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Maltodextrin/ascorbic acid stimulates wound closure by increasing collagen turnover and TGF-β1 expression *in vitro* and changing the stage of inflammation from chronic to acute *in vivo*

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ABSTRACT

It has been reported that carbohydrates confer physicochemical properties to the wound environment that improves tissue repair. We evaluated *in vitro* and *in vivo* wound healing during maltodextrin/ascorbic acid treatment. In a fibroblast monolayer scratch assay, we demonstrated that maltodextrin/ascorbic acid stimulated monolayer repair by increasing collagen turnover coordinately with TGF-β1 expression (rising TGF-β1 and MMP-1 expression, as well as gelatinase activity, while TIMP-1 was diminished), similar to *in vivo* trends. On the other hand, we observed that venous leg ulcers treated with maltodextrin/ascorbic acid diminished microorganism population and improved wound repair during a 12 week period. When maltodextrin/ascorbic acid treatment was compared with zinc oxide, almost four fold wound closure was evidenced. Tissue architecture and granulation were improved after the carbohydrate treatment also, since patients that received maltodextrin/ascorbic acid showed lower type I collagen fiber levels and increased extracellular alkaline phosphatase activity and blood vessels than those treated with zinc oxide. We hypothesize that maltodextrin/ascorbic acid treatment stimulated tissue repair of chronic wounds by changing the stage of inflammation and modifying collagen turnover directly through fibroblast response.

1. Introduction

Mono and polysaccharides contained in sugar and honey have been used for the treatment of acute and chronic wounds for millennia. Polysaccharide treatment for venous leg ulcers exerts a local osmotic effect by reducing edema [1], in consequence gas exchange improves and the characteristic hypoxia of the ulcer tissue diminishes [2,3]. Sugar based dressings also help prevent and/or control tissue infection frequently found in venous leg ulcers. The mechanism for microbial control is attributed to a decrease in wound pH and lowering water availability, both of which contributes to bacterial lysis [1]. Due to these benefits, polysaccharides have seen increased use in wound care; however research is limited primarily to honey [4,5] despite similar benefits existing for other polysaccharides, in particular maltodextrins [1,6–8].

Maltodextrin represents a polysaccharide that has been characterized for wound healing applications [7,8]. Maltodextrin is a D-glucose polysaccharide obtained by starch hydrolysis with an average molecular weight of 3 kDa that is composed of small quantities of glucose and maltose. In clinical studies, topical application of a maltodextrin/ascorbic acid to wounds and ulcers has shown to form a protective cover that regulates exudate and the invasion of microorganisms [7,8]. The moist wound environment established by maltodextrin promotes tissue granulation and

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with the ascorbic acid, the maltodextrin/ascorbic acid low pH exhibits a bacteriostatic activity [7]. Due to its gradual metabolism, maltodextrin can release some glucose to the wound environment providing topical nutrition [6]. Maltodextrin was shown to have a chemotactic effect on polymorphonuclear leukocytes *in vitro*; this finding explains in part, the mechanism of action for wound healing [7]. Administration of the maltodextrin/ascorbic acid dressing is simple due to its powder or gel formulation that can be applied daily to the wound by the patient with no discomfort. Despite these documented benefits and commercialization of maltodextrin as a wound dressing [6–8], very little scientific or clinical information has been provided. For this reason, we have investigated the wound healing mechanisms of maltodextrin effects in an *in vitro* wound healing model and evaluated the efficacy of maltodextrin clinically for treatment of venous leg ulcers in humans.

2. Materials and methods

2.1. In-vitro "wound healing" assay of fibroblast cultures exposed to maltodextrin/ascorbic acid

Human adult fibroblasts cultures were maintained in Dulbecco's Modified Eagle's Media (D-MEM; Gibco; Invitrogen, California, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 2 mM L-glutamine (Gibco), and 50 μg/mL gentamycin (Gibco) in an incubator at 37 °C with 5% CO_2. Cells on 4th passage were cultured on 96 well cell culture plates (Falcon, Becton Dickinson Labware, New Jersey, USA) at densities of 5×10^4 cells/cm^2 and they were incubated for 48 h to obtain confluent cultures. Then, cultures were synchronized in G_0 stage by maintaining them during 18 h with D-MEM, 1% FBS, 2 mM L-glutamine and antibiotic. Afterwards, we performed a single scratch in the middle of the culture, by means of a syringe needle (27G), enough to obtain a linear side-to-side damage [9].

Cultures were treated until they close with 1% of maltodextrin/ascorbic acid (Multidex® Powder, DeRoyal Inc. Tennessee, USA) in D-MEM supplemented with 1% FBS, 2 mM L-glutamine and antibiotic. D-MEM supplemented with 1% FBS or 10% FBS; both, in the presence of 2 mM L-glutamine and antibiotic were considered negative and positive controls, respectively. At 0, 8, 12, 24 and 30 h, images were acquired under a microscope (Axio Observer Z1; Carl Zeiss, Göttingen, Germany) fitted with a monochromatic high-speed camera (AxioCam camera Carl Zeiss).

Planimetry was performed by digital image analysis by assessing damaged area using AxioVision digital image processing software (AxioVision 4.8.1.0, Carl Zeiss). Each experiment was performed by triplicate, and data shown are the average from two different experiments.

2.2. Collagen turnover and transforming growth factor (TGF)-β1 expression in fibroblast damaged cultures exposed to maltodextrin/ascorbic acid

Following the previously mentioned model of damaged fibroblast culture, but in 24 well cell culture plates, we obtained supernatants from culture media at 12 and 24 h; control cultures were incubated in D-MEM supplemented with 10% FBS, 2 mM L-glutamine and antibiotic. Metalloproteinase (MMP)-1, tissue inhibitor of metalloproteinases (TIMP)-1 and TGF-β1 were assessed by ELISA (MMP-1and TIMP-1 from Amersham Biosciences. New Jersey, USA and TGF-β1 from R&D Systems, Minnesota, USA). In an independent experiment, homogenates from monolayer and supernatants of fibroblast scratched cultures after 12 and 24 h, were used for gelatinase activity assays [10]. Semiquantitative densitometric analysis was performed to zymograms with Quantity One 4.6.3 software (Bio-Rad Laboratories, Inc., California, USA).

2.3. Human chronic wounds treated with maltodextrin/ascorbic acid

We performed a bi-institutional, open, prospective, longitudinal, experimental, pilot study comparing the efficacy of maltodextrin/ascorbic acid treatment of venous ulcers of the lower limbs *vs.* zinc oxide. All patients were appropriately informed about their venous disease and the purpose and procedures of the study. All participants completed a consent form included in the trial document approved by Research and Ethics committees of the Centro Médico Nacional (C.M.N.) "20 de Noviembre", I.S.S.S.T.E of Mexico (Number 014/04).

The trial was performed by recruiting 21 patients (16 females and 5 males) from a pool of 230 individuals registered in the Wound Care Clinics, C.M.N. "20 de Noviembre" and Hospital Regional "General Ignacio Zaragoza", I.S.S.S.T.E. Mexico City, Mexico (see the consort chart). Only patients with venous ulcers, $C_6E_pA_sP_{r2\text{-}5}$ [11], normal ankle-brachial index ($\geq$1.0 but <1.4) and with lesion areas less than 25 cm^2 were included; all of subjects had a history of active lower limb ulcers $\geq$12 months, and the lack of complete healing despite previous nonsurgical treatment. None of the wounds had been treated within the last 2 months at the time of admission, except for daily washing. Patients with systemic infection, ischemia, deep vein thrombosis, heart failure or diabetes were excluded. Furthermore, none of the female participants in the study were pregnant or became pregnant during the treatment and evaluation period.

Patients were allocated by simple random sampling to the maltodextrin/ascorbic acid and zinc oxide groups by the staff of every wound care clinic. Treatment consisted of daily topical application for 12 weeks of maltodextrin/ascorbic acid powder or zinc oxide ointment (Industria Farmacéutica Andrómaco, Mexico City, Mexico). Then, wounds were covered with sterile gauze; in all cases the gauze was immobilized with a noncompressive bandage to prevent contamination. The daily treatment regimens were carried out by the patients themselves or with the aid of someone in their homes.

All patients were treated with diosmin/hesperidin (1 g/d, orally; Daflon® Laboratorios Sanfer, Mexico City, Mexico). Pain was treated with 500 mg of paracetamol up to 3 times per day as needed. Patients were recommended to engage regularly in moderate exercise and to raise legs periodically. Patients were checked every 4 weeks for follow-up evaluation of their healing progress.

2.4. Evaluation of wound closure and wound imaging

At the start of the study, 4, 8 and 12 weeks of treatment, the evolution of each patient's lesion was assessed. The area was measured by planimetry and all of the wounds were photographed and digitally processed to capture every lesion relief with the PhotoStudio 2000 software (ArcSoft, Inc.Fremont, California, USA) in order to distinguish the depth of each ulcer.

2.5. Bacteriological analysis

At the start of the study and at week 8, all wounds were swabbed to characterize the microorganisms colonized in each wound. This information was used to assess the hygiene of the patient and the microorganisms influence on the inflammatory response.

2.6. Collection and morphologic assessment of the biopsies

At week 0 and 8, full thickness biopsies (6 mm in diameter) were obtained from the middle of the ulcer and the tissue was divided

into two equivalent portions. The section to be used for evaluation of extracellular alkaline phosphatase (ALP) activity and proportions of type I and type III collagens, was frozen under liquid nitrogen and stored at −70 °C. The other tissue section was fixed in neutral formalin and used for the histologic and histochemical assays. Data were obtained from 8 of 11 patients and 7 of 9 patients in the maltodextrin/ascorbic acid and zinc oxide groups, respectively.

2.7. Histology and histochemistry analysis

Series of 6-μm and 10-μm thick sections were obtained from the frozen tissue samples with a cryostat and mounted directly onto slides. The 6-μm sections were stained by Herovici's method to identify proportions of type I and type III collagens [12] (type I and type III collagen fibers stain magenta and blue, respectively). Alkaline phosphatase activity was determined morphologically in 10-μm sections by *in situ* tetrazolium nitroblue/5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine (NBT/BCIP) enzymatic staining [13].

Cellularity and blood vessel quantification were assessed from 5-μm thick sections obtained from tissues fixed in neutral formalin and embedded in paraffin by staining with hematoxylin-eosin technique and by treatment with biotinylated lectin, *Griffonia simplicifolia* isotype B4 (GSL-IsoB4, Vector, California, USA). For lectin staining, endogenous peroxidase activity was blocked by treating the tissue with hydrogen peroxide/methanol (9:1) for 15 min. Subsequently, the tissue was held in lectin solution (1:100 in PBS) at room temperature for 1 h. The lectin-endothelial cell association was demonstrated by the avidin-biotin-peroxidase system which revealed aminoethylcarbazole labeling as a red colored precipitate.

Morphological evaluation was based on descriptive parameters and was expressed by the percentage of the patients with changes on each specific marker (type I and III collagens, ALP, fibroblasts, lymphocytes, neutrophils and blood vessels).

2.8. In-situ biochemical assay for alkaline phosphatase

Ex vivo ALP activity was assessed by a colorimetric method designed in our laboratory, in which reduction of 3-(4,5-dimethyltriazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by BCIP and tissue ALP generates a purple colored precipitate that was dissolved in absolute ethanol and quantified at $\lambda = 565$ nm. The quantity of extracted coloring was normalized with the amount of protein contained in the tissue where the reaction took place [14].

2.9. Statistics

For monolayer scratch assay, closure between controls *vs.* maltodextrin/ascorbic acid treatment was compared by Mann-Whitney test. MMP-1, TIMP-1 and TGF-β1 expression (intragroup 12 h *vs.* 24 h) were compared using Unpaired t test. Clinical, morphological and biochemical assessments at the beginning and after 12 weeks of treatment with maltodextrin/ascorbic acid or zinc oxide were compared according to Mann-Whitney test. p values ≤ 0.05 were considered significantly different.

3. Results

3.1. Scratch wound assay revealed that fibroblasts respond to maltodextrin/ascorbic acid stimulation by increasing the growth and collagen turnover

Since fibroblasts are some of the most important effector cells during wound repair, we evaluated fibroblast culture monolayer wound closure. After monolayer scratch, growth and reorganization of fibroblast depend on the environment. Cells require cytokines and a matrix that supports their movement; specifically, collagen turnover is a key condition to promote wound repair. After 24 h of culture, 1% of maltodextrin/ascorbic acid stimulated 20% more efficient monolayer repair than culture medium (negative control) ($p = 0.028$), but there was no difference when compared *vs.* D-MEM 10% FBS (positive control. Fig. 1a). Considering that fibroblast cultures were G_0 synchronized, this result indicates that maltodextrin/ascorbic acid promotes cell growth similar to optimal culture conditions. It is well known that *in vitro*, cells require growth factors and many other humoral elements present in the fetal serum for their appropriate metabolism, including proliferation [6,15]. Interestingly, when we compared by an intragroup analysis the fibroblastic behavior between 12 *vs.* 24 h, it was observed an improvement in wound closure after maltodextrin/ascorbic acid treatment and controls ($p = 0.009$ and 0.004, respectively. Fig. 1a). Nevertheless, TGF-β1 and MMP-1 expression only increased in a statistical significant fashion when scratched cultures were treated with maltodextrin/ascorbic acid ($p = 0.006$ and 0.017, respectively. Fig. 1 b and c), meanwhile TIMP-1 levels decreased ($p = 0.056$. Fig. 1d).

Zymographic analysis demonstrated that after 12 h, fibroblast cultures treated with maltodextrin/ascorbic acid expressed gelatinases with molecular weights between 55 and 97 kDa (55, 70 and 97 kDa) when compared to cultures treated with D-MEM 10% FBS (55 and 70 kDa), indicating that maltodextrin/ascorbic acid expressed an early broader spectrum of enzymes related to collagen turnover compared to D-MEM 10% FBS (Fig. 2a). After 24 h in both treatments, gelatinolytic activity increases were still detected but only for those corresponding to 55 and 70 kDa, cultures treated with maltodextrin/ascorbic acid expressed almost twice the activity than the control condition (Fig. 2b). This data is well associated to those from MMP-1 expression assessed by ELISA (Fig. 1c), suggesting that collagen turnover reaches a maximum when total closure has been achieved.

3.2. Maltodextrin/ascorbic acid controlled the growth of most of colonizing microorganisms hosted in the ulcer and improved chronic wound repair

Twenty one patients were recruited and followed for 12 weeks. At the beginning of the study, all patients were $C_{6s}E_pA_sP_{r4.}$ None of the patients manifested signs or symptoms of infection, though 80% of the ulcers hosted bacterial colonies of one or more microorganisms; some of which are commonly pathogenic and related to fecal contamination (see Table 1).

Demographic data, as well as the locations of the lesions and colonized microorganisms are presented in Table 1, and there were no significant differences between the two groups at the beginning of the study. After 8 weeks of treatment, only *Staphylococcus aureus* was retained in some ulcers of both groups. One patient from the zinc oxide group was withdrawn from the study due to rheumatoid arthritis diagnosis during the study.

Lesion assessments showed wound improvement in both groups until week 8, 41.6% ± 37.9 and 27.6% ± 41.1 (average ± standard deviation), maltodextrin/ascorbic acid and zinc oxide group, respectively. At week 12, only maltodextrin/ascorbic acid group maintained continued healing resulting in 57.4% ± 39.9 (average ± standard deviation) wound closure *vs.* 16% ± 41.6 in the zinc oxide group ($p = 0.034$). Maltodextrin/ascorbic acid improved the clinical quality of the granulation tissue after 12 weeks of treatment and this effect was qualitatively evident when compared to that observed in the zinc oxide group (Fig. 3). No adverse events were reported by patients from either group during the treatment period.

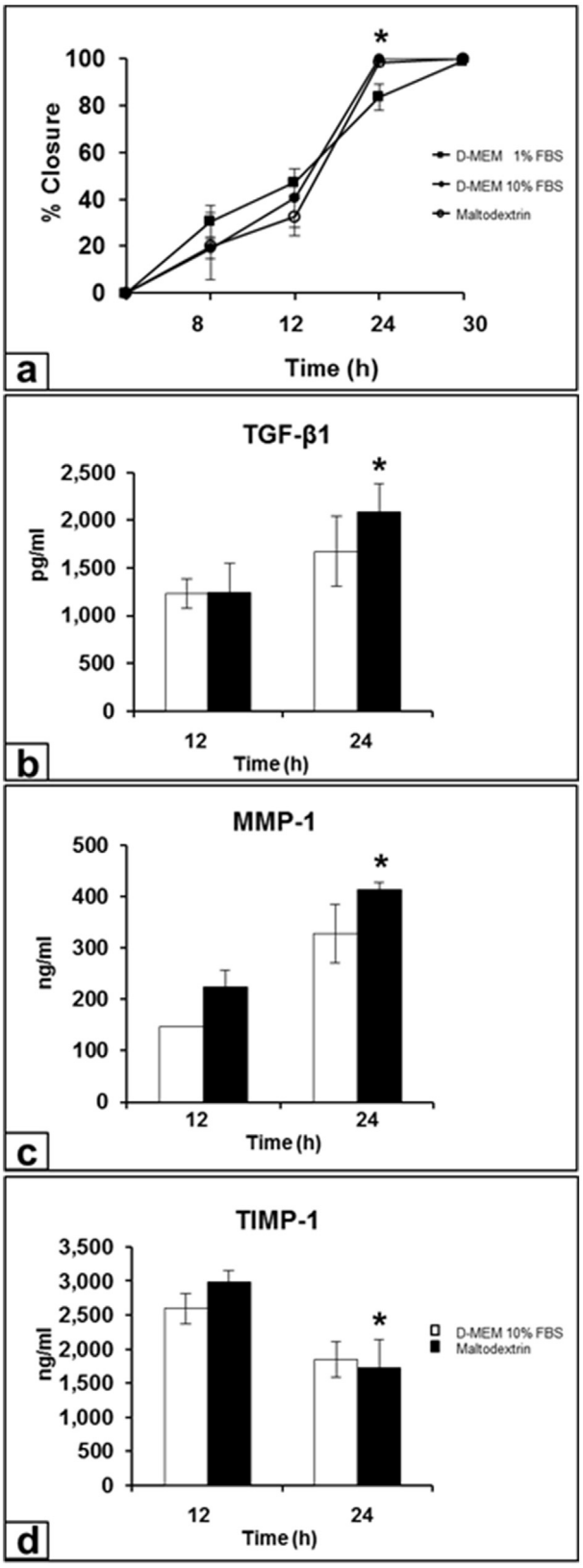

Fig. 1. *Percentage of "wound" closure, TGF-β1, MMP-1 and TIMP-1 expression in fibroblast damaged cultures exposed to maltodextrin/ascorbic acid.* Fibroblast cultures were scratched and monolayer closure was assessed by planimetry. Maltodextrin/ascorbic acid (open circles), D-MEM, 1% FBS (squares) and D-MEM, 10% FBS (filled circles). Difference between maltodextrin/ascorbic acid and D-MEM 1% FBS after 24 h of scratching was statically significant (*p = 0.049, Mann-Whitney test), data represent mean and error bars standard error of the mean (a). TGF-β1 (b), MMP-1 (c) and TIMP-1 (d) expressions were assessed by ELISA. Calculation of differences in protein expression was performed according Unpaired t-test. After 24 h, maltodextrin/ascorbic acid treated cultures increased TGF-β1 and MMP-1 levels, meanwhile decreased TIMP-1. *Statistical significant differences were observed when intragroup cultures from 12 h to 24 h were compared (TGF-β1, p = 0.006; MMP-1, p = 0.017; TIMP-1, p = 0.056), data represent mean and error bars standard deviation.

3.3. Morphological analysis

Blind morphological evaluation was performed in all tissue samples, where cellularity, type I and type III collagen fiber proportions in tissue sections were analyzed by hematoxylin-eosin and Herovici's picropolychromic technique, respectively. All the samples, at the beginning and after 8 weeks of treatment exhibited abundant collagen fibers. Fibroblasts, lymphocytes and neutrophils were the predominant cells in the wound, meanwhile hemosiderin deposits, as well as hemosiderophages were also present; however, no significant changes related to these data were observed when before-after treatment intragroup and intergroup were performed.

Increased presence of type III collagen fibers is particularly important during wound repair, because this type of collagen is related to the formation of granulation tissue that can mediate lesion repair. After 8 weeks of treatment, type III collagen fiber levels were increased in 44.4% and 42.9% of patients in maltodextrin/ascorbic acid and zinc oxide treated groups, respectively. Meanwhile, type I collagen fiber levels had increased by 55.6% and 71.4% in maltodextrin/ascorbic acid *vs.* zinc oxide-treated patients (Fig. 4a–d). This finding demonstrates a more fibrotic pattern in zinc oxide group, where dense packed type I collagen fibers were spread in the whole tissue, compared to the maltodextrin/ascorbic acid group.

In situ extracellular ALP activity in the biopsied tissue samples was evaluated at weeks 0 and 8 of treatment. We reported herein qualitatively and quantitatively ALP activity since the enzyme plays a role associated to inflammation [16–18].The results indicated that extracellular ALP activity had increased from week 0 to week 8 in 55.6% of the patients treated with maltodextrin/ascorbic acid meanwhile it was diminished in 71.4% of the patients treated with zinc oxide (For morphological reference see Fig. 4e–h). Blood perfusion is essential during wound repair. Important treatment group differences in vasculature were observed at the 8 week time point. Vessels increased in the 71.4% of the patients treated with maltodextrin/ascorbic acid, and only in the 40% of the zinc oxide group (Fig. 4i–l).

The clinical, morphological and biochemical results showed no significant differences within-patient correlations between lesion closure and the non-parametric assessments. However, we did observe distinctive trends between the maltodextrin/ascorbic acid and zinc oxide groups in extracellular ALP activity (55.6% *vs.* 28.6%, respectively) and blood vessels (71.4% *vs*. 40.0%).

4. Discussion

Wound repair is a multifactorial process involving cells, extracellular matrix and humoral responses. From the plethora of substances and devices that stimulate wound healing, polysaccharides are some of the most ancient and popular materials, frequently administered from natural extracts, where the mechanism of action cannot be attributed to a particular molecule [1]. On the other hand, maltodextrins are highly specific carbohydrates with an interesting potential to improve wound healing [7]. In this work we assessed maltodextrin/ascorbic acid in a preclinical *in vitro* wound healing model, as well as *in vivo* in venous leg ulcers. *In vitro* we found that cultures treated with maltodextrin/ascorbic acid mimics the *in vivo*

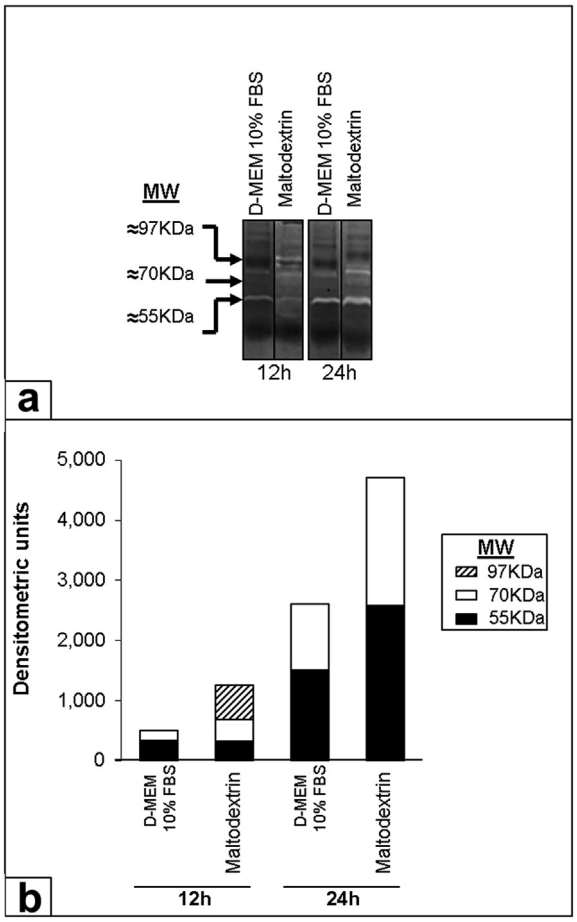

Fig. 2. *Zymographic analysis of gelatinolytic activity in fibroblast damaged cultures exposed to maltodextrin/ascorbic acid.* Gelatinase activity was evidenced by zymography performed on polyacrylamide gel electrophoresis, a representative image from two separate experiments (a) and its densitometric analysis (b). MW = molecular weight, kDa = kilo daltons.

wound repair behavior related to MMP-1 and TIMP-1 expression, which are well known to be regulated by TGF-β1 [19]. Additionally, gelatinolytic activities, specifically 55 and 70 kDa, increased after 24 h, following repair of the fibroblast monolayer, suggesting that maltodextrin/ascorbic acid stimulates fibroblast metabolism in order to promote collagen turnover (collagenase and gelatinases). *In vivo* evaluation demonstrated that patients treated with the maltodextrin/ascorbic acid dressing experienced 3.7 times wound healing at 12 weeks with improvements in tissue morphology and cellular biomarkers compared to control treatment. These findings support the general efficacy of maltodextrin as a viable option for the treatment of chronic wounds.

Although the scratch assay model used in this study is a limited two dimension wound bed model, this model has been employed by other researchers to help describe cellular mechanisms and processes associated with wound healing observed *in vivo* [20]. The use of the *in vitro* scratch assay allowed us to relate the qualitative morphological changes observed *in vivo* to quantifiable changes for specific biomarkers in a simplified wound environment. The collagen turnover observed in fibroblast culture scratch assays is related, at least in part, to the architectural changes observed in venous leg ulcers treated with maltodextrin/ascorbic acid, where an important remodeling of the stroma was observed (Fig. 4a–d). Further we observed an up-regulation of tissue ALP in patients treated with maltodextrin/ascorbic acid. Other studies have indicated that fibroblasts are an important source of ALP [21,22] and that ALP is associated with improved wound healing [17]. This marker exhibits differential expression during the acute scar formation process, and correlates with collagen levels [23]. Initially, we hypothesized that maltodextrin/ascorbic acid-induced healing effect could be associated to the enzyme activity derived from neutrophils and other cell types [24,25], nevertheless we were unable to ascertain this hypothesis because neutrophil/ALP proportions were not clearly related. A possible explanation is that the second biopsy was taken too late to demonstrate wound healing induction (8 weeks after treatment) or because it is known that chronic venous ulcer healing is multifactorial, since many systemic and local factors play roles in wound repair.

Our findings are in line with these published reports and our study provides additional evidence to support ALP's role in wound healing. Combined analysis *in vitro* and *in vivo* allowed us to conclude that the maltodextrin/ascorbic treatment was beneficial for wound healing.

Microbial contamination of ulcers is common and the cohort of microorganisms on the lesion surface is a key indicator of the patient's living conditions, hygiene habits, and tendency for infection [26]. Therefore, the use of topical therapies with antiseptics or astringents, such as silver and zinc oxide ointments, can be important in the early stages of treatment [27]. Here, we found that both, maltodextrin/ascorbic acid and zinc oxide treatments were able to

Table 1
Demographic, microbiological and cytokine expression data.

Treatment	n	Age Years (range)	Sex F/M	Evolution time Years (range)	Ulcer location	LDS	Average size (cm^2)		Initial bacterial presence
							Week 0	Week 12	
Maltodextrin/ascorbic acid	11	68.7 (39–90)	7/4	6.1 (1–20)	Medial malleolus. Lateral malleolus.	4/11	6.5 ± 5.0	2.8 ± 1.1	*S. cohnii* *S. aureus* *E. aerogenes* *E. cloacae* *P. aeruginosa* *E. coli* *S. agalactie* *S.epidermidis*
Zinc oxide ointment	9	60.6 (42–77)	7/2	5.3 (1–20)	Medial malleolus. Lateral malleolus. Tibia.	4/9	6.8 ± 5.1	5.7 ± 2.4	*S. aureus* *E. coli* *S.epidermidis* *P. vulgaris* *E. fecalis* *Leuconostoc* sp. *S. simulans*

n = sample size, F = Female, M = Male, LDS = Lipodermatosclerosis, numbers between parentheses means the range and numbers after "±" means standard deviation.

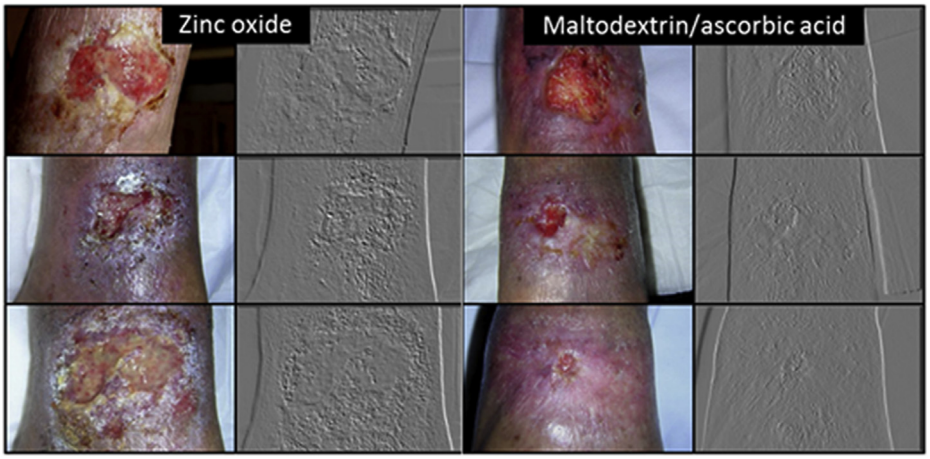

Fig. 3. *Clinical images of two representative patients treated with zinc oxide ointment and maltodextrin/ascorbic acid.* The panels on the left show wounds treated with zinc oxide at weeks 0, 8 and 12 and on the right wounds treated with maltodextrin (inside each panel, left images are photographs of wounds and right images show relief of the ulcer, in which the depth of the wound at each time point can be observed).

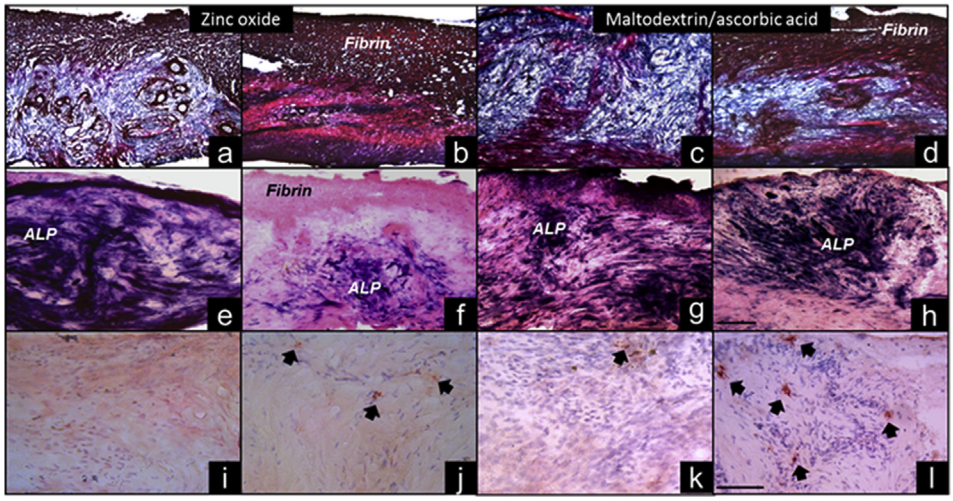

Fig. 4. *Histological images of biopsies from the wound of a patient treated with zinc oxide ointment and a patient treated with maltodextrin/ascorbic acid.* The two panels with figures **a, e, i, c, g, k**, belong to two patients before treatments. The two panels with figures **b, f, j, d, h, l** belong to the same two patients after 8 weeks of treatment with zinc oxide ointment and maltodextrin/ascorbic acid, respectively. **(a–d)** Photomicrographs of Herovici stained sections revealing type I (red) and type III (blue) collagen fibers. **(e–h)** Photomicrographs of extracellular alkaline phosphatase activity (ALP, purple). **(i–l)** Photomicrographs of blood vessels revealed by Isolectin B4 histochemistry (arrows show vessels in red). Bar represents 100 μm; Herovici and ALP stained sections are in the same magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

help control bacterial contamination, which should be associated with improvement of the wound in the majority of cases. This effect is most likely associated with the drying effect of zinc oxide and the pH diminution in exudate, increased osmotic gradient and the formation of a film over the wound in patients treated with maltodextrin/ascorbic acid [1,7]. Differences between the first 8 weeks of treatment for both groups were not statistically significant; however by week 12, wounds treated with maltodextrin/ascorbic acid had significantly improved compared against the zinc oxide group. The initial similar healing rate could be associated to several conditions, 1) daily cleaning of the wound, 2) venotonic treatment, 3) microorganism control, 4) granulation tissue induction by treatments, and 5) psychological considerations that involve attitude and wellness. However, after week 8, there was a dramatic change in the evolution of the healing process between both groups of treatment, only maltodextrin/ascorbic acid treatment group maintained the trend, perhaps closely related with decreasing wound depth (Fig. 3) associated to better tissue architecture.

In conclusion, the present observations indicate that maltodextrin/ascorbic acid treatment favors wound repair *in vitro* and *in vivo*. We hypothesize that maltodextrin/ascorbic acid improves healing by changing the wound environment from chronic to acute, promoting wound healing through stimulating the formation of granulation tissue and consequently epithelialization. At the rate of closure observed with 12 weeks of treatment, it can be projected through linear regression analysis (data not shown), that complete closure should be achieved in all maltodextrin/ascorbic acid-treated patients by week 20 of treatment. In comparison, wound healing in patients treated with zinc oxide wound occur at week 28. Further research is still required to fully establish clinical efficacy of maltodextrin/ascorbic acid treatment as well as research to investigate other wound healing markers (i.e. IL-10, TNF-α, and PDGF-AB) to fully understand the mechanisms of wound healing associated with maltodextrin/ascorbic acid treatment. While further research is still required, the results from this study are the first to clearly relate clinical wound healing seen with maltodextrin/

ascorbic acid to specific established biomechanisms for wound healing.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtv.2017.01.004.

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