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The combination of hyaluronic acid and collagenase in the treatment of skin ulcers: an open, multicenter clinical study assessing safety and tolerability of Bionect Start®

P. FINO[1], C. CHELLO[1], C. LATINI[2], S. OCCHIONORELLI[3], M. MORUZZI[4], N. SCUDERI[5], G. PELLACANI[1]

[1]Department of Clinical Internal, Anesthesiological and Cardiovascular Sciences, Dermatology Clinic, University of Rome "La Sapienza", Rome, Italy
[2]Department of Plastic and Reconstructive Surgery, Ospedale San Giovanni Addolorata, Rome, Italy
[3]Department of Emergency Surgery, Arcispedale Sant'Anna, Ferrara, Italy
[4]Fidia Farmaceutici S.p.A., Abano Terme, Padua, Italy
[5]Department of Plastic and Reconstructive Surgery, University of Rome "La Sapienza", Rome, Italy

Abstract. – OBJECTIVE: Several clinical studies have shown that hyaluronic acid collagenase is well-tolerated and very effective in managing chronic venous ulcers. The aim of the present study is to confirm the safety and tolerability of daily application in patients suffering from cutaneous ulcers of different etiologies. The efficacy of the treatment and its impact on patients' quality of life are also assessed.

PATIENTS AND METHODS: Patients with a clinical diagnosis of skin ulcer with devitalized/fibrinous/slough tissue that could delay the healing process were enrolled in the study. The hyaluronic acid/collagenase ointment was applied topically until wound closure or total debridement of non-viable tissue was achieved, however, with a limit of 30 days. Monitoring was performed weekly, either through outpatient visits or telephone surveys. Assessments included adverse events, local irritation reactions, pain at dressing changes, and wound bed status. Patients were also requested to complete a quality-of-life questionnaire.

RESULTS: The study involved 96 patients with a mean age of 71 years. The patients suffered mainly from traumatic (21.9%), venous (15.6%), or pressure ulcers (12.5%); in 26% of cases, ulcers had mixed etiology. In approximately 32% of patients, the ulcer had been present for more than 6 months, and 18.1% of subjects had previously undergone surgical wound debridement.

CONCLUSIONS: Daily application of hyaluronic acid-collagenase achieved the following results: i) absence of adverse events related to the use of the product; ii) significant reduction in the degree of localized irritation and pain at dressing changes; iii) significant support to wound bed preparation; iv) trend towards improvement in the quality of life and health status of the patients.

Key Words:
Hyaluronic acid, Collagenase, Wound care, Skin ulcers.

Introduction

Non-healing skin ulcers have a huge impact on patients' quality of life and high social/health care costs, which are expected to escalate with the population's increasing age and comorbidity profile.

A recent survey[1] analyzing data covering over 1,000 lesions across multiple community care providers in Europe revealed that 35.6% of them are classified as chronic or hard-to-heal wounds. This term refers to lesions that do not follow normal repair processes and do not respond to standard treatment within a reasonable period of time (usually 30 days). This condition often occurs in patients with underlying systemic disorders, including diabetes, arterial and venous insufficiency, or systemic inflammatory disease, and is supported by local factors such as tissue hypoxia, exudates, excessive or prolonged inflammation, necrosis,

Corresponding Author: Pasquale Fino, MD, Ph.D; e-mail: pasquale.fino@gmail.com

and persistent bacterial infection[2]. The goal in the management of hard-to-heal wounds is to perform a series of procedures that lead, on the one hand, to the removal of barriers to healing and, on the other hand, to the stimulation of the repair process. Four different aspects need to be addressed for optimal wound bed preparation. They are identified by the TIME acronym: Tissue refers to the non-viable or deficient tissue that impairs the healing process; Infection or Inflammation indicates the presence of contaminated areas or excessive inflammation; Moisture denotes the need to balance the wound exudate to avoid excessive fluid or desiccation; Edge evaluates the presence of factors preventing the advancement and closure of wound edges. By getting rid of necrotic, damaged, or infected tissue, debridement contributes to controlling inflammation, promoting the formation of granulation tissue, reducing excess moisture, and stimulating non-advancing wound edges[3,4]. Several methods of wound debridement are available: surgical, mechanical, autolytic, biological, and enzymatic debridement. The selection of the optimal method depends on different factors, including wound features, patient comorbidities, pain limitations, and setting (e.g., an outpatient clinic or home).

Autolytic debridement involves the use of moisture-donating or moisture-retentive dressings, such as hydrogels, hydrocolloids, films or alginates, that are placed on the wound for 2-3 days to allow endogenous enzymes to eliminate non-viable tissue; this method exploits the patient's immune system, but is slow, requires multiple cycles of dressing application/wound irrigation, and is not appropriate for patients with compromised immune system or infected wounds[5]. Enzymatic debridement uses topical application of natural proteases to digest necrotic tissue in the wound bed. Among enzymatic debridement agents, collagenase is characterized by high selectivity because it specifically degrades collagen, a key component of the extracellular matrix, accelerates necrotic plug removal, thus preparing the wound bed for granulation tissue formation and healing process acceleration.

Hyaluronic acid (HA) is an endogenous non-sulfated glycosaminoglycan with specific biological and nonbiological properties, present in large amounts in the skin, synovial fluid, and umbilical cord, among others. It is the main component of the extracellular matrix[6]. It plays a critical role in cell signalling and tissue homeostasis and is particularly concentrated in areas of rapid tissue proliferation, regeneration, and repair. Its biological functions include the maintenance of water homeostasis: it is characterized by the ability to retain large amounts of water molecules due to its multiple negatively charged subunits, controlling tissue hydration[7]. Hyaluronic acid also plays a fundamental role in the wound healing process: due to its pores and hydrated organization, it allows for the rapid diffusion of water-soluble molecules and contributes to maintaining an optimal moist environment in the wound that results in reduced scar, discomfort, and pain[8]. Hyaluronic acid exists in various forms that differ in molecular weight [high molecular weight hyaluronic Acid (HMW-HA) and low molecular weight hyaluronic Acid (LMW-HA)] and physicochemical properties, such as viscoelasticity and lubrification[9]. In contact with the skin, hyaluronic acid forms a film that provides natural protection against abrasion, friction, and dehydration. Moreover, the association of HA with collagenase increases the debridement rate of chronic hard-to-heal ulcers, as demonstrated by clinical studies[10-12].

Among the other components present in the product, we find bacterial collagenase derived from aerobic non-pathogenic *Vibrio Alginolyticus* strain, a proteolytic enzyme with high specificity for native and denatured collagen. *Vibrio alginolyticus*-derived collagenase is a component whose action is ancillary to that of the device. The substance aids sodium hyaluronate in preparing the wound bed by removing necrotic tissues that interfere with the wound-healing process in chronic wounds. The collagenase incorporated in hyaluronic acid was added to the HA-based device as a wound bed preparation agent to make the treatment more adequate for the management of ulcers in the first phase of wound bed preparation.

The safety and efficacy of low molecular weight hyaluronic acid-collagenase (LMW-HA-collagenase) were previously assessed in several clinical studies[10-12] where the combination of hyaluronic acid and collagenase were shown to be safe and very effective in promoting both debridement and healing of chronic venous ulcers. The purpose of this study is to evaluate the safety and tolerability of LMW-HA-collagenase in the management of skin ulcers of different etiology, in order to

identify any undesirable side effects related to the treatment.

Patients and Methods

Patients

This was an interventional, multicenter, open-label, uncontrolled clinical investigation performed on 107 patients. The study was conducted in compliance with the protocol approved by the Italian Ministry of Health by the Coordinator Ethics Committee "Sapienza" (Prot. 1082/13), and the Local Ethical Committees of the 16 participating centers. The study was carried out in accordance with the GCP guidelines, the regulation on medical devices, and the Declaration of Helsinki.

Adult patients with a clinical diagnosis of cutaneous ulcer with the presence of devitalized/fibrinous/ slough tissue preventing or slowing the healing process were included.

All subjects signed a written informed consent form before being included in the study.

Inclusion and Exclusion Criteria

The inclusion criteria foresaw the inclusion of adult subjects diagnosed with skin ulcers with the presence of devitalized/fibrinous/ sloughy tissue.

Bones' exposure, tendons, and fascia in the area target, use of local antibiotics, use of detergents, acid solutions, antiseptic with metallic ions, concomitant use of detergents with quaternary ammonium and hypersensitivity to collagenase were criteria of exclusion.

Experimental Protocol

The treatment was carried out with LMW-HA-collagenase (Bionect Start®, Fidia Farmaceutici S.p.A., Abano Terme, Italy), a topical ointment based on hyaluronic acid sodium salt (0.2% w/w) and bacterial collagenase obtained from non-pathogenic *Vibrio alginolitycus* (>2.0 nkat/g).

The application consisted of topical administration of a 2-mm-thick layer of ointment to the wound bed once daily after cleaning with saline solution. Then, the wound was covered with a suitable secondary dressing. Treatment was performed until the wound was closed, or the nonviable tissue was completely debrided, or up to 30 days, whichever condition was met first. Patients were trained to apply the product correctly at home.

Patients were evaluated every 2 weeks starting from the baseline visit (V2, V4), while telephone calls were made in the intermediate weeks (V1, V3). During the baseline visit, the investigator collected clinical data, including demographics, medical history, medications, and wound characteristics such as wound bed status, pain during dressing, degree of irritation, burning, and itching. All subjects were also asked to complete the EQ-5D quality of life questionnaire at baseline and during the final visit.

During Visit 1 (days 7) and Visit 3 (days 21), patients were contacted by telephone and asked to complete a survey to assess the presence of pain during medication, burning or itching, adverse events since the previous visit, changes in comorbidities, and/or concomitant medications. At Visit 2 (day 15), the same evaluations were performed by medical staff, who also assessed the wound bed status. In case of complete wound closure or absence of devitalized/fibrinous/ slough tissue, the investigator could decide to discontinue the treatment, and the evaluation of the final visit was performed. Patients were asked to complete the EQ-5D quality of life questionnaire. In the event of incomplete wound closure or incomplete debridement, patients continued the treatment until Visit 4 (day 30) at the latest, when their participation in the study was concluded.

Study Endpoints

The primary objective of the study was the evaluation of the short-term safety and tolerability of LMW-HA-collagenase daily application in patients with devitalized/fibrinous/ slough skin ulcers. The primary outcome was evaluated by measuring the degree of irritation in perilesional skin, which was quantified by the investigator using an 8-point scale. Itching and burning were reported by the patient based on a 4-point scale (absent, minimal, moderate, severe). Local and systemic tolerability were assessed by monitoring the patients throughout the duration of the study for the occurrence of unexpected serious and nonserious adverse events, both reported by the patients and observed by the investigator. Pain at dressing changes (absent, minimal, moderate, severe)

was also assessed as a secondary safety parameter.

Secondary endpoints evaluated in the study were the efficacy of the treatment and its impact on patients' quality of life. The wound bed status was assessed by considering the amount of viable tissue (very poor, poor, satisfactory, good, very good), the ulcer size (healed, small, medium, large), and clinical signs such as odor (absent, minimal, tolerable, intense, repellent).

Statistical Analysis

Qualitative variables were expressed as frequencies and percentages. Statistically significant differences were measured over time (baseline/follow-up) of the main variables and were analyzed using univariate tests (paired samples *t*-test) and nonparametric tests (Wilcoxon). The data analysis was generated using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA); results were considered significant if $p<0.05$.

Results

During a period of 9 months, 107 patients were enrolled in 16 Italian centers. Due to difficulties in recruiting and considering that the interim analysis of data confirmed the absence of treatment-related adverse events, recruitment was closed early, with a lower number of cases than initially planned.

Eleven subjects were subsequently excluded from the analysis due to early dropouts, absences at control visits, or failure to meet study requirements.

Three parameters – perilesional irritation, burning, and itching – were analyzed to evaluate the occurrence of local irritative reactions. The patients' quality of life was evaluated using the EQ-5D questionnaire.

Ninety-six patients (96) completed the study: 54 were males (56.3%), and a mean age of 71.3±14.9 years. Most lesions (97.2%) were located on the lower limbs. Wound etiology was distributed as follows: 21.9% traumatic, 15.6% venous, 6.3% arteries, 12.5% pressure ulcers, diabetic ulcers, 10.4%, and 26% of ulcers had mixed etiology (Table I). In 37.4% of the cases, the lesion had been present for less than a month, while in about 32% of the patients, it was more than 6 months old; 18.1% of patients had already experienced surgical debridement.

Table I. Wounds typologies.

Wound typologies	% (N=96)
Venous ulcers	15.6
Pressure ulcers	12.5
Traumatic ulcers	21.9
Mixed etiology	26
Diabetic ulcers	10.4
Arterious ulcers	6.3
Other	7.3

Safety and Tolerability Analysis

The safety and tolerability of LMW-HA-collagenase daily application were assessed by monitoring the occurrence of adverse events, the onset of local irritative reactions, and pain at dressing changes. During the entire study duration, 4 patients experienced adverse events, one of which was severe and had a fatal outcome. None was related to the use of LMW-HA-collagenase product.

Daily topical administration resulted in a progressive reduction in the degree of perilesional irritation: the percentage of subjects with "no evidence of irritation" (grade 0) increased from 15.6% at baseline to 35.4% after 15 days of treatment and 43.8% at the final visit.

The overall improvement in the degree of irritation was statistically significant both at 15 days (44.8%, $p<0.0001$) and at 30 days (61.5%, $p<0.0001$) (Table II).

Similarly, treatment with LMW-HA-collagenase achieved a progressive decrease in perceived burning: the percentage of patients reporting "absence of burning" increased from 38.5% at baseline to 51% at 15 days and 61.5% at the end of the treatment (Table III); the improvement was statistically significant at both time points (30.2%, $p=0.0015$ at 15 days; 45.8%, $p=0.0001$ at 30 days) as shown in Table IV.

Instead, the effect on itching was fluctuating, with the same number of patients experiencing a decrease (n=19) and an increase (n=20) in the perception of itching; only at the end of the treatment period, 25% of patients reported an improvement ($p=0.0318$) (Table V).

A significant reduction in pain at dressing changes was also observed: 83.3% of patients

Table II. Peri-lesional irritation grade.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
0. No evidence of irritation	15	15.6	34	35.4	42	43.8
1. Minimal erythema, barely noticeable	28	29.2	24	25.0	29	30.2
2. Definite, clearly visible erythema, minimal edema or minimal presence of papules	30	31.3	23	24.0	15	15.6
3. Erythema and papules	9	9.4	5	5.2	1	1.0
4. Definite edema	5	5.2	4	4.2	5	5.2
5. Erythema edema and papules	5	5.2	3	3.1	3	3.1
6. Vesicular rash	.	.	.	.	.	.
7. Strong reaction widely spread around the lesion	4	4.2	3	3.1	1	1.0
Change from baseline visit						
Improving			43	44.8	59	61.5
Not changing			44	45.8	27	28.1
Worsening			9	9.4	10	10.4
Wilcoxon Test			$p<0.0001$		$p<0.0001$	

Table III. Burning.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
Absent	37	38.5	49	51.0	59	61.5
Minimal	29	30.2	31	32.3	24	25.0
Moderate	27	28.1	12	12.5	10	10.4
Severe	3	3.1	4	4.2	3	3.1
Overall	96	100.0	96	100.0	96	100.0

Table IV. Percentage of improvement in burning at 15 and 30 days *vs.* baseline.

	Changing at 15 days *vs.* baseline		Changing at 30 days *vs.* baseline	
	N	%	N	%
Improved	29	30.2	44	45.8
Not changed	58	60.4	38	39.6
Worsened	9	9.4	14	14.6
Overall	96	100.0	96	100.0
Wilcoxon Test	$p=0.0015$		$p=0.0001$	

reported absent or minimal pain at 30 days of treatment (Table VI and Table VII), with an improvement of pain in 40.4% and 47.9% of patients at 15 days and at the end of the treatment, respectively ($p<0.0001$). No significant correlations were found between changes in perilesional irritation, burning, itching, or pain and ulcer characteristics (size, site, and type).

Table V. Itching.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
Absent	59	61.5	55	57.3	71	74.0
Minimal	22	22.9	28	29.2	16	16.7
Moderate	11	11.5	12	12.5	6	6.3
Severe	4	4.2	1	1.0	3	3.1
Change from baseline visit						
Improving			19	19.8	24	25.0
Not changing			57	59.4	60	62.5
Worsening			20	20.8	12	12.5
Wilcoxon Test			p=n.s.		p=0.0318	

n.s.: not significant.

Table VI. Reduction in pain at dressing changes.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
Absent	29	30.2	43	44.8	53	55.2
Minimal	22	22.9	31	32.3	27	28.1
Moderate	24	25.0	16	16.7	10	10.4
Severe	19	19.8	6	6.3	6	6.3
Overall	96	100.0	96	100.0	96	100.0

Table VII. Percentage of change in pain at 15 and 30 days *vs.* baseline.

	Changing at 15 days *vs.* baseline		Changing at 30 days *vs.* baseline	
	N	%	N	%
Absent	38	40.4	45	47.9
Not changing	47	50.0	42	44.7
Worsening	9	9.6	7	7.4
Overall	94	100.0	94	100.0
Wilcoxon Test	p<0.0001		p<0.0001	

Table VIII. Tissue vitality.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
Not determined	21	21.9	2	2.1	1	1.0
Very poor	15	15.6	12	12.5	8	8.3
Poor	22	22.9	18	18.8	11	11.5
Satisfying	27	28.1	39	40.6	33	34.4
Good	10	10.4	13	13.5	21	21.9
Very good	1	1.0	12	12.5	22	22.9
Changing from baseline visit (N=75)						
Improved			33	44	48	64.0
Not changing			38	50.7	25	33.3
Worsening			4	5.3	2	2.7
Wilcoxon Test			$p<0.0001$		$p<0.0001$	

Table IX. Mean percentage of devitalized tissue.

	Baseline	Changing at 15 days	Changing at 30 days
N	95	96	96
Average	62.4	38.4	26.0
Std. Dev	32.7	31.5	31.0
Max	100.0	100.0	100.0
3rd Quartile	90.0	65.0	40.0
Median	70.0	30.0	20.0
1st Quartile	30.0	10.0	0.0
Minimum	5.0	0.0	0.0
Missing	1	0	0

Wound Bed Assessment

The effect of LMW-HA-collagenase application on wound bed status was monitored by different parameters: dimension of the wound, odor, presence of vital tissue, and total area of vital tissue.

A significant increase in the vitality of injured tissues was detected (Table VIII): the percentage of lesions with satisfactory, good, or very good tissue vitality at baseline was 28.1%, 10.4%, and 1%, respectively, for a total amount of 39.5%. At 15 days, the percent-

Table X. Dimension of the wound.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
Not determined	1	1.0				
Healed	.	.	2	2.1	9	9.4
Small ≤6 cm^2	49	51.0	58	60.4	60	62.5
Medium 6-10 cm^2	33	34.4	23	24.0	19	19.8
Big >10 cm^2	13	13.5	13	13.5	8	8.3
Change from baseline visit (N=95)						
Improving			16	16.8	32	33.7
Not changing			75	78.9	59	62.1
Worsening			4	4.2	4	4.2
Wilcoxon test			$p=0.0118$		$p<0.0001$	

Table XI. Evaluation of odor.

	Baseline		Changing at 15 days		Changing at 30 days	
	N	%	N	%	N	%
Not determined	10	10.4	2	2.1	.	.
Absent	49	51.0	74	77.1	80	83.3
Minimum	23	24.0	13	13.5	11	11.5
Tollerable	9	9.4	5	5.2	4	4.2
Intense	5	5.2	2	2.1	.	.
Repellent	.	.	.	.	.	.
Change from baseline visit (N=86)						
Improving			25	29.1	29	33.7
Not changing			57	66.3	52	60.5
Worsening			4	4.7	5	5.8
Wilcoxon Test			$p<0.0001$		$p<0.0001$	

Table XII. Quality of life improvement assessed with the EQ-5D questionnaire.

Items	Improved subjects		Wilcoxon Test
	N	%	
Movement skills	10	16.1	p=n.s.
Personal care	8	12.9	p=n.s.
Habitual activities	7	11.3	p=n.s.
Pain and discomfort	21	33.9	$p<0.0001$
Anxiety and depression	9	14.8	p=n.s.

n.s.: not significant.

age of lesions showing satisfactory, good, or very good tissue vitality were 40.6%, 13.5%, and 12.5%, respectively, totaling 66.6%. At 30 days, there was a further increase in the percentages: 34.4%, 21.9%, and 22.9% of patients exhibited satisfactory, good, or very good tissue vitality, respectively, making up a total of 79.2%. At the end of the treatment, 64% of the subjects showed an improvement in tissue viability ($p<0.0001$).

Moreover, the mean percentage of devitalized tissue (Table IX) dropped significantly from 62.4% at the baseline to 38.4% at 15 days ($p<0.0001$) and 26.0% at the last examination ($p<0.0001$). An effect on ulcer size (Table X) was also detected, with a reduction in 33.7% of patients at the end of the treatment period ($p<0.0001$).

Wound odor (Table XI) also progressively decreased, with 29.1% and 33.7% of subjects showing an improvement at 15 days ($p<0.0001$) and the end of the study ($p<0.0001$), respectively. No significant correlations were found between changes in tissue viability or wound odor and ulcer characteristics (size, site, and type).

Effect on Quality of Life

The effect of LMW-HA-collagenase treatment on patients' quality of life was evaluated with the EQ-5D questionnaire, which was filled in by patients at the baseline and at the final visit (Table XII). All items showed a trend towards amelioration, but only the item "pain/discomfort" improved significantly ($p<0.0001$). Assessment of the quality of life at follow-up based on the score (Table XIII) calculated by the Time Trade-Off method showed a slight but statistically significant improvement in 49.2% of patients ($p=0.0097$).

Finally, a significant enhancement in the health status, assessed by the VAS scale, was reported for 59.6% of patients ($p=0.0005$) (Table XIV).

Table XIII. EQ-5D questionnaire scores.

	Baseline	Changing at 30 days	Variation
N	61	61	61
Average	0.52	0.60	0.08
Dev. Std	0.34	0.36	0.22
Maximum	1.00	1.00	0.53
3rd quartile	0.73	0.85	0.20
Median	0.62	0.73	0.00
1st quartile	0.52	0.52	0.00
Minimum	-0.43	-0.33	-0.57
	***t*-test**		***p*=0.0097**
Improving	30	49.2	
Not changing	23	37.7	
Worsening	8	13.1	

Table XIV. Change in Visual Analogue Scale (VAS) pain from baseline at 30 days.

	Baseline	Changing at 30 days	Variation
N	57	57	57
Average	49.4	60.3	10.9
Dev. Std	29.4	30.8	28.1
Maximum	100.0	100.0	81.0
3rd quartile	70.0	90.0	25.0
Median	50.0	70.0	5.0
1st quartile	30.0	40.0	0.0
Minimum	0.0	5.0	-72.0
	***t*-test**		***p*=0.0005**
Improving	34	59.6	
Not changing	11	19.3	
Worsening	12	21.1	

Discussion

The results of this multicenter, open-label, uncontrolled clinical investigation revealed that the daily application of Bionect Start® in patients affected by skin ulcers of different etiology was safe and well tolerated despite the reduction of the sample size population. The treatment was also effective in improving the wound bed status, reducing the percentage of nonviable tissue and wound odor. Finally, a beneficial effect on the patients' quality of life was observed. These results confirm those obtained in previous studies[11] that demonstrated the safety and efficacy of Bionect Start® in the management of chronic venous ulcers. In a preliminary pilot study, Gravante et al[11] showed that the topical application of Bionect Start® significantly increased the percentage of patients who experienced a reduction in both the necrotic component and the total ulcer area.

Likewise, the results of a randomized controlled clinical study[12] demonstrated the efficacy of the collagenase/hyaluronic acid combination over placebo for the treatment of chronic venous ulcers: patients treated with Bionect Start® in addition to compression therapy achieved significantly higher debridement rates than patients receiving compression therapy alone.

In our study, Bionect Start® was used for the treatment of patients suffering from ulcers of different etiology, including traumatic, venous, arterial, pressure, and diabetic ulcers. All these lesions share the characteristic of being "hard-to-heal" wounds. In this situation, all the physiologic processes that usually lead to wound healing are delayed. Therefore, a two-fold approach that removes infected and non-viable tissue from the wound bed while simultaneously stimulating mechanisms involved in tissue regeneration can be crucial for promoting wound healing. This approach would allow healthy tissue to be exposed, which in turn would facilitate the regeneration process. One of the most widely used methods to achieve wound bed cleansing is the employment of enzymes, and collagenase is often the preferred choice due to its high selectivity, possibility of prolonged application, and lack of pain after treatment. However, some adverse effects are described in the literature. In particular, the onset of contact dermatitis following application of clostridiopeptidase has been reported[13]. This bacterial collagenase originates from a notably virulent strain of *Clostridium histolyticum*. Despite its frequent usage, preparations containing clostridiopeptidase exhibit diminished selectivity. Non-specific proteases found as impurities in *C. histolyticum* collagenase preparations are accountable for breaking down non-collagenous elements of the extracellular matrix, such as decorin or fibronectin[14,15]. Otherwise, the enzymatic component of Bionect Start® is a bacterial collagenase derived from a non-pathogenic bacterial strain, *Vibrio alginolyticus*. This highly pure (>98%) collagenase does not contain non-specific proteases and displays the maximum of its activity at alkaline pH values that are characteristic

of chronic wounds (8.0-9.5), while it loses its activity in the mild acidic range (pH 5.6-6.0), which are typical of normal healthy skin and spontaneously healing, or acute wounds[16]. Unlike *C. histolyticum* collagenase, these two characteristics, the high selectivity for collagen degradation and the specificity of action on necrotic tissues, make the *V. alginolyticus*-derived enzyme less aggressive on healthy skin[17].

The results of our study showed that Bionect Start® is safe and very well tolerated: no treatment-related adverse events were detected after daily application of the device for up to 30 days, while progressive and significant reduction in perilesional irritation, burning and pain at dressing change were evidenced.

Hyaluronic acid has been known to play an important role in wound healing process. The effect of exogenous HA has a well-known beneficial effect on healing wounds. Due to his role in water homeostasis, it favours tissue hydration, which is of primary importance for helping and accelerating healing process.

However, *in vitro* and *in vivo* studies suggest that HA promotes cell proliferation and migration, and the molecular weight of exogenously administered HA has an effect on the wound healing process. Kawano et al[18] examined the effect of HA addition in full-thickness wound model in mice and the gene expression related to wound healing. They found that the proliferation and migration of HaCaT cells increased with the increase of MW and concentration of HA.

HA may also contribute to improved tolerability: thanks to its protective action on the peri-wound skin, it may help prevent irritation and discomfort[11]. Among the symptoms analyzed to assess local irritation, itching displayed a seesawing trend: after 15 days of treatment, the same number of patients reported a decrease (n=19) and an increase (n=20) in itching perception, and only at the end of the treatment an improvement in discomfort was achieved in 25% of cases. A possible explanation for this fluctuation may be related to different patient sensitivities or the onset of itchiness associated with the healing process.

As a secondary outcome, our study also emphasized the efficacy of combined collagenase/hyaluronic acid in the treatment of skin ulcers of diverse etiology. Complete wound debridement was achieved already at the first control visit by 18.8% of patients, and the percentage increased to 40.6% at the end of treatment. These results are in accordance with those presented in the retrospective study on 70 patients with chronic wounds of different etiologies by De Francesco et al[10].

A significant effect on the size of the lesions was also observed; it should be noted that this effect is probably underestimated due to the high percentage of small ulcers in the population. Finally, a trend towards improvement in the quality of life and health status of patients was highlighted. Although all items on the EQ-5D questionnaire showed improvement at the final evaluation, only pain/discomfort changed significantly; the age and general status of the sample population may have contributed to limiting patients' perception of a better quality of life.

Limitations

A limitation of the present study may be the lack of a long-term follow-up, which would allow evaluation of the safety and tolerability of Bionect Start® in case of prolonged treatments. Moreover, the observed variability in patients' response to the perceived itching could not be addressed in the present study. Either a larger cohort or a more targeted study would be required to shed light on this particular issue.

Though the present study opens the possibility of using Bionect Start® collagenase/hyaluronic acid for more diverse etiologies of cutaneous ulcers, it could not address a differential treatment protocol for each. Further studies will be required to establish better, tailored treatments for the different types of skin ulcers, improving from the current "one size fits all" approach.

Conclusions

Bionect Start® a combination of hyaluronic acid sodium salt (0.2% w/w) and bacterial collagenase derived from *Vibrio alginolyticus* (>2.0 nkat/kg), was safe and well-tolerated, significantly reducing pain and perilesional skin irritation. It was also effective in removing necrotic tissue, significantly supporting wound bed preparation. Thus, the results of this study confirm previously obtained data on chronic venous ulcers and endorse the use of this topical device for the treatment of chronic lesions of diverse origins, including traumatic, pressure, diabetic, and mixed-etiology ulcers.

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Conflict of Interest
The authors P. Fino, C. Chello, C. Latini, S. Occhionorelli, N. Scuderi, and G. Pellacani declare that they have no potential conflict of interest. The author M. Moruzzi is a clinical project manager employed by Fidia Farmaceutici, the sponsor of the study.

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Availability of Data and Materials
Data used in this study are collected in a specific database and available upon request to the corresponding author.

Ethics Approval
This clinical study was approved by the Sapienza Ethics Committee (N. of Protocol. 1082/13) and by all local Ethics Committees of the 16 participating centers: Centro Azienda Ospedaliera S.S. Antonio e Biagio e C. Arrigo di Alessandria Prot. ASO.ChirP.13.01 del CE 10/12/2013. Comitato Etico Lazio 2 Prot. CE/77838 del 19/12/20213 Centro Ospedale S. Eugenio. Comitato Etico Lazio 2 Prot. CE/77837 del 19/12/20213 S. Giovanni Addolorata Roma. Comitato Etico Lazio 2 Prot. CE/77840 del 19/12/20213 Ospedale Isrealitico. CE CEIIAV Meldola Prot. 1100/2013 I.5/238, seduta del 19/02/2014. Roma 14 Novembre 2013 Prot. n. 575/2013 CE Lazio 1. Prot. 4/2014 4 Febbraio 2014 CE Lazio l. Prot. 1656 - 27/01/2014 Azienda Ospedaliera Alessandria Regione Piemonte. Prot. 1176/CE CE-BIF 23 dicembre 2013 Centro Bologna. Prot. 444/CE 05/03/2014, seduta del 26/02/2014 Comitato Etico Indipendente AO U Policlinico consorziale Bari. Prot. R39-CCM 44, seduta del 03/02/2014 Comitato Etico degli IRCCS Ilstltuto Europeo di Oncologla e Centro Cardiologlco Monzíno. Prot. 1176/CE, 23/12/2013, Comitato Etico Interaziendale Bologna-Imola-Ferrara CE-BIF. Prot. 5A/CESC, seduta del 25/3/2014, Comitato Etico Per La Sperimentazione Clinica Della Provincia Di Venezia e IRCCS San Camillo (CESC). Comitato Etico della Provincia di Bergamo, con sede presso Azienda Ospedaliera Papa Giovanni XXIII. Prot. HQC6-13-01 21/02/2014, Registro sperimentazioni 162/13, Comitato Etico Indipendente Fondazione PTV Policlinico Tor Vergata.

Informed Consent
Each subject signed a written informed consent for inclusion before participating in the study.

Authors' Contributions
P. Fino and N. Scuderi designed the study. C. Latini, S. Occhionorelli, actively participated to the clinical investigation. M. Moruzzi and G. Pellacani analyzed and interpreted data. P. Fino and C. Chello wrote the manuscript. Each author participated sufficiently in the work to take public responsibility for the content. All authors contributed to drafting the work and revised and approved the final version of the manuscript.

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