collagenase.
(Di Pasquale et al., 2019).

The comparative results demonstrate how *V. alginolyticus* collagenase exhibits a better safety profile in pharmaceutical/biomedical applications, with a considerably higher purity grade compared with other commercially available collagenase from *C. histolyticum*.

The specific enzymatic activity of the two collagenases on selected dermal substrates was compared. The results showed that while the *V. alginolyticus* enzyme is fully active on collagen filaments that anchor the necrotic tissue to the wound bed, it does not degrade other minor, but structurally important, components of the dermal ECM. This feature could explain why collagenase preparation from *V. alginolyticus* has been reported to be much gentler on perilesional, healthy skin.

Moreover, the influence of pH on collagenase activity was investigated. Normal healthy skin is characterized by pH values in the mildly acidic range (5.0-5.6), and similar pH values are noted in spontaneously healing, acute wounds. Non-healing, chronic wounds are characterized by higher pH values in the mild basic range (8.0-9.5).

V. alginolyticus collagenase loses 90% activity at pH 5.6 (compared with 100% activity at pH 7). In contrast, the *C. histolyticum* collagenase preparation still retains 60% and 30% activity at pH values of 5.6 and 5.0, respectively, therefore implying that this enzyme is still able to degrade the ECM matrix in healthy skin. At mild basic pH values (7.4-8.5), typical of chronic, non-healing wounds, both preparations are equally effective on the collagen substrate.

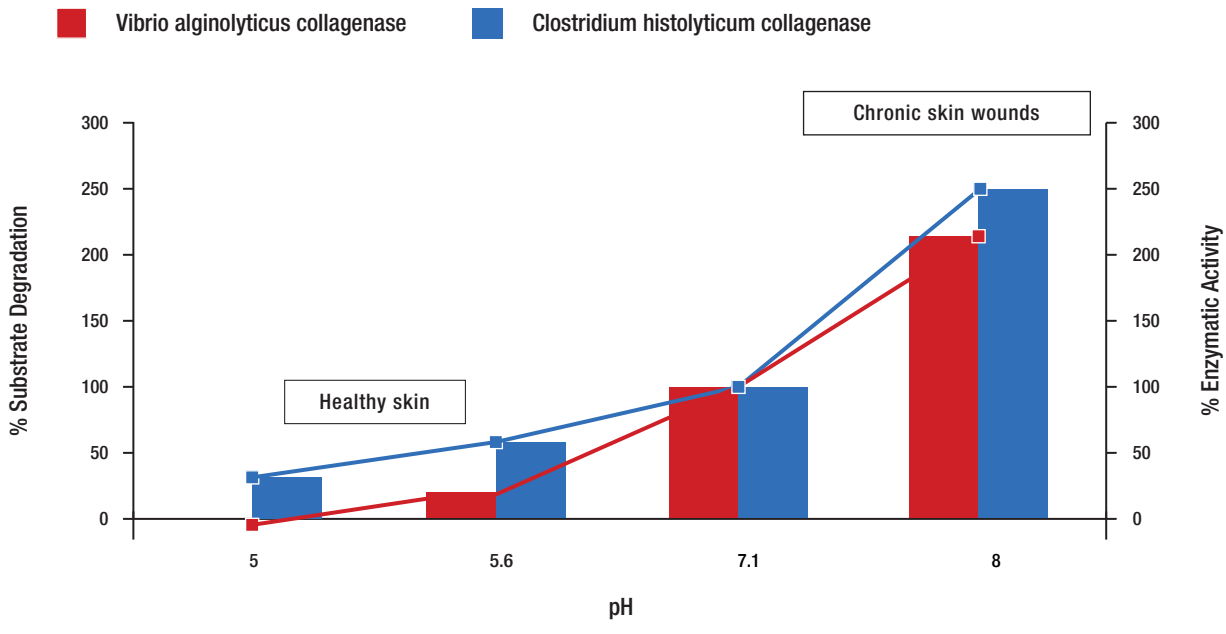


Figure 3. In vitro collagenase activity was determined at different pH values by quantifying the fragment produced by the enzymatic reaction according to the Wünsch method. (Di Pasquale et al., 2019).

In conclusion, a difference in aggressiveness between the two products containing different collagenases that act on periwound skin was demonstrated. Actually, the wound edges in patients treated with *V. alginolyticus*-derived collagenase were in better condition than the wounds treated with the *C. histolyticum*-derived enzyme. In fact, the *V. alginolyticus* enzyme is gentler on healthy tissue and allows improvement of the surrounding skin.

Clinical studies with HYALO4 Start

Multicenter clinical trial on the performance and tolerability of the Hyaluronic acid-Collagenase ointment for the treatment of chronic venous ulcers: a preliminary pilot study

Gravante G, Sorge R, Giordan N, et al. *Eur Rev Med Pharmacol Sci. 2013;17(20):2721-7.*

This clinical trial involved 100 patients with class 6 venous ulcers (CEAP classification) of at least 6-month duration. HYALO4 Start was administered daily and follow-up visits were conducted on days 5, 10, 15, and 20.

All patients achieved complete preparation of the necrotic area and a significant reduction of the total ulcer area by day 20, while other parameters improved significantly over time.

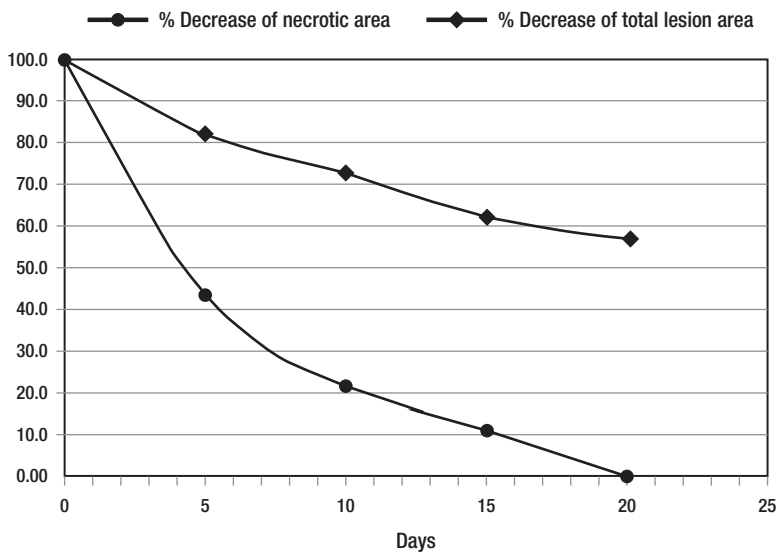


Figure 4. Trends of the percentage of necrotic area and total ulcer area during the treatment. (Gravante et al,, 2013)

As shown in Figure 4, the necrotic component of the ulcer decreased at a higher rate compared to the total area. This demonstrates the clear dual action of Hyalo4 Start:

- Wound bed preparation by collagenase (more than 50% decrease in the necrotic area after only 5 days)
- Stimulation of healing by hyaluronic acid (20% decrease of total lesion area)

In addition, all the other parameters associated with the ulcer (odor, erythema, tissue viability and moisture balance) showed significant improvement over time. The safety of the device was globally satisfactory and no hypersensitivity reactions were reported.

Comparison between two ointments containing collagenases versus mechanical wound bed preparation on chronic hard-to-heal wound

Onesti MG, Fioramonti P, Fino P, Sorvillo V, Carella S, Scuderi N. *Int Wound J. 2016;13(6):1111-5.*

This study compared two ointments with collagenase from *V. alginolyticus* and *Clostridium histolyticum* to mechanical wound bed preparation alone. There were 30 patients in each group.

After 8 weeks, 33% of patients in the HA + *V. alginolyticus* group, 27% of patients in the *Clostridium histolyticum* group and 20% of patients in the mechanical preparation group achieved complete healing (Table 1).

	Hyaluronic acid + Collagenase from Vibrio anginolyticus	Collagenase from Clostridium histolyticum	Mechanical preparation
Complete wound healing (% of patients) over 8 weeks	33%	27%	20%
Adequate wound bed preparation (% of patients) at 4 weeks	67%	57%	30%
Reduction of lesion area (%) at 8 weeks	84%	77%	52%
Reduction of mean pain score (VAS) (%) at 8 weeks	-3.9% °§	-2.6°	-1.8
Integrated wound margins at 8 weeks (%)	80%	73%	54%

Table 1. Results with Hyaluronic acid + Collagenase from *Vibrio anginolyticus* vs. collagenase from *Clostridium histolyticum* and mechanical preparation. °p<0.0002 vs. mechanical preparation, §p<0.0093 vs. *Clostridium histolyticum.*

While at T8, the difference in the wound cleansing percentage of group B was not statistically significant compared with the M group, at T4, the wound cleansing percentage was greater in the B group with respect to the N and M groups, suggesting a more rapid wound bed cleansing process.

This result could be attributed to the synergy between HA and *V. aginolyticus* collagenase. In fact, Hyaluronic Acid maintains an optimal moist environment and promotes the healing process, thereby reducing crusting, discomfort, erythema and swelling.

Hyaluronic Acid + *V. alginolyticus* also performed favourably compared to the other two methods studied in terms of better condition of wound edges, reduction of pain and persistence in the wound.

Based on these results, HA + collagenase derived from *V. alginolyticus* demonstrated chemical and physical properties that make it a product that is easy to manage and effective in the preparation of the wound bed, and ensures the protection of peri-wound skin.

Is Hyaluronic acid-Collagenase the right synergy? Randomized double-blind controlled clinical trial in venous leg ulcers

Scalise A, Campitiello F, Della Corte A, et al. *Eur Rev Med Pharmacol Sci. 2017;21(6):1421-31.*

This was a double blind, multicenter, controlled clinical trial conducted to demonstrate the superiority of the Hyaluronic acid-Collagenase ointment from the non-pathogenic *V. alginolyticus* applied once a day over placebo in mean reduction of devitalized/fibrinous/slough tissue after 15 days of treatment. A total of 113 patients with venous ulcers were enrolled and randomized to receive active treatment therapy or vehicle preparation. Both arms also received compression therapy.

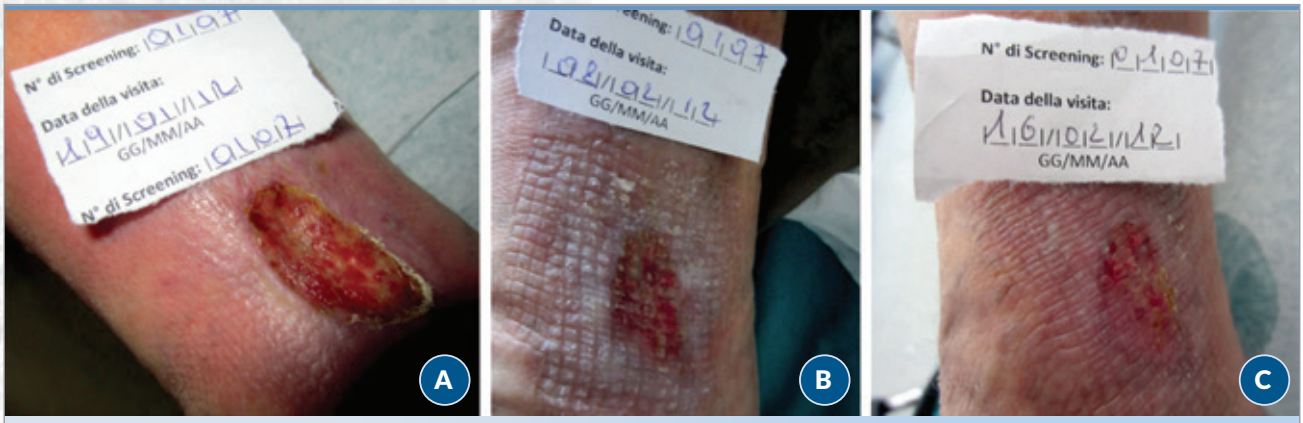


Figure 5. Treatment group. **A**, Pre-treatment aspect of the wound; **B**, Aspect after 15 days of HA-Collagenase treatment; **C**, Aspect after 30 days of treatment.

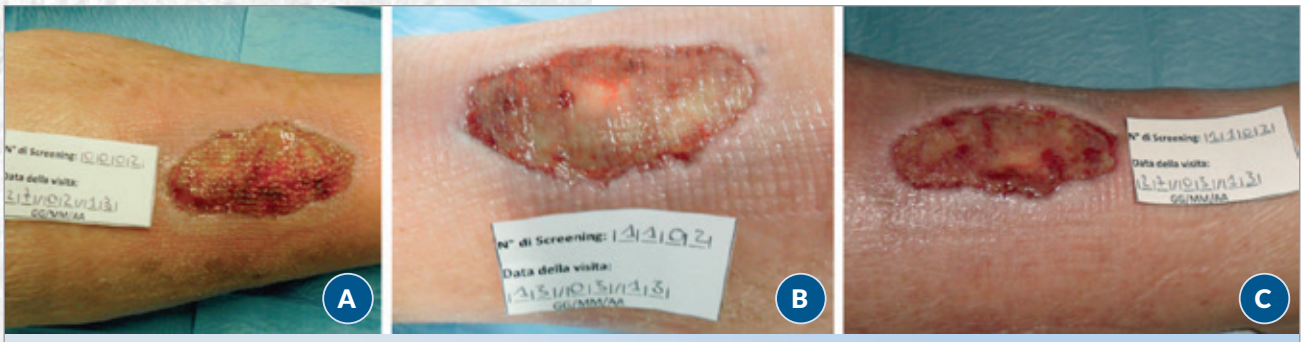


Figure 6. Placebo group: **A**, Pre-treatment lesion; **B**, Lesion after 15 days of treatment with placebo ointment; **C**, Lesion after 30 days of treatment.

The percentage reduction in devitalised tissue was significantly greater in patients randomised to HA-collagenase compared to vehicle after 15 days of treatment, suggesting a rapid onset of action.

The wound characteristics after 15 days of treatment with placebo (Figure 6B) presented more fibrinous tissue than lesions in patients after 15 days of HA-collagenase treatment (Figures 5B and 5C). Consequently, after 30 days wounds treated with placebo ointment (Figure 6C) showed delayed healing.

The overall suitable wound bed preparation rate was greater with HA-collagenase than with placebo (Figure 7).

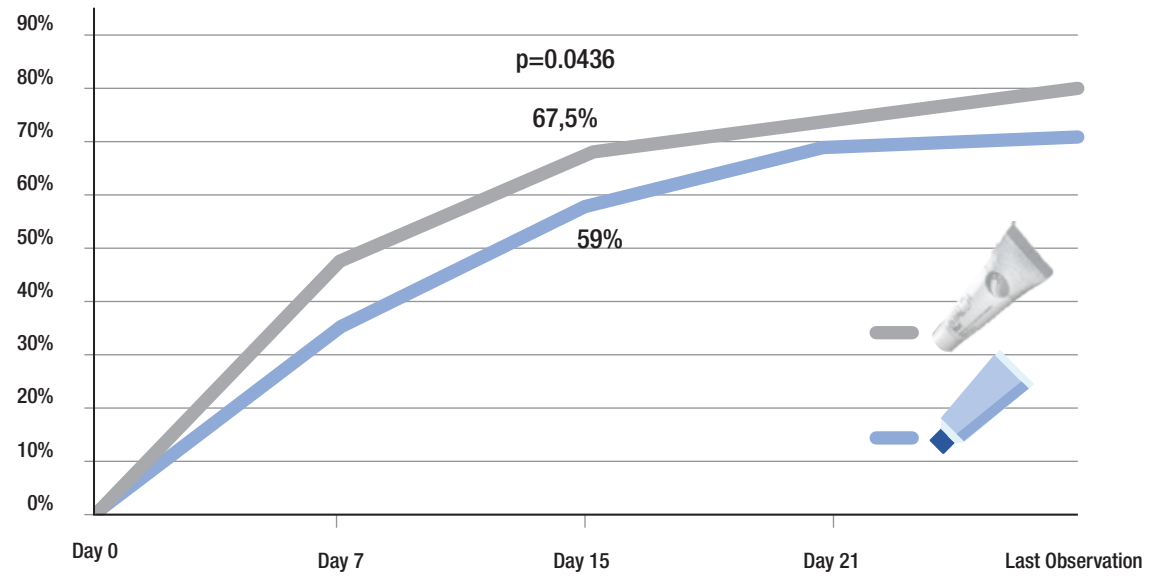


Figure 7. Overall suitable wound bed preparation at day 15, ITT population. Revisited from Scalise et al., 2017.

Moreover, a significantly higher number of patients in the treatment group achieved 100% wound bed preparation by day 15 (p = 0.0002) than in the control group, and a higher percentage also demonstrated complete preparation at all time points (Figure. 8).

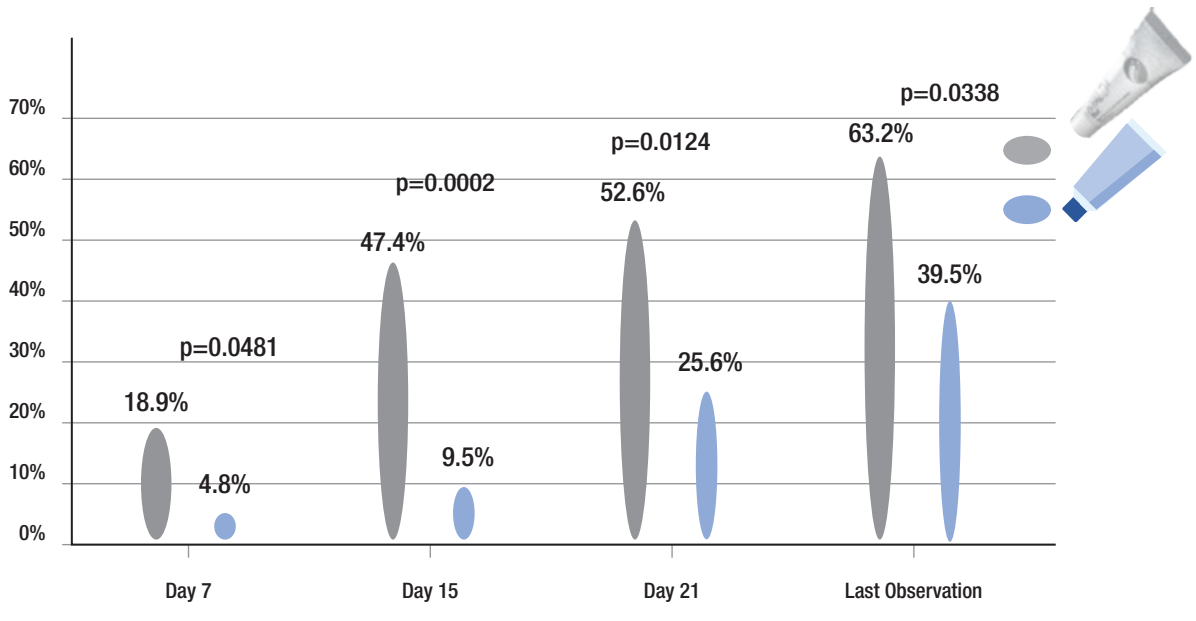


Figure 8. Proportion of patients with complete wound bed preparation. Revisited from Scalise et al., 2017.

Performing fast and effective wound cleansing, as well as avoiding pain and periwound skin irritation, Hyalo4 Start can be considered the first step towards successful healing with better effectiveness, good tolerability and less pain for patients.

Application of Hyaluronic acid and Collagenase ointment in the treatment of chronic wounds: a case series

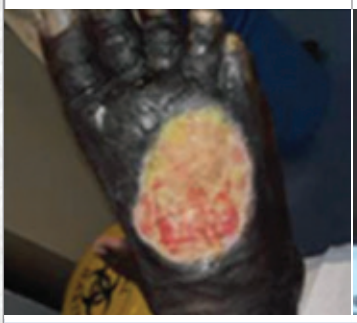
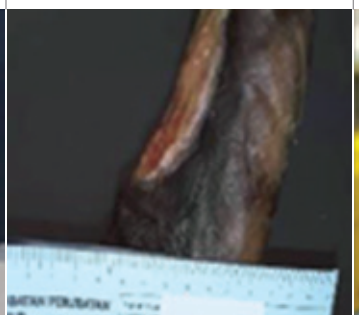
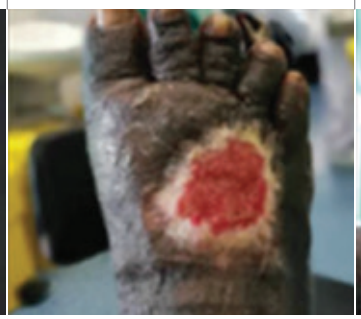
Nair HKR, Sylvia Chong SSY, Khamis SAB, Eruthayaraj A.
Wounds Asia. 2020;3:49.

This case series reviews 8 patients with chronic wounds of different aetiologies including diabetes mellitus (n=6), bullous cellulitis (n=1), thigh abscess (n=1). All patients had sloughy, necrotic tissue in the wound bed. Wounds were evaluated using the TIMES concept before cleansed with polyhexamethylene biguanide with betaine (PHMB) solution.

CASE STUDY 1

- A **70-year-old male**: diabetes mellitus, hypertension, dyslipidemia, benign prostatic hyperplasia and bullous cellulitis;
- Diabetic foot ulcer on the right, on the dorsal and lateral heel. Wound presented with 70% slough and 20% granulation tissue with heavy yellowish exudate. Dry skin was observed in the periwound area.

Total duration of treatment: 54 days

BEFORE: 16th July 2019		AFTER: 5th September 2019	
Wound A	**Wound B**	**Wound A**	**Wound B**
			
TOTAL WOUND AREA			
63.8 cm^2	**7.5 cm^2**	**20 cm^2**	**4 cm^2**
PAIN SCORE			
3		**0**	

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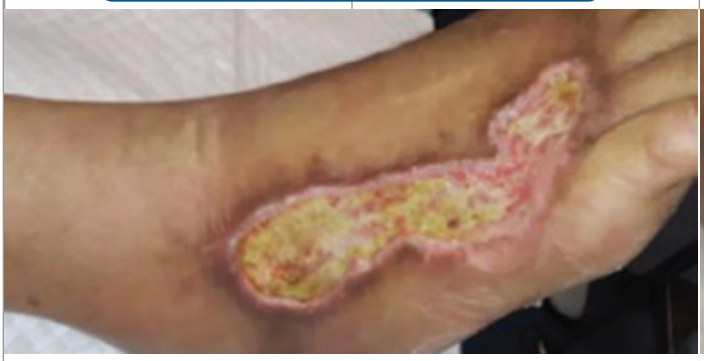
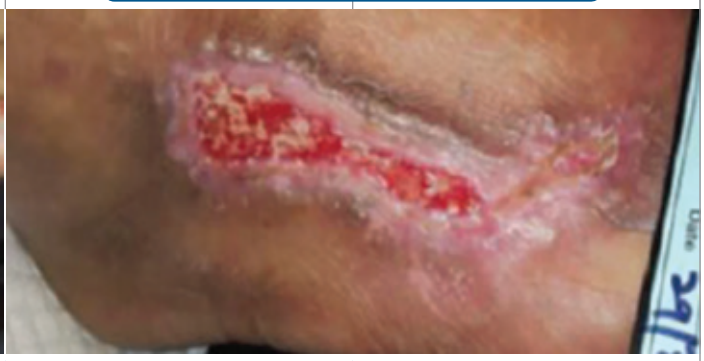
CASE STUDY 2

- A **56-year-old female**;
- Bullous cellulitis on right dorsal foot. Ultrasound revealed heterogeneous subcutaneous hypoechoic collection over lateral aspect representing an early abscess. Wound presented with moderate 50% slough, 25% granulation tissue.

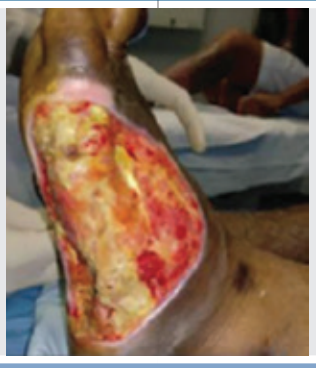
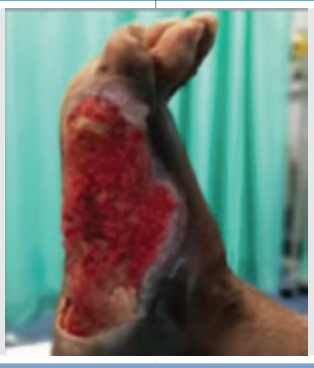
Total duration of treatment: 44 days

BEFORE: 17th July 2019	AFTER: 29th August 2019
	
TOTAL WOUND AREA	
30 cm^2	**9.8 cm^2**
PAIN SCORE	
1	**1**

CASE STUDY 3

- A **48-year-old male**: diabetes mellitus, hypertension. History of Ray's amputation of 3rd right toe;
- Left lateral diabetic foot ulcer. Wound presented with exposed tendon, 50% sloughy tissue and 10% granulation tissue.

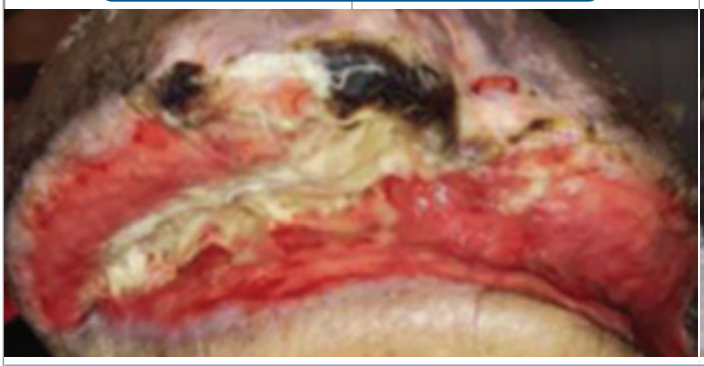
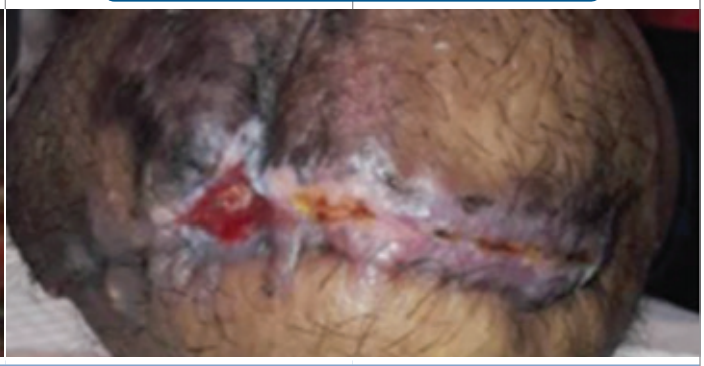
Total duration of treatment: 46 days

BEFORE: 25th July 2019	AFTER: 5th September 2019
	
TOTAL WOUND AREA	
96 cm^2	**51 cm^2**
PAIN SCORE	
0	**0**

CASE STUDY 4

- A **48-year-old male**: diabetes mellitus, hypertension. History of ray amputation of 3rd left toe;
- Left lateral diabetic foot ulcer. Wound presented with exposed tendon, 50% sloughy tissue and 10% granulation tissue.

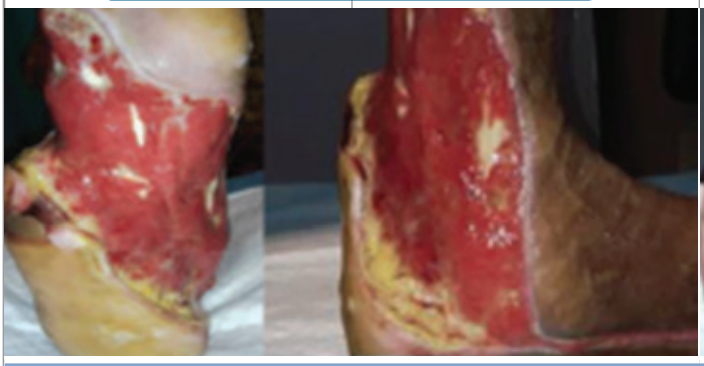
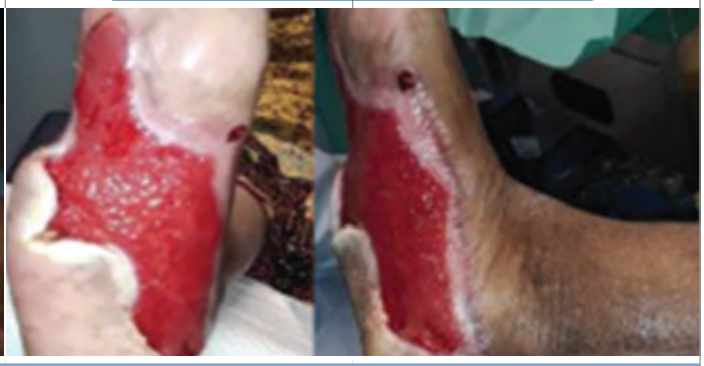
Total duration of treatment: 48 days

BEFORE: 25th July 2019	AFTER: 10th September 2019
	
TOTAL WOUND AREA	
72 cm²	**4 cm²**
PAIN SCORE	
1	**0**

CASE STUDY 5

- A **69-year-old female**: diabetes mellitus, hypertension and dyslipidemia;
- Initial wound on 4th left toe followed by severe infection, which resulted in extensive wound debridement and amputation of 4th and 5th toes. Wound presented with 50% thick sloughy tissue, 40% granulation tissue. Maceration tissue was observed in the periwound area.

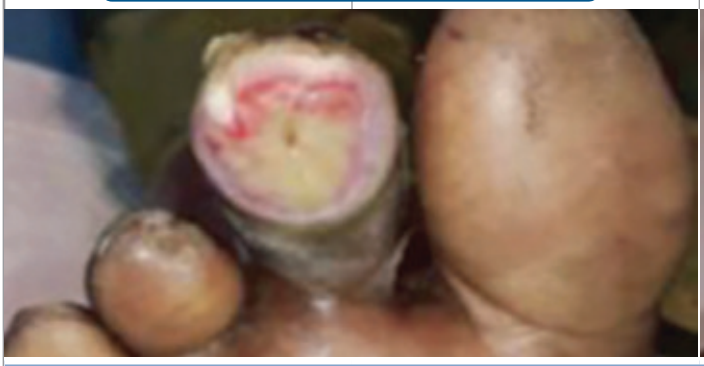
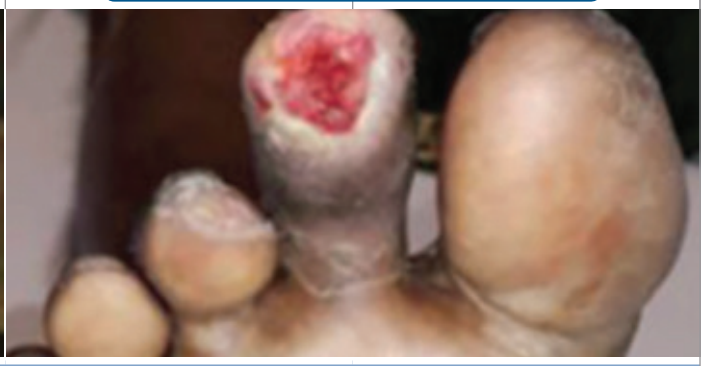
Total duration of treatment: 41 days

BEFORE: 25th July 2019	AFTER: 3rd September 2019
	
TOTAL WOUND AREA	
158 cm²	**98 cm²**
PAIN SCORE	
3	**0**

CASE STUDY 6

- A **75-year-old female**: diabetes mellitus;
- Diabetic wound on 2nd right toe. Wound was 70% sloughy, with 20% granulation tissue and 10% epithelization of skin surrounding the wound.

Total duration of treatment: 10 days

BEFORE: 25th July 2019	AFTER: 3rd September 2019
	
TOTAL WOUND AREA	
3 cm^2	**1.2 cm^2**
PAIN SCORE	
1	**1**

In this case series, the combined action of hyaluronic acid and collagenase favors the reduction of healing time while improving healing quality.

Clinical studies have shown that the combination of **hyaluronic acid** and collagenase from ***Vibrio alginolyticus*** **(HYALO4 Start) is associated with a number of significant benefits for supporting wound healing and patients.**

Conclusions

- Synergic action of hyaluronic acid with the proteolytic action of collagenase from *Vibrio alginolyticus*: HYALO4 start prepares the wound bed to allow faster healing.
- Promotes the healing process by anticipating the transition to the re-epithelialisation phase.
- More rapid wound bed cleansing and healing clinically-demonstrated versus a *Clostridium histolyticum-based* product[5].
- Less pain referred by patients versus a *Clostridium histolyticum-based* product[5].
- Long persistence in wound site, allowing for less frequent dressing changes[5].
- HYALO4 Start is very fluid, smooth and simple to apply, with neither discomfort nor pain.

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