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A toolkit for the quantitative evaluation of chronic wounds evolution for early detection of non-healing wounds

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ABSTRACT

Background: Chronic wounds resulting from a number of conditions do not heal properly and can pose serious health problems. Beyond clinician visual inspection, an objective evaluation of the wound is required to assess wound evolution and the effectiveness of therapies.
Aim: Our objective is to provide a methodology for the analysis of wound area vs. time for the early prediction of non-healing wounds evolution.
Methods: We propose a two-step approach consisting of: i) wound area quantification from planimetries and ii) classification of wound healing through the inference of characteristic parameters. For the first step, we describe a user-friendly software (Woundaries) to automatically calculate the wound area and other geometric parameters from hand-traced planimetries. For the second, we use a procedure for the objective classification of wound time evolution and the early assessment of treatment efficacy. The methodology was tested on simulations and retrospectively applied to data from 85 patients to compare the effect of a biological therapy with respect to general basic therapeutics.
Results: Woundaries provides measurements of wound surface equivalent to a validated device. The two-step methodology allows to determine if a wound is healing with high sensitivity, even with limited amount of data. Therefore, it allows the early assessment of the efficacy of a therapy.
Conclusion: The performance of this methodology for the quantification and the objective evaluation of wound area evolution suggest it as a useful toolkit to assist clinicians in the early assessment of the efficacy of treatments, leading to a timely change of therapy.

1. Introduction

The term chronic wound generally refers to an ulcer that does not progress through the normal stages of healing and often stall in the inflammatory phase [1]. Chronic wounds frequently occur in adults with vascular disease or diabetes and are generally classified as vascular ulcers, pressure ulcers (UPP), and diabetic ulcers [2]. Due to the societal and economic burden associated with chronic wounds and their increasing incidence, extensive efforts toward the development of advanced therapies, including plasma-derived products have been deployed [3,4].

In spite of the application of advanced therapies, chronic wounds often do not respond to treatments. In these cases, an early detection of unresponsiveness, followed by wound re-assessment and change of treatment, can reduce the risk of complications and lead to an improved outcome [5]. Usually, the evolution of the wound is only assessed through visual inspection, e.g. by monitoring surface granulation and size. In this scenario, a quantitative characterization of wound geometry as a function of time can help to detect subtle variations before a visually-observable change occurs, leading to a change of therapy at an

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early stage [6]. For this reason, several approaches have been proposed for the measurement of wound size [6–11]. Among them, digital planimetry is considered a reliable and cost-effective method for area measurement, particularly in wounds with irregular shapes [6]. However, its accuracy is affected by the camera lens orientation [7,9] and by the three-dimensional curvature of the wound. When the direct contact with the wound does not constitute a matter of concern, these problems can be simply overcome by manually tracing its outline on a transparent film placed over the wound. The ensuing evaluation of the area, performed by counting the number of squares falling within the outline, makes this method error-prone and tedious [7]. More complex methods, based on 3D reconstruction, volume filling or laser scanners have also been proposed but are not routinely used due their cost or invasiveness [10,12].

Besides the measurement of the wound area, the prediction of its evolution is essential to assist clinicians in timely therapeutic decisions. This prediction can be obtained through the proper modelling of the kinetic of wound area and the inference of parameters that quantify the effect of treatments [13–17]. While the kinetic of wound area has been extensively described and approximated by means of nonlinear models [13–15,17], the number of attempts aimed at using size and shape information to predict wound evolution have been so far rather limited [16]. Recently, methods based on artificial intelligence have shown outstanding results but they still require further validation before being accepted for routine clinical uses [18].

In this work, we describe and validate a simple toolkit for the measurement and quantitative evaluation of wounds evolution. We further apply it to a set of clinical data from patients with chronic wounds treated with different therapies. In addition, we assess its capability for the early detection of unresponsive wounds, with the objective to support professionals in clinical decision making.

2. Methods

2.1. Wound area and shape quantification

At each weekly visit, after wound cleaning, wounds were photographed with a digital camera (Fig. 1A). Thus, a sterile transparent adhesive film with a measurement grid (1 cm pace; OpSite flexigrid, Smith & Nephew) was placed over the wound and the wound margin was directly traced, obtaining the wound planimetry (Fig. 1B). All the clinical procedures used were in accordance with the institutional guidelines and were approved by the ethics committee of the hospital. Patients gave written informed consent.

The wound margin was digitalized through either a scanner or a digital camera and processed using a custom software named Woundaries (freely available on the github repository https://github.com/qubilab/woundaries) written in Matlab (The MathWorks, Inc., Natick, Massachusetts, United States) to calculate the surface area, perimeter, and several other shape descriptors. The software consists in a graphic user interface (Fig. 1C) that allows the upload and the visualization of the digitalized planimetry and the selection of the area of interest. The user can adjust two sliders controlling the values of parameters of the image processing algorithm. The first slider controls a threshold value used to transform the image in a black and white map, and it is aimed at removing the grid texture from the image, while highlighting the wound planimetry. In the case of a not completely closed planimetry, the second slider can be used to join gaps in the wound boundary by an edge linking function [19]. The steps of the image processing algorithm are visualized and updated at each sliders' movement, together with the overlay

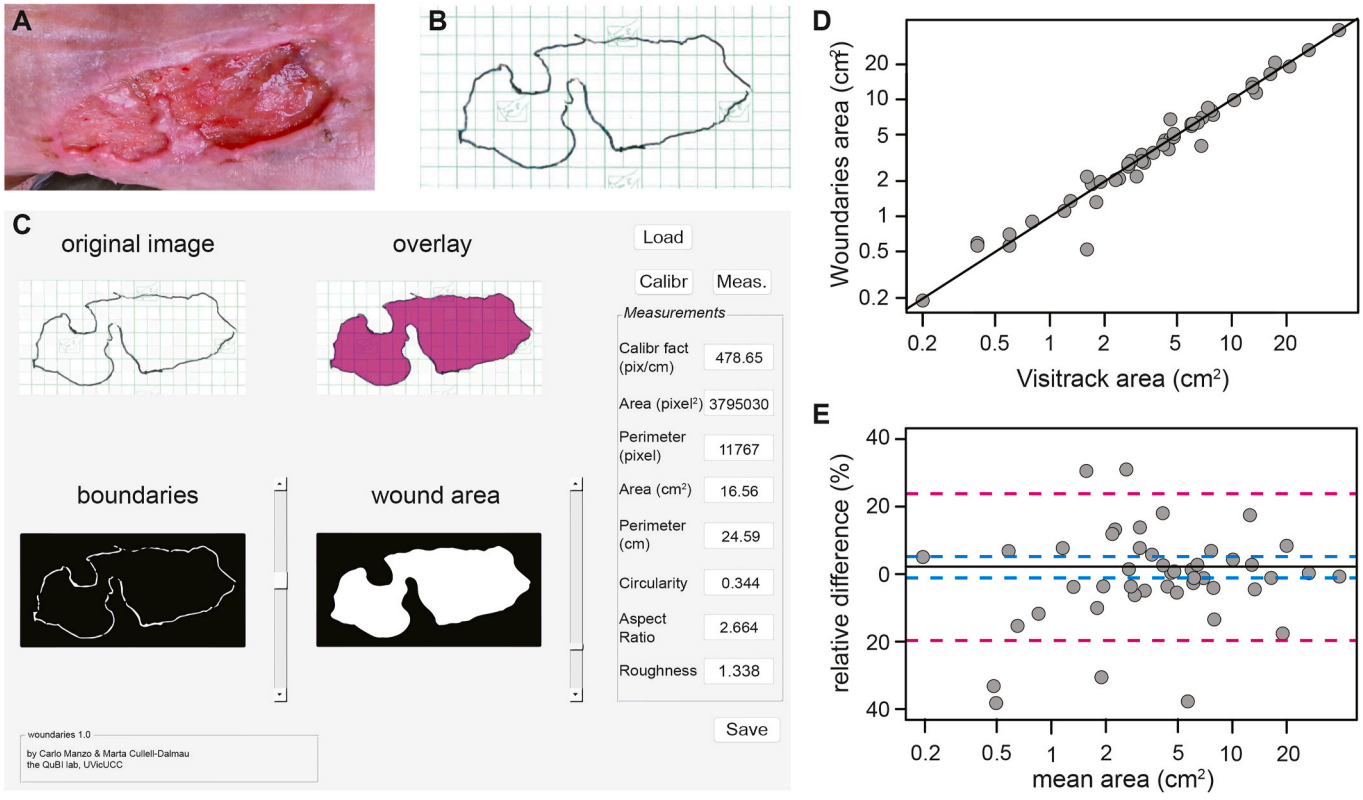

Fig. 1. Schematic and performance of the Woundaries software. (A) A digital photograph of a representative wound and (B) the corresponding planimetry. (C) Screenshot of the Woundaries software during the quantification of the wound represented in panels A–B. (D–E) Comparison of the quantification of 23 representative wounds performed by means of Woundaries and Visitrack. (D) A log-log scatter plot of the data (circles) and a straight line with unitary slope and null intercept, used as a visual reference. (E) A Bland–Altman semilog plot of the percentage of relative difference between the two methods of measurement (circles). Continuous black line corresponds to the mean difference. Dashed blue lines correspond to the standard error of the mean and dashed magenta lines to the standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of the original planimetry with the wound area (Fig. 1C). Once the adjustment of the parameters produces a closed contour faithfully matching the planimetry outline and completely filled (Fig. 1C), then the "Measure" button enables the calculation of wound area, shape and other geometry descriptors. The software allows users to define a calibration area based on the measurement grid for unit conversion. By means of this calibration, the unknown area of a pixel can be calculated and thus the total number of pixels (and their fractions) contained within the contour are converted into area units. All the measurements can be saved in a text file for further analysis.

To validate our method, area measurements obtained for a subset of planimetries with different shapes and sizes were measured with Woundaries (by the same operator) and with a previously-validated device (Visitrak, Smith & Nephew, United Kingdom, measurements were performed by two members of the sanitary staff of the hospital) [7]. The subset was composed by 48 wounds (mean area = 6.32 cm^2, median area = 3.87 cm^2, IQR = 5.05 cm^2, minimum area = 0.19 cm^2, maximum area = 39.17 cm^2). The equivalence of both measurements was assessed through a Passing-Bablock regression, providing results compatible with null intercept (−0.02 cm^2, 95% confidence interval −0.19 to 0.12 cm^2) and unitary slope (1.00, 95% confidence interval 0.96 to 1.04).

The results can be visually confirmed through the scatter plot of the data and a Bland–Altman plot of the percentage of relative difference between the two methods (Fig. 1D–E). The relative difference was calculated as the absolute difference between the two measures divided by their average and multiplied by 100 to be transformed in a percentage. To assess the repeatability and reproducibility of Woundaries, 3 wounds having small (6.54 cm^2), medium (13.38 cm^2), and large size (20.05 cm^2), were repeatedly measured (n = 10) by three different operators. A statistical analysis performed through a Gage R&R ANOVA showed non-significant differences in wound quantification due to different operators (p-value = 0.923) and interactions (p-value = 0.29) and was used to calculate repeatability (standard deviation = 0.27 cm^2, corresponding to 4.1% percent study variation) and reproducibility (standard deviation = 0 cm^2).

2.2. Wound evolution classification routine

We developed a routine for wound classification, based on model selection, parameter inference, and a statistical test. Time series corresponding to at least 3 wound area measurements collected at different times were analysed using a custom routine written in R 3.5.0 [20] as schematically represented in Fig. 2A.

Time series composed by at least 5 measurements were first analysed through a fitting procedure aimed at establishing whether it was possible to identify a delay time, produced by an initial stall or increase in wound area [17]. This analysis is based on the comparison of the results obtained by fitting the data with two models: a first model, consisting in a simple exponential behaviour with 2 free parameters; and a mixed model, composed by an initial linear behaviour followed by an exponential decay, with 4 free parameters in total (Fig. 2B and C). The best model was determined as the one providing the smallest reduced χ^2. In order to avoid overfitting, no attempt was made to calculate an initial delay for time series composed by less than 5 measurements; moreover, the delay parameter was constrained to be smaller than the time at which the $(n-2)^{th}$ data point was collected. In the cases in which the mixed model provided a better fitting, only the data collected at times larger than the calculated delay were considered for the following calculations. For time traces composed by 3 or 4 points, the analysis described above was bypassed, and data were directly fed into the ensuing step. At this point, we performed a linear regression of the logarithm of the area vs. time, $logA = logA_0 - t/\tau$, where A represent the surface area, t is the time at which the area was measured, A_0 is the area at time zero, and τ is a characteristic time. Based on the fitting results obtained for the characteristic time and its 95% confidence interval, wounds were classified as healing if the null hypothesis, corresponding to $\tau \leq 0$, could be rejected. It must be noticed that a negative τ produces an area increasing with time.

2.3. Data simulations

To test the classification/regression routine performance, we simulated data reproducing area vs. time for both healing and non-healing wounds. The traces were composed by a varying number of data

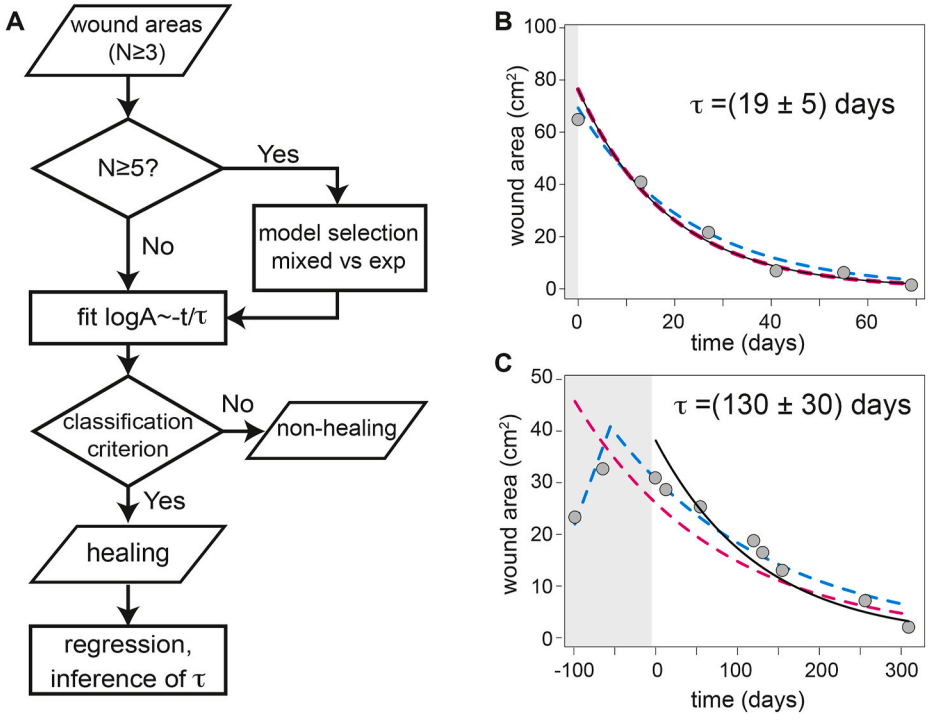

Fig. 2. Schematic and performance of the classification routine. (A) Flow diagram of the procedure used to classify wound as healing or non-healing and calculate the characteristic parameter. (B–C) Representative data sets of wound areas vs. time (circles) and corresponding fits by means of simple exponential (dashed magenta line) and a mixed model (dashed blue line), composed by an initial linear behaviour followed by an exponential decay. The fit comparison was used to determine the best fit and calculate the initial delay. The data in panel (B) were best fitted with a simple exponential and no delay was determined (continuous black line). In contrast, the data in panel (C) were best fitted with a mixed model. Only the data collected at times larger than the delay were considered for further calculations (continuous black line). The characteristic time errors correspond to the 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

points and had a different frequency of collection. To mimic the actual conditions, the logarithm of the area vs. time was simulated as to follow a linear behaviour with different slopes. The data were further corrupted with Gaussian noise with zero mean and standard deviation $\sigma = 0.125$, as estimated from the clinical data (Fig. 3A).

2.4. Methodology application to clinical data

The methodology described above was retrospectively applied to clinical data of patients to evaluate its capability to determine the wound healing kinetics upon different treatments. Patient socio-demographic characteristics, wounds' aetiologies, and wound treatment were collected. Patients were classified between those receiving autologous poor-platelet plasma (PPP) therapy [21] and those receiving general basic therapeutics [4]. The characteristic time of healing was used to classify the wounds as healing ($\tau > 0$) or non-healing. For the healing ones, this parameter was further used for the statistical comparison of wound healing rate upon different treatments.

2.5. Data treatment and statistical analysis

Unless differently specified, data were analysed using parametric tests to compare the mean of two (t-test) or more groups (ANOVA). Shapiro-Wilk and Levene tests were first applied to check for normality and homoscedasticity. Non-normal and heteroscedastic data were Box-Cox or logarithmically transformed. Post-hoc analyses were performed by means of Tukey's honest significant difference test. The tests were considered significant if their p-value was smaller than 0.05.

3. Results

3.1. Wound area measurement

We first validated the tool for wound area and shape characterization. The area of 23 wound planimetries was quantified through the Woundaries (Fig. 1A–C). These results were thus compared to those obtained through the validated device Visitrak [22] applied to the same dataset.

The scatter plot of the results obtained with both methods of measurement (Fig. 1D) display a good agreement. A Bland–Altman plot [23] of their relative differences with respect to the average (Fig. 1E) and a paired t-test ($p = 0.152$) were used to exclude significative differences between both methods. As a further verification, a paired-samples test of equivalence [24] also showed the equivalence of both methods at a level of significance of 5%.

3.2. Wound evolution assessment

We developed and validated a routine for the classification of wound area time-series and the inference of kinetic parameters. The routine is based on a model selection scheme, followed by a regression for the inference of the characteristic time of healing.

To test its performance, the routine was applied to simulated data corresponding to healing and non-healing wounds. The method shows a high specificity, with a false positive rate well below the nominal type I error (5%). Expectedly, the method sensitivity was found to depend on the number of points available for the fit and to the extent of time these points cover with respect to the healing characteristic time (Fig. 3B). As an example, while with 4 data points covering ~0.87 τ (corresponding to an expected reduction of the initial area of the ~60%) it is possible to classify a wound as healing with 90% sensitivity, reaching the same conclusion within a time of ~0.3 τ (corresponding an area reduction of ~25%) requires 21 measurements (Fig. 3B). Therefore, our results show that a quantitative inspection of the wound at high frequency can further improve the determination of its evolution.

As an *a posteriori* validation of the model used to describe the data, the logarithm of areas and times were plotted after rescaling by the fitting parameters A_0 and τ, respectively (Fig. 3B), showing a very good agreement.

3.3. Application to clinical data

We carried out a retrospective study on observational data corresponding to 120 wounds from 85 patients treated in our wound clinical outpatient unit between 2015 and 2017. Table 1 shows patients' characteristics.

Fig. 4A and B shows the distribution of wounds with respect to their aetiology and the sex of the patient (4A), and respect to patient age group and sex (4B). Ulcers were treated with PPP therapy (47.5%) or general basic therapeutics (52.5%).

Table 1
Baseline patients' characteristics.

Characteristic		Number of patients (%)
Sex	Males	36 (42.4%)
	Females	49 (57,6%)
Number of ulcers	1 ulcer	59 (69,4%)
	2 ulcers	19 (22,4%)
	3 or 4 ulcers	7 (8,2%)
Aetiology	Venous	35 (29,2%)
	Arterial	17 (14,2%)
	Traumatic/surgical	23 (19,2%)
	Pressure	12 (10%)
	Others	33 (27,6%)
Age groups	50–59 years	9 (10,6%)
	60–69 years	21 (24,7%)
	70–79 years	25 (29,4%)
	80–89 years	24 (28,2%)
	90 years or older	6 (7,1%)

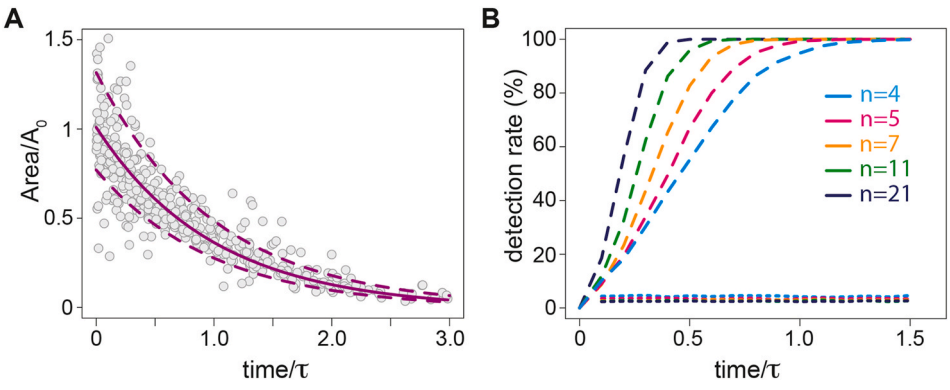

Fig. 3. Performance of the classification routine on simulated data. (A) Plot of all the measured wound areas vs. time (circles) rescaled on the basis of the parameters A_0 and τ calculated by the fitting procedure and for times larger than the respective delays. The agreement with an exponential decay with unitary parameters validates the choice of the model. Dashed magenta lines correspond to standard errors. (B) Detection rate (dashed lines) and false positive error rate (dotted lines) as a function of the normalized characteristic time of healing, as determined from simulations with a varying number of data points (different colours). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

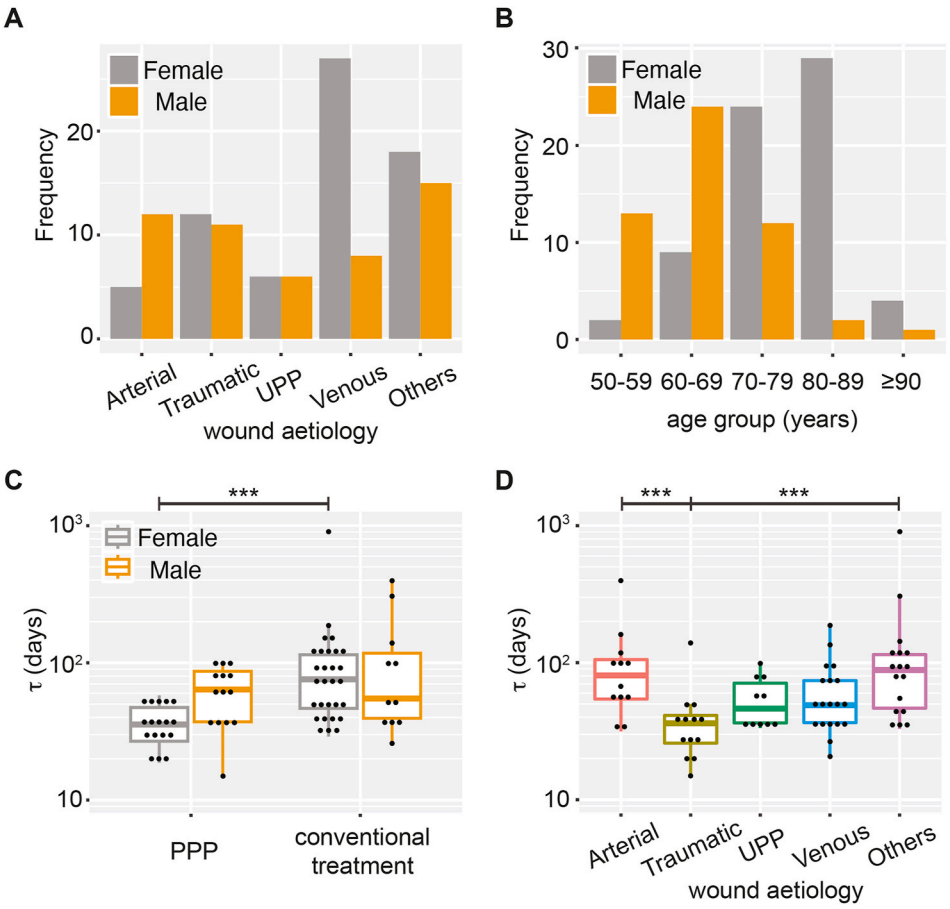

Fig. 4. Statistical analysis of chronic wounds. (A–B) Frequency histogram of the analysed wounds as a function of aetiology and patients' sex (A) and of age group and sex (B). (C–D) Box and whiskers plot of the healing time determine by the fitting algorithm as a function of wound treatment and patients' sex (C) and of wound aetiology (D). *** corresponds to *p*-value smaller than 0.05.

According to the criterion of classification described above, the PPP therapy did not produce significative differences in the probability of healing (Fisher's test, $p = 0.355$), despite a slightly lower percentage of healing (54.4%) observed in PPP-treated wounds with respect to wounds treated conventionally (63.5%). For healing wounds, the fitting provided the characteristic time of healing τ, corresponding to the time at which the wound area reduces to ~37% of its initial value. This parameter did show a significative difference between groups undergoing different treatments, with a significantly faster healing (lower characteristic time of healing τ) for the PPP-treated ones. This effect can be almost entirely attributed due to female patients (Fig. 4C, $p = 2.5 \times 10^{-4}$), whereas wounds from male patients do not seem to heal faster when treated with PPP. To investigate whether this difference was related to other factors, e.g. a higher incidence of a given type of wound in a sex group, we analysed the healing time with respect to wound aetiology and treatment (Fig. 4D). This analysis shows that traumatic wounds heal significantly faster than arterial ($p = 4.5 \times 10^{-4}$) and unclassified wounds ($p = 6.2 \times 10^{-5}$). Similar analyses were also carried out to explore whether patients' age, the presence of health conditions, and wound location had any influence on the wound healing time but did not show significative effects.

We attempted to find correlation between healing and other measured geometrical parameters (initial wound circularity, roughness, and aspect ratio) as a function of the main factors involved in the study, however we could not find any conclusive evidence.

4. Discussion

Monitoring the evolution of ulcers is a complex process in which quantitative variables such as wound area are essential. Despite emerging methods to measure the area based on photography or 3D scanners, planimetries are still widely used. In this scenario, we reckoned it could be useful to develop a graphic user interface (Woundaries) for wound area measurements. Woundaries aims to provide a user-friendly tool that allows the automatic calculation of wound area together with several shape descriptors from digitalized planimetries. To help the user to intuitively adjust the image, all the steps of the image processing algorithm are visualized. The code works also for partly open curves that can result from an incomplete planimetry drawing. The output data are easily saved in a text file for further processing (e.g., the wound classification step) or batch analysis. Importantly, we demonstrated that measurements obtained by Woundaries are equivalent to those provided by the validated device Visitrack. Being based on digitized planimetry images, our method does not present fundamental limitations with respect to the size of wound to be assessed. However, we must point out that large wounds (area larger than 400 cm^2) cannot be easily traced on planimetry. Therefore, alternative strategies for the tracing must be used to subsequently enable the use of Woundaries. Additionally, we observed that measurements obtained by Woundaries show a good repeatability and reproducibility in the explored range of wound sizes. The wound descriptors provided by the Woundaries can be further processed by means of other routines for prognostic use. Along this line, we developed a classification routine that uses wound area vs. time traces to sort wound as healing or non-healing. For the healing case,

the characteristic parameter of wound area evolution was also inferred, thus predicting the dynamics of wound closure. With the help of simulations, we analysed the performance of our routine in providing a reliable prognosis as a function of the number and the frequency of measurements. As an example, for a wound with a nominal characteristic healing time of 2.5 months, predicting its evolution with ~70% classification sensitivity requires collecting measurements at least once a week for 5 consecutive weeks. The same sensitivity can be reached earlier if the frequency of measurement is increased. In addition to the need of an accurate methodology, this result further stresses the importance of a regular and frequent clinical follow up of the wound. In fact, wound healing characteristic time should be estimated as soon as possible in order to verify if a specific treatment is being effective.

Since it has been previously reported that other wound and patient factors also have prognostic value [16], the classification routine could provide a more accurate prediction by the simultaneous evaluation of additional wound descriptors as, e.g., the geometrical parameters provided by the Woundaries. However, the implementation of these analyses requires further efforts in studying and modelling their time evolution in healing and non-healing wounds.

The rapid determination of the efficacy of a specific treatment is particularly crucial when applying advanced therapies for chronic wounds [25]. In fact, biological therapies are expensive and time-consuming to prepare; they usually require several applications before their effect could be visually observed. Therefore, a method able to determine the effect of a treatment in a shorter time could significantly contribute to improve wound management.

We applied our methodology to analyse 120 wounds of different aetiologies from patients receiving conventional therapeutics or PPP therapy, a well-established autologous therapy in our clinical facilities. The statistical analysis of the data showed that traumatic wounds heal significantly faster than other aetiologies such as venous wounds. Moreover, while the PPP therapy does not seem to increase the probability of healing as compared to conventional treatment, it shows a significative effect in reducing the wound closure time in female patients. However, assessing whether this is a direct effect of patient's sex or a consequence of other factors, such as the higher incidence among female patients of wounds with a shorter healing time (e.g. venous wounds), requires further verification.

In addition, we didn't report any relation with healing kinetics and initial morphology although other authors reported some correlations studying a unique aetiology wounds [26,27].

5. Conclusion

In this study, we demonstrated a reliable and user-friendly method for the quantification of area and shape descriptors from digitalized hand-traced planimetries. Based on our results, the proposed method has a straightforward implementation and could be easily adopted in routine wound monitoring. In this sense, we are currently developing a phone/tablet app including the Woundaries capabilities.

Our methodology provides information on wound healing evolution and estimates kinetic parameters of the process. It is based on an objective evaluation of parameters of wound evolution and provides the early identification of non-healing wounds, thus facilitating the assessment of the efficacy of therapies. Therefore, we believe that our methodology can assist sanitary staff in clinical decision making, thus improving chronic wounds management and early prediction of wounds evolution.

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Conflicts of interest

The authors have no conflict of interest.

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