



RESEARCH ARTICLE

Regenerative collagen biomembrane: Interim results of a Phase I veterinary clinical trial for skin repair [version 1; referees: 2 approved, 1 approved with reservations]

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Abstract

Background: The availability of commercial tissue engineering skin repair products for veterinary use is scarce or non-existent. To assess features of novel veterinary tissue engineered medical devices, it is therefore reasonable to compare with currently available human devices. During the development and regulatory approval phases, human medical devices that may have been identified as comparable to a novel veterinary device, may serve as predicate devices and accelerate approval in the veterinary domain. The purpose of the study was to evaluate safety and efficacy of the biomembrane for use in skin repair indications.

Methods: In the study as a whole (3 year total length), 15 patients (animals), dogs and cats (male/female, <8 years) with skin lesions of different etiologies considered difficult to heal (size, >2 cm), with a wound depth equivalent to 2nd/3rd degree burns are to be studied from Day 0 to Day 120-240, post-application of the biomembrane. This interim report covers the 5 patients assessed to date and deemed eligible, of which 3 enrolled, and 2 have completed the treatment. Wound beds were prepared and acellular collagen biomembranes (Eva Scientific Ltd, São Paulo, Brazil) applied directly onto the wounds, and sutured at the margins to the patient's adjacent tissue. Wound size over time, healing rate, general skin quality and suppleness were assessed as outcomes. Qualitative (appearance and palpation) and quantitative (based on Image Analysis of photographs) wound assessment techniques were used.

Results: Both patients' wounds healed fully, with no adverse effects, and the healing rate was comparable in both, maxing out at approximately 1 cm²/day.

Conclusions: Early results on the biomembrane's safety and efficacy indicate suitability for skin repair usage in veterinary patients.


Keywords

biomembrane, biofabrication, tissue engineering, medical device, collagen, wound healing

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Referee Status: ✓ ? ✓

	Invited Referees		
	1	2	3
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Competing interests: The first author (A.K.) is the founder and shareholder of Eva Scientific Ltd, where the biomembrane used in this clinical trial was developed and is sold. The co-authors are veterinary practitioners of Sorocaba Veterinary Hospital (J.F.L.N., J.A.M.F. and M.H.S.C.) and full-time employees of University of Campinas (A.L.J. and P.K.). All co-authors read and agreed to the manuscript and had full access to the data, and attest to the study's impartiality as a whole, as well as declare no competing interests.

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Editorial note:

12th June 2018: F1000Research operates an author-led publishing model where the authors are typically asked to suggest reviewers. However, as one of the authors of this article, Andreas Kaasi, has declared competing interests with the company that has developed the biomembrane used in this study, the F1000Research editors have agreed with the authors that in the interest of ensuring a fair and unbiased peer review process, the selection of the peer reviewers will instead be the responsibility of the F1000Research editorial team.

Introduction

Ever since the pioneering work of [Rheinwald & Green \(1975\)](#), on skin epidermis (keratinocyte) cultures, [Yannas & Burke \(1980\)](#) on hybrid (biologic/synthetic) dermis/epidermis skin substitutes, and [Bell *et al.* \(1981\)](#) on fully biologic dermis and epidermis skin substitutes, a number of companies and products engaged with tissue engineering technologies have developed and consolidated in the market. In particular, early clinical research (Phase 1 and Early Phase 1) has tended to be characterized by a tight relationship between the industry-academia-hospital stakeholders of this archetypical medical device triad.

The tissue engineered devices that have made the most headway, clinically and commercially, include Epicel®, Integra®, Dermagraft®, Apligraf® and Alloderm®. These products have all had to make decisions and compromises whether to mimic only the dermis, only the epidermis, or both. Whether to employ only synthetic biomaterials, only natural biomaterials, or both. And whether to include only living dermis-type (fibroblast) cells, only epidermis-type (keratinocyte) cells, or both, or alternatively, to let the device be acellular. Review articles written by [Eisenbud *et al.* \(2004\)](#) and [Groeber *et al.* \(2011\)](#) provide a more in-depth comparison of these products and others.

Tissue engineered devices for veterinary use are non-existent, or scarce, at best. The same can be said about veterinary clinical trials in general, which lack a global consensus concerning the rules and regulations governing them. Usually, veterinary clinical trials with *patients having spontaneously occurring diseases* are treated analogously to animal experimentation with *experimental subjects with induced diseases* ([Füirdös *et al.*, 2015](#)), requiring Ethics Committee approval for the use of Animals for Experimentation (the same way that this is required for “traditional” animal experimentation). By contrast, countries may not regard animal clinical trials as animal experiments, thus not requiring an Ethics Committee approval by law. This would present a *less* restrictive approach. A third possibility would be to implement a set of rules and regulations specific for veterinary clinical trials, with a separate Ethics Committee for this purpose. This would present a *more* restrictive approach.

Many researchers debating on the topic of veterinary clinical trials ([Füirdös *et al.*, 2015](#); [Vail, 2007](#)) acknowledge the attractiveness of letting veterinary clinical trials pave the way for human use of the same therapy. To make the cross-applicability of veterinary/human data as smooth as possible, and while more specific veterinary clinical trial rules and regulations remain

absent or non-enforced, it may be advantageous to frame veterinary clinical trials to conform with rules, regulations and standards governing human clinical trials. One aspect of interest is the sample size and terminology of the “phase” of the trial.

In the human domain, the U.S. Food and Drug Administration (FDA) defines the number of participants appropriate for each phase as follows: Phase 1, 20–100 participants; Phase 2, “several hundred” participants; Phase 3, 300–3000 participants; Phase 4, “several thousand” participants (<https://www.fda.gov/ForPatients/Approvals/Drugs/ucm405622.htm>, retrieved on 12 Jan 2018).

The U.S. National Institutes of Health (NIH) has a similar definition for Phases 1 (n= 20–80) and 3 (n=100–3000), but offers a precise definition for Phase 2, at 100–1000 participants. The NIH, unlike the FDA, does not provide any specific participant number for Phase 4 (<https://www.nih.gov/health-information/nih-clinical-research-trials-you/basics>, retrieved on 12 Jan 2018).

A so-called “Early Phase 1”, previously known as Phase 0, is defined as an “exploratory study involving very limited human exposure to the drug, with no therapeutic or diagnostic goals” (<https://clinicaltrials.gov/ct2/help/glossary/phase>, retrieved on 12 Jan 2018). Again, the descriptor “very limited human exposure” does not offer precise guidance on numbers, but a figure of <15 participants appears to be the consensus for this type of study ([Gawai *et al.*, 2017](#)).

We believe a sample size of 15, being the borderline number between the above mentioned “Early Phase 1” and “Phase 1” (as defined by FDA and NIH, above) is a suitable sample size for veterinary clinical trials, being an economically and practically feasible number to execute in a veterinary medicine context (usually not backed up by heavy industry or institutional funding), whilst at the same time offering the desired property of translatability of veterinary clinical trial findings to human applications.

In this study, we set out to treat skin lesions in dog and cat patients referred to our clinical practice, where conventional clinical treatment had proved itself incapable of satisfactorily healing the lesions. In order to form suitable exclusion criteria with respect to wound size, we considered the following reference parameter: maximum lesion/defect size for a spontaneous and unconfined tissue regeneration to occur *in vivo*, without special treatment = 1 cm ([Dorin *et al.*, 2008](#)). The same value is also given in the literature in a burn context, when faced with a full thickness, third degree burn ([Papini, 2004](#)). Thus, we chose to concentrate on wounds larger than this reference size.

In assessing the wounds and progression of healing, for qualitative data, we ordered that photographs be taken as often as possible, and, inasmuch possible, using certain reference angles of photography that were easy for the patient owners to employ when sending photographs from home. These same photographs formed the basis for a quantitative wound healing assessment, using the photographs as raw data for an image analysis powered calculation of wound area/healed area over time. The approach chosen required minimal or no investment, as opposed to commercial wound image scanner devices (see e.g., [Bills *et al.*, 2016](#); [Davis *et al.*, 2013](#)) that can cost upwards of US\$10,000. In addition to the experiences sought

through this clinical trial, pertaining to the clinical outcome of the study, the experiences obtained with this image analysis method were seen as secondary benefits of the study that also would be useful to perfect the methodological aspects of the study for the remainder of the study period.

Methods

This study has been reported using CONSORT guidelines (see [Supplementary File 1](#) for a completed checklist).

Study design

The protocol to this ongoing, 36-month, phase I, quasi-experimental, interventional clinical trial is available as [Supplementary File 2](#).¹

Results in this interim report include data from animals who were assessed and eligible (n=5) and enrolled (n=3) in the trial, with treatment completed (n=2), at the time of writing ([Figure 1](#)). Therefore this article presents interim results, spanning 1 year of the 3 year study length.

The study protocol abided by the principles of the WMA Declaration Of Helsinki – Ethical Principles For Medical Research Involving Human Subjects (<https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/>), adapted to veterinary practice.

The Ethics Committee of Sorocaba Veterinary Hospital approved the study protocol before the study began (Ref. no. 20161012-1), and all patient (animal) owners provided written informed consent for their pets' participation before undergoing any procedure related to the present study. The study was carried out at Sorocaba Veterinary Hospital.

Study objective

The objective of the present study was to obtain clinical experiences with the use of a proprietary collagen biomembrane medical device for skin repair in dogs and cats, and to assess its safety and efficacy for this indication.

Eligibility criteria

Dogs and cats, of both sexes, aged up to 8 years, with an etiology of skin lesion of different causes, such as burns, traumas, surgical excisions, were deemed eligible for treatment with the collagen biomembrane. The lesions should be equivalent to second or third degree burns, i.e., extending into the dermis or subcutaneous layers of the skin, or loss of skin tissue altogether. The size of the lesion should measure 2 cm or more

(length/width/diameter as per the lesion geometry). This figure is double the reference maximum lesion/defect size (1 cm) for spontaneous and unconfined tissue regeneration *in vivo* to occur, without special treatment, as noted above. The rationale of setting the size criterion at *double* that figure, considered the maximum size for spontaneous healing (1 cm), was that the healing of a lesion/defect size of this magnitude (2 cm) would be unquestionably attributed to the treatment regime. Key exclusion criteria included lesions only of the epidermis (equivalent to a first degree burn), in addition to skin lesions stemming from chronic disease. These criteria are summarized in [Table 1](#).

Surgical regimen

Each patient was photographed on the operating table, just prior to the first surgical procedures, from a variety of angles. Inasmuch possible, a decision on the most suitable angle and focal distance for future photographs, would take place during these initial photographs. Ideally, a single angle and focal distance would provide for a general wound healing assessment, as supposed to requiring multiple angles, especially since later follow-up time points depend on owners' own photographs, with little or no control by study researchers. A ruler would be placed adjacent to the treatment area for later dimensional analyses, or, alternatively, direct measurements would be taken of reference distances such as eye-midline, knee-hip joint, and recorded on file.

Under general anesthesia (preanesthetic agents: acepromazine 0.02 mg/kg and meperidine 3 mg/kg, both administered intramuscularly; induction agent: propofol, 3 mg/kg, administered intravenously; maintenance agent in cats: propofol, 0.2 mg/kg/min; maintenance agent in dogs: oral isoflurane administered through tracheal intubation with a universal anesthetic vaporizer, dose being subject to anesthetist's clinical assessment and varied as required during the surgery), the treatment area was prepared by shaving away fur adjacent to the treatment area and cleaned with saline and iodine. If the affected area had been subject to natural scarring, with the formation of hard, unhealthy, fibrotic scar tissue, this was surgically removed, exposing subcutaneous tissue. In this case, the tissue would be fixed in 10% formalin and submitted to histological analysis, using standard procedures. If the treatment area was an unhealed, deepithelialized wound bed, but with no fibrotic scar tissue formation, then shallow scalpel incisions were made scattered throughout the treatment area, causing local bleeding, with the aim of promoting regeneration and integration with the later application of the collagen biomembranes. In any case, debridement of lacerated, devitalized or contaminated tissue was performed prior to the application of the collagen biomembranes.

Acellular collagen biomembranes measuring 8 × 3 cm were obtained from Eva Scientific Ltd, São Paulo, SP, Brazil (<http://www.evascientific.com/products/biomembranespet/>). According to the manufacturer, the biomembranes are made aseptically from collagen type I sourced from rat tail tendon (Ref. E-003RT050M1), using the manufacturer's proprietary biofabrication apparatus. The resulting biomembranes possess a mean thickness of 200 to 300 µm, with a regular pattern of local depressions/elevations of approximately 100 µm and 2.5 mm apart center-to-center (see [Figure 2](#)).

¹There are as yet no widely known veterinary clinical trial registries, apart from the American Veterinary Medicine Association's (AVMA) Animal Health Studies Database. This database is the first of its kind, and was only 4 months old at the time the present trial was conceptualized and commenced (October 2016). The authors only became aware of its existence in subsequent months, and the study details were submitted to the database thereafter; however, the AVMA informed the authors that they were unable to accept studies from outside the U.S.A. and Canada, with the reason being they do not have the resources to consider study listings in the context of animal use laws and regulations that vary from country to country. As such, it was decided to include the study protocol as a [Supplementary File](#) to this article. It is also available on the Eva Scientific website (<http://www.evascientific.com/products/a161012001/>).

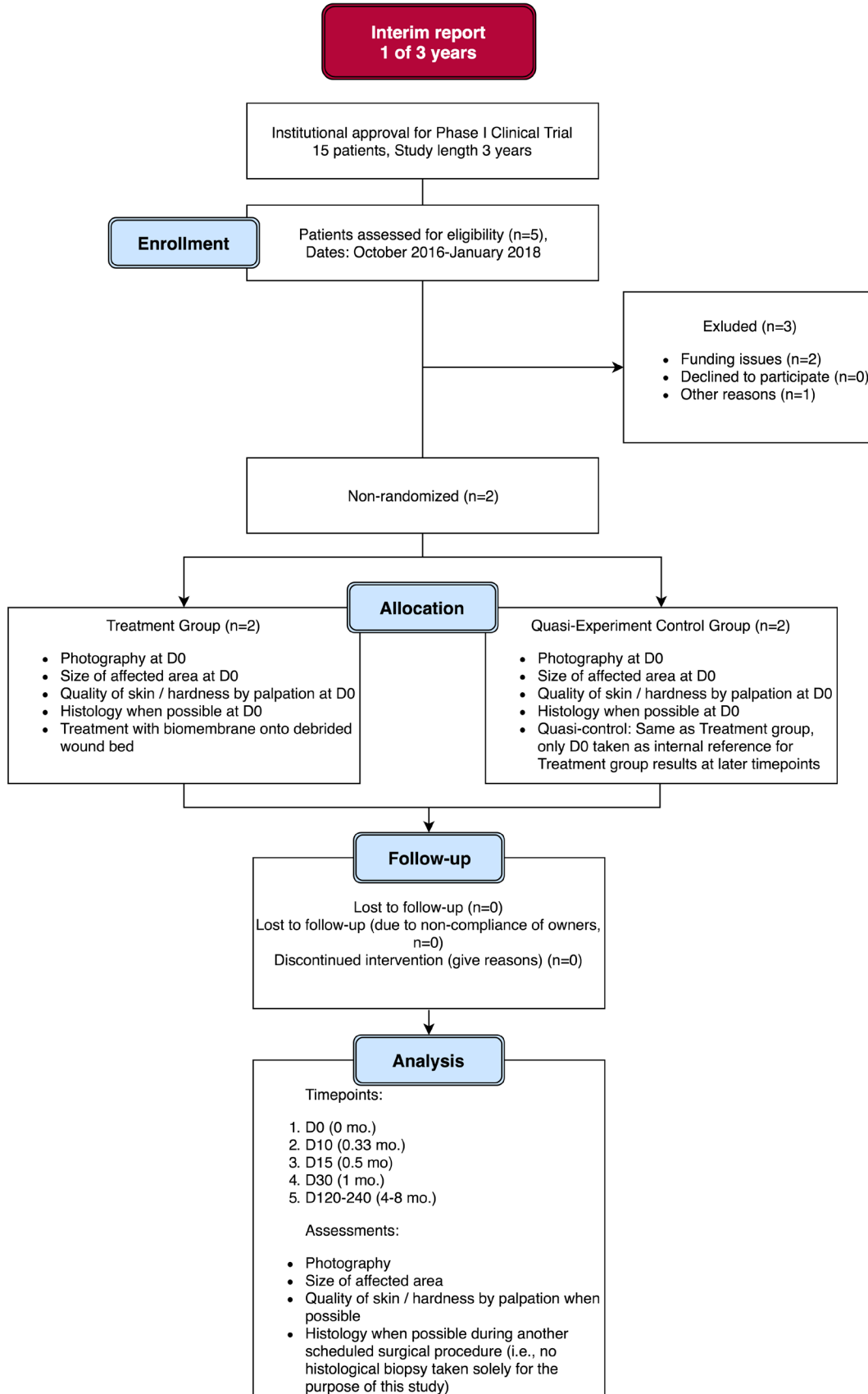


Figure 1. Study flow diagram.

Table 1. Eligibility criteria for the present study.

Inclusion criteria	Exclusion criteria
Species: dog or cat Sex: male or female Age: <8 years Skin lesion etiology: various Skin lesion severity: equivalent to 2nd or 3rd degree burn Skin lesion size: > 2 cm (L/W/diameter as per geometry)	Epidermis-only lesions (equiv. 1st degree burn) Skin lesions stemming from chronic disease

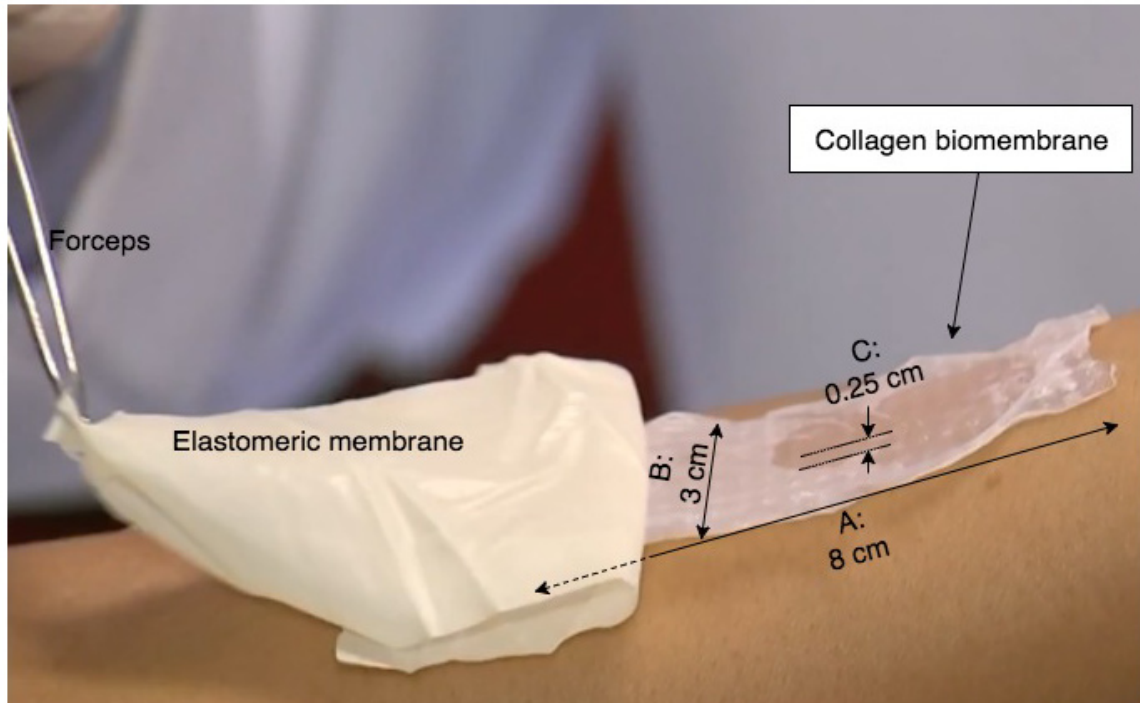


Figure 2. Regenerative collagen biomembrane (Eva Scientific Ltd, São Paulo, Brazil). The biomembrane measures 8×3 cm, and is scattered with small ridges throughout the structure, measuring approximately 100 μm in height, and a spacing of 0.25 cm between each elevated ridge. The standard presentation of the biomembrane is with a synthetic elastomeric membrane underlying the biomembrane itself; when applied to the treatment area, the device (double-layer synthetic membrane / biomembrane structure) is placed biomembrane-side down onto the treatment area, and the synthetic membrane carefully peeled away with a forceps, leaving behind the biomembrane (see [Supplementary File 3](#) for footage of this application technique).

Depending on the size and shape of the treatment area, one or several collagen biomembranes were used to cover the area, extending over the wound margin by a few millimeters. At the margins, the biomembranes were sutured to non-treatment tissue using the simple interrupted suture technique. If one or more biomembrane edges were facing away from the non-treatment tissue margin (i.e., into the wound, kissing or overlapping another biomembrane's non-treatment tissue margin), the surgeon would make a decision on whether or not to make an internal suture; the biomembrane's capacity to adhere to the wound bed and commence integrative regeneration discourages the use of the internal suture.

After applying and suturing all biomembranes, alginate hydrogel (Curatec®, LM Farma, São José dos Campos, SP, Brazil) was

generously applied onto the treated areas. At this stage, the patients' general anesthesia would be retracted. The patients were then medicated with a long-duration injectable antibiotic drug (cefovecin sodium, 8 mg/kg), and the treatment areas covered up with gauze tape.

Wound treatment regimen

Upon awakening from the anesthesia, the first dose of an antifungal oral drug (ketoconazole, 10 mg/kg or itraconazole 5 mg/kg) was administered, with the treatment carried through for the duration as prescribed by the drug manufacturer.

The sutures were removed upon attesting that the biomembrane had adhered well to adjacent tissue structures and when the sutures would not provide any useful additional mechanical

support to the host tissue-wound-biomembrane system integrity. This could happen as early as Day (D)3 after the surgery.

Approximately every two days, the gauze tape was removed, and the wound cleaned with physiological saline and extra alginate hydrogel applied, as needed, and covered up again with fresh gauze tape, to prevent infection. This care was continued until the wound had completely healed.

Patient evaluation and follow-up

Follow-up time points for patient evaluation were defined as D0 post-surgery, D10, D15, D30 and a long-term time point at D120–D240 (4–8 months). At these time points, patients had photographs taken of the treatment area at roughly identical angles and distance to those taken immediately prior to the surgery. These photographs were used as basis for quantitative size assessments, as detailed below. When possible, a qualitative assessment of skin tissue quality would be done, either by a researcher associated with the study or by the owner. If another surgical procedure was scheduled for a study participant, for any reason, a small skin biopsy was attempted to be taken from the treatment area, but the study's premise was that end-point histology would not be necessary to provide assertive conclusions to the treatment effectiveness, based on clinical assessment and healing rates.

The wounds were monitored for adverse effects such as infection, stagnation of healing for more than 15 days, and necrosis. If any adverse effects were confirmed, in the first 1–2 days, then the biomembranes were excised or scraped off host tissue. If adverse effects were confirmed in later stages (>D3), the biomembranes were not excised due to the fact that by this time they had absorbed significantly into the host tissue, rendering removal difficult or impossible. In this case, the patients were treated symptomatically and added to the “Discontinued intervention” group, as indicated in the Study Flow Diagram (Figure 1).

Quantitative wound size assessments based on Image Analysis

In order to use a quantitative wound size measurement method based on two-dimensional image analysis, the photographs would ideally depict the wounds at an angle as normal (90°) as possible to the idealized, hypothetically two-dimensional wound area.

Using ImageJ 1.49v (Schneider *et al.*, 2012) and the “Straight line” tool, a straight-line was traced for a known distance. Usually, the tool was applied to trace the long dimension of a biomembrane, using sutures applied at either end as cue points for the extremities, and, assuming the sutures were placed some 2 millimeters from the edges of the biomembranes, using the “Set scale” tool that distance in *pixels* (as traced in the previous step) was taken to be equivalent to a distance in *centimeters*, 7.5 cm, slightly less than the nominal 8.0 cm biomembrane length (Figure 3). Subsequent uses of ImageJ's “Measure” tool then output a distance in cm (distance) or cm^2 (area), which were used for the analyses.



Figure 3. ImageJ software image analysis approach for quantitative size measurements of wounds - Part 1. The first part of the method is to use the Set scale function on the image analyzed for a known distance, visible on the photo. In this case, the distance chosen was the length of the biomembrane (with the sutures used for cue points of approximate termini of the biomembrane, useful for later time points when the original biomembrane had been completely absorbed into the patient tissue), with a correction applied to account for the inclination of the target plane, using a trigonometric approach.

Such analysis assumes the wound plane to be in the same plane as the observer (camera). Whenever photographs diverged from this standard photographing procedure, a semi-quantitative assessment was made to estimate the approximate *inclination angle* relative to the observer (camera). This angle was then used to estimate the true distance of wound plane by projection of the observer (camera) plane distance onto the inclined wound plane, using a simplified trigonometric approach, as outlined in Figure 4.

This method was applied to the photographs collected for every time point, for every individual, and every wound region, and a total wound area recorded in a table. The whole process was repeated, albeit using the same photograph, but redoing the set scale, the approximate polygon wound area/margin delineations, and measure function, thus producing a duplicate measurement for statistics. For each individual and time point, the mean wound area and the standard deviation were calculated, along with the coefficient of variation.



Figure 4. ImageJ software image analysis approach for quantitative size measurement of wounds - Part 2. (A) The known distance (b) traced was, for photographs captured at an angle exactly normal to the wound plane, used for the “Set scale” function as-is, to trace the wound margin for use with the “Measure” function to obtain the wound area. (B) For photographs captured at an inclined angle, a semi-quantitative estimate of the inclination angle (theta) was made and a simplified trigonometric system used to approximately determine the known distance’s (c) adjacent cathetus b’ (b’≈b; the unknown distance in the camera plane) measurement in the *observer (camera) plane*. Then, using “Set scale”, the distance traced in b was taken as equal to b’=c*cos(theta). (C and D) Thereafter, the wound margins were traced using the “Polygon selection” tool for subsequent measurement. This patient’s wound at this time point had formed a “pinch point” whereby the wound area above the eye had become discontinuous with the wound area adjacent to the eye (originally, these two were continuous) due to the advancing epithelialization, thus forming two distinct wound areas, 1 and 2, that were measured (using “Analyze-Measure”) as shown in C and D, respectively, and the sum of this *observer (camera) plane* wound area projected onto the true inclined *wound plane* using the trigonometric system explained above. The total wound area (whether a single area or the sum of distinct areas) was calculated and recorded.

Another inferred parameter, the “Wound healing rate” was calculated using the mean wound areas, defined as:

$$\dot{A}_x = \frac{\Delta A_x}{\Delta t_x}$$

where

$$\Delta A_x = A_{\text{time point } x} - A_{\text{time point } x-1}$$

is the current wound area ($A_{\text{time point } x}$) measurement minus the previous wound area ($A_{\text{time point } x-1}$) measurement, and

$$\Delta t_x = t_x - t_{x-1}$$

is the absolute time difference in days between the current wound measurement’s nominal time point and the previous’ nominal time point.

The calculated values for total wound area ($A_{\text{time point } x}$) and wound healing rate ($\dot{A}_{\text{time point } x}$) were tabulated and plotted graphically against nominal time points. A third parameter, wound healing area at a given nominal time point, $\%A_{\text{time point } x}$, was defined as:

$$\%A_{\text{time point } x} = \frac{(\max(A) - A_{\text{time point } x})}{\max(A)}$$

where

$$\max(A)$$

is the maximum wound area for the series.

The choice of using the maximum value of the series, rather than the initial value, counteracted the effects of the imprecision of the method; at times, the second (D10) and third (D15) time point measurements, ended up having higher values than the initial value. Albeit not theoretically impossible (wound growing instead of shrinking/healing), the clinical, qualitative assessment did not suggest this was the case in any individual studied, and the choice was therefore made to use the maximum value as the common reference point for the series, as explained.

Statistical analysis

The quantitative wound area measurements, using Image Analysis as the method, were obtained in duplicate. Basic statistical analysis was performed on these n=2 samples to provide a mean value (“AVERAGE function”), the standard deviation (“STDEV function”) and coefficient of variation (defined as the standard deviation divided by the mean value, for each sample). The software used for analysis was Excel 2011 (Microsoft Corporation, Redmond, WA, USA).

Histology protocol

In the cases where pre-surgery and/or post-surgery biopsies of the affected area were collected, these were fixed in 10% formalin, embedded in paraffin, sectioned at 4 μm and subjected to the hematoxylin and eosin stain (H&E). The same protocol was applied to an unused biomembrane of identical specification to those used in the treatment group, to serve as a comparator for patient tissue histological samples. The stained tissue sections were visualized in an upright microscope with automatic scale bar incorporation.

Results

Patient characteristics

Patient N01 was approximately 6 years old (rescued animal, exact age unknown) at the time of surgery, female dog, with a skin lesion due to trauma (dog fight), disfigured fibrotic overgrowth of area adjacent to left eye with an area of approximately 10 cm².

Patient N04 was approximately 1 year old (rescued animal, exact age unknown) at the time of surgery, male cat, loss of skin equivalent to 2nd degree burn after car accident trauma with an area of approximately 120 cm² (note: approximately 40 cm² of the total lesion area was treated with the biomembranes, the remainder was treated using conventional wound treatment methods and healed at a slower rate than the biomembrane-treated areas).

Qualitative wound healing: Photography and clinical assessment

The photography series presented a favorable outcome in terms of wound closure with the formation of healthy skin in the areas treated, in both patients studied in this interim report. In both patients, vascularization of the biomembrane-treated areas was observed clinically by the marked redness of the areas treated. The *neo*-skin that was formed presented itself as supple and robust, as opposed to a tissue that could form itself as hard and/or fragile, respectively. Moreover, in both patients, the areas treated ended up, at the end of the follow-up period, having a partial or complete coverage of fur, indicating the possible formation of new hair follicles in the treated areas, or,

alternatively, a migratory mechanism whereby hair follicles in adjacent healthy areas were tugged toward the center of the wound/treated area, as part of the gradual wound closure process. The photographs collected are shown in **Figure 5**.

Worthy of an additional explanation, N04’s fur coat color/tonality changes significantly from D30 to the last time point, D120–240. Such changes are not uncommon in cats, especially in individuals that have not yet reached adult age, as was the case here. Other variables, such as temperature/season change (here, fall-winter), sunlight exposure, diet, hormones and stress also play a role, as do genetics and illness (here, the significant change may be due to the transition from a “sick” or “recovering” phenotype to a “healthy” phenotype).

Quantitative wound healing: Image analysis

The photographs collected were analyzed as described in the *Methods*, giving rise to values for wound area, healed area and healing rate, as tabulated in **Table 2**. Graphs for *Wound area vs Time*, *Healing rate vs Time* and *Healing rate vs Healed area*, based on the values shown in **Table 2**, are shown in **Figure 6**. The wounds of the two patients studied differed somewhat in size, which in turn made it more difficult to generalize features of healing in the wounds and individuals treated. However, it was noted that, in general, the *healing rate* accelerates during the first phase of the wound healing, reaching a maximum at D15–30. When looking at the *Healed area percentage* at maximum *Healing rate*, the results indicate that maximum *Healing rate* coincides with a *Healed area percentage* of around




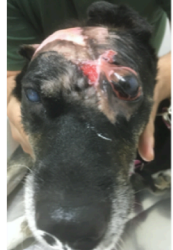


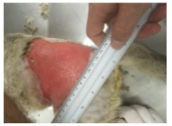

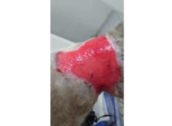



Individual	Time point	D0 (pre-surgery)	D0 (post-surgery)	D10	D15	D30	D120-240
N01	Data type: Photography						
N04	Data type: Photography						

Figure 5. Photographs of areas treated with the biomembrane. In patient N01, old fibrotic scar tissue was removed surgically and two biomembranes applied and sutured to adjacent tissue. By time point D30, the wound had fully healed. In patient N04, only part of the lesion (approximately 1/3) was treated on D0 with the biomembrane (as seen in the D0 post-surgery photograph). The non biomembrane-treated area (without biomembrane in the D0 post-surgery photograph) had in fact been treated with another wound dressing two weeks before, but this treatment proved to be ineffective. The subsequent regeneration of the biomembrane-treated areas, opportunely led to a snowball effect that eventually would lead to the complete healing also of the non biomembrane-treated areas. The photograph in the last time point shown here, having a very wide bracket of Day 120 to 240, was in fact taken on day 129; follow-up visits were also done at the end of the D120-240 time point interval, but photographs are not available. Nonetheless, the clinical data obtained at this final assessment confirm complete wound closure with clinically excellent parameters, such as suppleness, robustness, lack of contracture and with unrestricted mobility in the region in question (as seen on examination of the patient). The photographs provided basis for these *qualitative* assessments, in addition to the *quantitative* assessment aided by image analysis. In the case of N04, only the areas treated with the biomembrane *de facto* were used in the statistical analyses (even though the snowball effect led to healing of non biomembrane-treated areas, but at a slower rate).

Table 2. Quantitative wound healing parameters. Obtained from analysis of data collected at the time points as per the study protocol, from Day 0 to Day 120–240. The values for parameter A, wound area, were calculated using an image analysis approach of photographs taken at the different time points, from which the two other parameters: %A, healed area; and \dot{A} , healing rate, were calculated. \bar{x} , mean; SD, standard deviation; CV, coefficient of variation.

Patient	Parameter	Statistical descriptor	Units	Time point				
				D0	D10	D15	D30	D120–240
N01	A wound area	\bar{x}	cm ²	10.87	5.90	2.02	0.00	0.00
		SD	cm ²	0.16	0.21	0.25	0.00	0.00
		CV	-	1.5%	3.6%	12.4%	n/a	n/a
	%A healed area	\bar{x}	-	0%	46%	81%	100%	100%
		SD	-	0.0%	1.1%	2.6%	0.0%	0.0%
		CV	-	0.0%	2.4%	3.2%	0.0%	0.0%
	\dot{A} healing rate	\bar{x}	cm ² /day	n/a	0.50	0.78	0.13	0.00
		SD	cm ² /day	n/a	0.01	0.09	0.02	0.00
		CV	-	n/a	2.0%	11.5%	15.4%	0.0%
N04	A wound area	\bar{x}	cm ²	37.54	40.40	38.22	22.37	0.00
		SD	cm ²	5.74	3.79	0.69	4.20	n/a
		CV	-	15.3%	9.4%	1.8%	18.8%	0.0%
	%A healed area	\bar{x}	-	7.3%	0.0%	5.1%	43.9%	100.0%
		SD	-	5.5%	0.0%	7.2%	15.6%	0.0%
		CV	-	75.3%	n/a	141.2%	35.5%	0%
	\dot{A} healing rate	\bar{x}	cm ² /day	n/a	-0.29	0.44	1.06	0.15
		SD	cm ² /day	n/a	0.20	0.62	0.33	0.03
		CV	-	n/a	-69.0%	140.9%	31.1%	20.0%

50% or more. Upon investigating the general shape of the curves, in particular in Figure 6B curve depicting *Healing rate vs Time*, the first part of the curves (from the beginning of the data set to maximum value) have a comparable, accelerating shape, but the latter part (from maximum value to the last data point) have differing shapes, possibly owing to lack of suitable complementary time/data points between D30 and D120–240.

Histology

As described in the *Methods*, pre-surgery histology would only be done with the patient’s pre-existing scar tissue, removed during the first stage prior to the application of biomembranes. Moreover, post-surgery histology of a biopsy taken from the biomembrane-treated healed wounds would only be done in conjunction with another surgical procedure executed at a later date, i.e., no biopsy would be taken solely for the purpose of obtaining post-surgery histology data.

In this interim report, patient N01’s pre-existing scar tissue was removed, and the corresponding histomicrograph is shown in Figure 7 (A and C). In the case of patient N04, no pre-existing scar tissue was available for histology and the biomembranes were applied directly to the wound bed as described above. At the time of writing, no later surgical procedures

were scheduled for either of the two individuals covered by this interim report, and, as such, no post-surgery histology data is available. Figure 7 (B and D) shows a histomicrograph of a biomembrane representative of the biomembranes applied in the study.

Dataset 1. Biomembrane data files. This dataset contains the unedited photographs from the study

<http://dx.doi.org/10.5256/f1000research.15138.d206373>

Discussion

The present clinical trial did not have a randomized sample assigned to a treatment and a placebo arm; rather, the sole sample group was the treatment group, and a quasi-experimental control group (pseudo-placebo arm; Figure 1) was taken as the clinicians’ assessment of how the individual in question would fare *without* the treatment with the biomembrane. In both patients reported in this interim report, the biomembranes were capable of stimulating formation of quality skin tissue and complete wound closure in the time frame studied, indicating efficacy. The clinicians’ assessment (J.F.L.N. and M.H.S.C) was that the wounds would take approximately 3 × longer to heal compared to the time it took using the biomembrane treatment, or, possibly, the wounds would not have healed

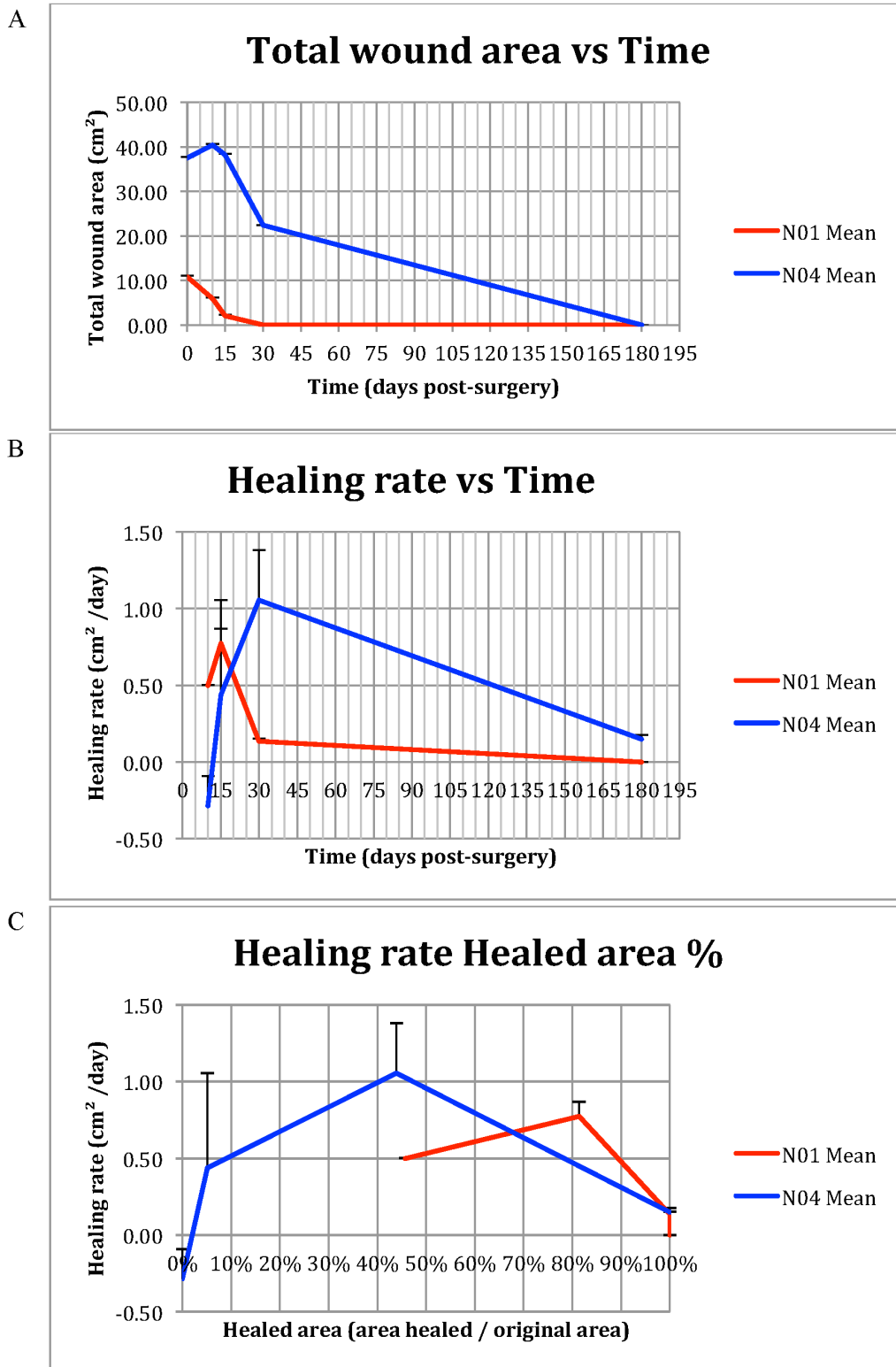


Figure 6. Graphs depicting data from **Table 2.** (A) Total wound area vs Time. (B) Wound healing rate vs Time. (C) Wound healing rate vs Wound healing percentage. Error bars indicate +1 SD from mean data point.

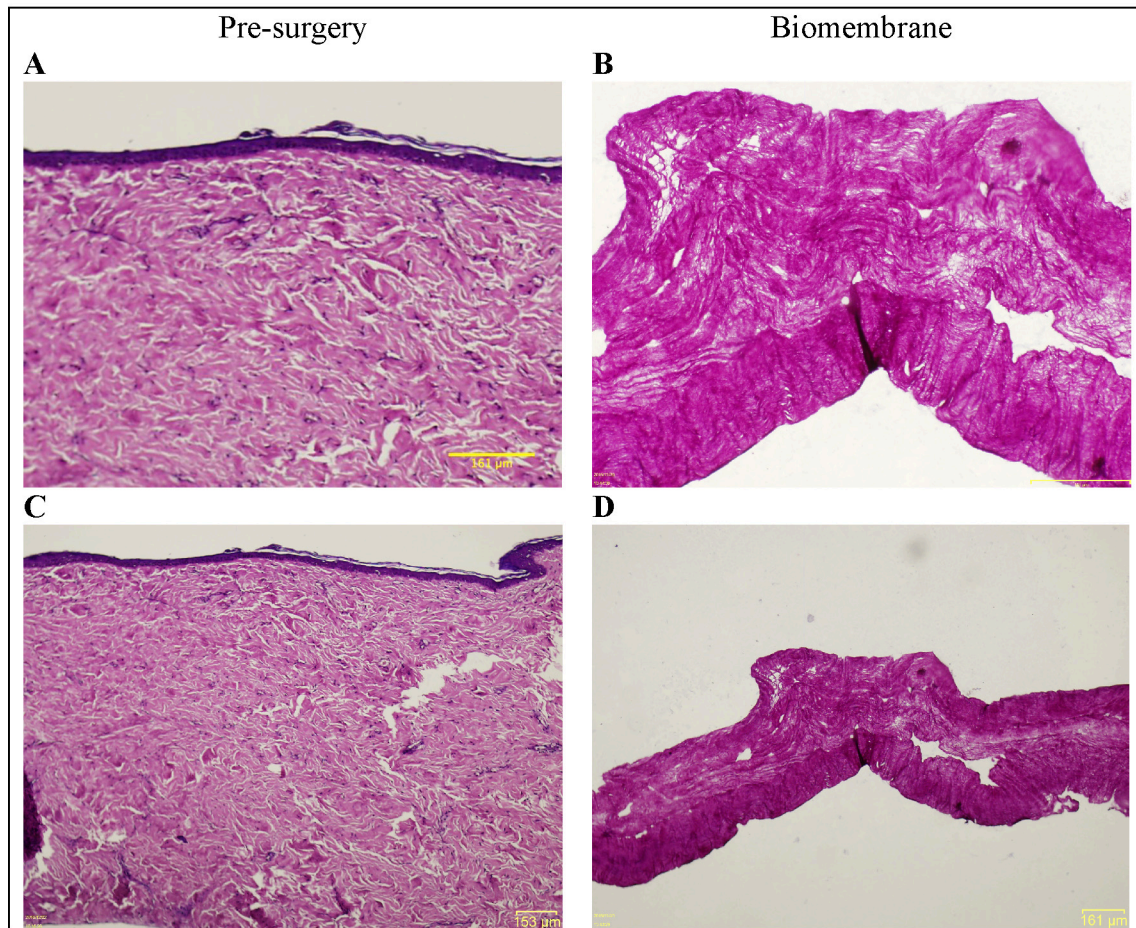


Figure 7. Hematoxylin and eosin (H&E) histomicrographs. (A and C; 20 × and 4 × magnification of same specimen) Hematoxylin and eosin (H&E) histomicrograph of N01's pre-surgery tissue in the treatment area, exhibiting numerous hallmarks of fibrotic scar tissue. Epidermis is flattened, with no undulation on the surface, no epidermal rete ridges extending into the dermis, and no dermal papillae. The uppermost papillary dermis presents a dense collagenous matrix, and the lowermost, reticular dermis exhibits highly acidophilic regions with denser bundles of collagen. (B and D; 20 × and 4 × magnification of same specimen) H&E histomicrograph of a representative collagen biomembrane (i.e., equivalent to the implanted biomembranes prior to their utilization). Horizontally aligned thin collagen fibers with some larger pores interspersed throughout the structure, along with smaller pores with a more uniform distribution. The lowermost part of the structure presents increased acidophilicity and collagen density. This particular part of the histomicrograph shows the local elevation of approximately 100 μm in height. These elevations are part of the characteristics of the biomembrane, and are present throughout the structure, spaced approximately 2.5 mm from one another.

at all, or formed fibrotic scar tissue, indicating superiority compared to conventional wound therapy. No adverse effects were noted, indicating safety. In addition to the objectives of the study pertaining to skin repair, an additional apparent benefit of the biomembrane treatment was seen with the apparent stimulation of fur growth, a characteristic that will be investigated in more detail for subsequent patients enrolled in the study. The image analysis method for quantitative size assessments is robust and accurate for photographs taken perpendicular to the wound (commercial systems also exclusively mandate perpendicular image capturing), but when taken at an angle, even with the trigonometric correction as detailed herein, the final size will suffer and not present good accuracy. Ideally, all photographs should be taken completely perpendicularly to the wound to maximize accuracy (and precision).

The enrolment of patients has been slow, due to cost considerations, but agreements have been made with new partner clinics to ensure the total number of 15 patients is reached by the end of the study period. We believe the biomembrane is well suited for wound healing in veterinary patients, and foresee other uses as well, e.g. as a periosteum substitute. Moreover, due to the similarities between the veterinary medical device studied in this report and human medical devices based on tissue engineering technologies, we expect that the study may have repercussions beyond the veterinary domain and into the human domain, and vice versa.

Data availability

Dataset 1: Biomembrane data files. This dataset contains the unedited photographs from the study. DOI, [10.5256/f1000research.15138.d206373](https://doi.org/10.5256/f1000research.15138.d206373) (Kaasi *et al.*, 2018).

Competing interests

The first author (A.K.) is the founder and shareholder of Eva Scientific Ltd, where the biomembrane used in this clinical trial was developed and is sold.

The co-authors are veterinary practitioners of Sorocaba Veterinary Hospital (J.F.L.N., J.A.M.F. and M.H.S.C.) and full-time employees of University of Campinas (A.L.J. and P.K.). All co-authors read and agreed to the manuscript and had full access to the data, and attest to the study's impartiality as a whole, as well as declare no competing interests.

Grant information

This work was supported by the Council for Scientific and Technological Development (CNPq 573661/2008-1) and São Paulo Research Foundation (FAPESP 2008/57860-3) grants awarded to Biofabris (The National Institute of Biofabrication) and by a FAPESP grant awarded to Eva Scientific Ltd (FAPESP 2014/22799-3).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Supplementary material

Supplementary File 1: CONSORT checklist.

[Click here to access the data.](#)

Supplementary File 2: Study protocol.

[Click here to access the data.](#)

Supplementary File 3: Footage of application of double-layer regenerative collagen biomembrane.

[Click here to access the data.](#)

References

- Bell E, Ehrlich HP, Buttle DJ, *et al.*: **Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness.** *Science*. 1981; **211**(4486): 1052–1054.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Bills JD, Berriman SJ, Noble DL, *et al.*: **Pilot study to evaluate a novel three-dimensional wound measurement device.** *Int Wound J*. 2016; **13**(6): 1372–1377.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Davis KE, Constantine FC, Macaslan EC, *et al.*: **Validation of a laser-assisted wound measurement device for measuring wound volume.** *J Diabetes Sci Technol*. 2013; **7**(5): 1161–1166.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dorin RP, Pohl HG, Filippo RE, *et al.*: **Tubularized urethral replacement with unseeded matrices: what is the maximum distance for normal tissue regeneration?** *World J Urol*. 2008; **26**(4): 323–326.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Eisenbud D, Huang NF, Luke S, *et al.*: **Skin Substitutes and Wound Healing: Current Status and Challenges.** *Wounds*. 2004; **16**(1): 2–17.
[Reference Source](#)
- Fürdös I, Fazekas J, Singer J, *et al.*: **Translating clinical trials from human to veterinary oncology and back.** *J Transl Med*. 2015; **13**(1): 265.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Gawai AA, Shaikh F, Gadekar M, *et al.*: **A review on: Phase '0' clinical trials or exploratory investigational new drug.** *Turkish Journal of Pharmaceutical Sciences*. 2017; **14**(1): 84–89.
[Publisher Full Text](#)
- Groeber F, Holeiter M, Hampel M, *et al.*: **Skin tissue engineering--*in vivo* and *in vitro* applications.** *Adv Drug Deliv Rev*. 2011; **63**(4–5): 352–366.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kaasi A, Lima-Neto JF, Matiello-Filho JA, *et al.*: **Dataset 1 in: Regenerative collagen biomembrane: Interim results of a Phase I veterinary clinical trial for skin repair.** *F1000Research*. 2018.
[Data Source](#)
- Papini R: **Management of burn injuries of various depths.** *BMJ*. 2004; **329**(7458): 158–160.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Rheinwald JG, Green H: **Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells.** *Cell*. 1975; **6**(3): 331–343.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schneider CA, Rasband WS, Eliceiri KW: **NIH Image to ImageJ: 25 years of image analysis.** *Nat Methods*. 2012; **9**(7): 671–675.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Vail DM: **Cancer clinical trials: development and implementation.** *Vet Clin North Am Small Anim Pract*. 2007; **37**(6): 1033–1057; v.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Yannas IV, Burke JF: **Design of an artificial skin. I. Basic design principles.** *J Biomed Mater Res*. 1980; **14**(1): 65–81.
[PubMed Abstract](#) | [Publisher Full Text](#)

Open Peer Review

Current Referee Status:



Version 1

Referee Report 11 January 2019

<https://doi.org/10.5256/f1000research.16492.r41549>



Kalan Bastos Violin 

Material Science and Technology Center, Nuclear and Energy Research Institute (IPEN) , São Paulo, Brazil

The article seems to provide enough evidence for the proposed goal, which basically was to induce skin repair in a critical size wound equivalent to 2nd to 3rd degree burns in depth, using an engineered biomembrane based on collagen type I.

The biggest challenge was to gather enough subjects eligible to the study, presenting only 2 cases isn't ideal, but expected since real cases for clinical trials are difficult to respond to all criteria to be included. For further studies these criteria should be flexed to maximize the number of subjects included, specially if the exclusion of those were related to unsuccessful results, cut to not compromise the product. A larger number of subjects is desirable and for clinical trials mandatory. Nevertheless for this level of initial trial and concept it is acceptable and the results were remarkable.

Another point to be kept for the following research is to give a molecular approach to the biopsies. Basic histology just present the morphological aspects of the tissue and it would be interesting to gather from these same samples an immunohistochemistry analysis to characterize the molecular content of the formed tissue looking for proteins related to repair and healing, but also for inflammatory and regulatory markers that play an important role in those process. This would give a enormous depth to the consolidation of the material and present more reliable and straightforward data for comparison.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Biomaterials, Experimental and Comparative Pathology, Pre-clinical Trials, Histopathology, Veterinary Medicine, Immunohistochemistry, Lectin histochemistry, Image Analysis, Microscopy

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 27 September 2018

<https://doi.org/10.5256/f1000research.16492.r34932>

**Iris Ribitsch**

Department for Companion Animals and Horses, Veterm, University Equine Hospital, Vetmeduni Vienna, Vienna, Austria

Introduction:

The introduction focuses on an important topic which is drawing more and more attention in medical sciences: The idea of “one health” - the collaborative effort of multiple disciplines – to attain optimal health for people, animals and the environment. The authors point out the necessity to conduct veterinary clinical trials in a standardized manner in order to obtain valid and meaningful results to facilitate the translation into human medicine. Currently there is a lack of global consensus concerning the rules and regulations governing veterinary clinical trials.

However, this discussion (although very important) should not make the bigger part of the introduction. Instead please include a discussion of solid scientific evidence on the similarities and discrepancies of human, feline and canine wound healing and why it may be valid to extrapolate results from one species to the other. Furthermore, please offer an overview of the research question, the state of the art in the field, how the current state may be improved, what is new about the collagen membrane used in this study compared to other similar products and how and why the collagen membrane employed in this study may therefore contribute to an improvement of the current state of the art. Currently the collagen membrane, which is the core of this paper, is not described whatsoever.

Methods:

The materials and methods section offers a description of the study design of the envisaged phase 1 clinical trial. It gives in detail guidelines how to proceed at certain stages in the course of the study and based on findings which may be encountered within the scope of the study (e.g. “If the affected area had been subject to natural scarring, with the formation of hard, unhealthy, fibrotic scar tissue, this was surgically removed, exposing subcutaneous tissue. In this case, the tissue would be fixed in 10% formalin and submitted to histological analysis, using standard procedures. If the treatment area was an unhealed, deepithelialized wound bed, but with no fibrotic scar tissue formation, then shallow scalpel incisions were made scattered throughout the treatment area, causing local bleeding, with the aim of promoting regeneration and integration with the later application of the collagen biomembranes.”

“If any adverse effects were confirmed, in the first 1–2 days, then the biomembranes were excised or scraped off host tissue. If adverse effects were confirmed in later stages (>D3), the biomembranes were not excised due to the fact that by this time they had absorbed significantly into the host tissue, rendering removal difficult or impossible. In this case, the patients were treated symptomatically and added to the “Discontinued intervention” group...”).

Also detail on how data should be obtained and by whom are included (“When possible, a qualitative assessment of skin tissue quality would be done, either by a researcher associated with the study or by the owner. If another surgical procedure was scheduled for a study participant, for any reason, a small skin biopsy was attempted to be taken from the treatment area....”).

However, the materials and methods should provide detailed information on methods and analysis carried out particularly for the current 2 patients included into the study.

The anaesthesia regimen which is described in great detail is not so relevant for the interpretation of the study results as e.g. the preparation of the skin prior to surgery which may have considerable influence on the healing process. Please provide more information.

Results:

Following up clinical patients especially long term is very challenging and depends on the cooperation of the animal owners and their willingness and time to take their animals to the follow up examinations etc. Using series of pictures which can be taken either by the owners or the treating veterinarian at defined time points to document the healing process will certainly facilitate cooperation. Detailed instructions when and how (distance camera to animal, angle camera to wound etc.) are offered in the methods section. However, the photos in the paper (Figure 5) do unfortunately not look standardised even when comparing only the pictures taken from one patient, which hampers comparability.

- Is the work clearly and accurately presented and does it cite the current literature? No.
- Is the study design appropriate and is the work technically sound? No.
- Are sufficient details of methods and analysis provided to allow replication by others? Yes
- If applicable, is the statistical analysis and its interpretation appropriate?

Meaningful statistical analysis on $n=2$ is impossible.

A detailed description of the results is more useful in this case.

- Are all the source data underlying the results available to ensure full reproducibility? Yes
- Are the conclusions drawn adequately supported by the results? Partly

The results of the study are interesting and encouraging and (so I am convinced) worth further research efforts.

However, in order to improve the quality of the clinical study standardisation should be increased, the photo documentation should be improved, mandatory re-checks with the treating veterinarian should be implemented during which a healing score is obtained based on healing rate and a qualitative assessment of newly formed skin tissue. Histologic evaluation pre and post-surgery should be implemented. Also inclusion of a control group would considerably increase the quality of the study. The clinicians assessment (“The clinicians’ assessment was that the wounds would take approximately 3 × longer to heal compared to the time it took using the biomembrane treatment, or, possibly, the wounds would not have healed at all, or formed fibrotic scar tissue, indicating superiority compared to conventional wound therapy.) and a ‘quasi-experimental control group’ are not sufficient. I certainly agree with the author’s that it wouldn’t be possible and ethically debateable to include a control group receiving no treatment, but a control group receiving the current gold standard of wound treatment would be

feasible and still increase the quality of the study.

I strongly suggest publishing the results from the two patients as case reports because they are valuable and interesting for the scientific community and veterinary clinicians, but at the current stage unfortunately not sufficient for an original research article. However, I am looking forward to read more and see more results to come from further patients enrolled into this interesting and promising clinical trial and wish the authors all the best for the continuation of their study.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Veterinary medicine, regenerative medicine, tissue engineering

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 19 July 2018

<https://doi.org/10.5256/f1000research.16492.r35668>



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² Orthopaedic Research UK, London, UK

³ Mechanical Engineering Department, University College London, London, UK

⁴ Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

The manuscript entitled, "Regenerative collagen biomembrane: Interim results of Phase I veterinary clinical trial for skin repair" by Kaasi et al explores the application of acellular collagen membranes for veterinary wound healing purposes in dogs and cats. The study provides the preliminary results to what

may be considered a phase I clinical study on animal subjects that had been victims of injuries with the immediate need of care. The manuscript is well written with a good structure and the data presented is interesting and worth publishing. There are, however, some minor clarifications and modifications that are suggested to improve the manuscript before publication.

- 1: The study presents an interesting idea of letting veterinary clinical trials pave the way for human use and equivalent therapy. Currently, there is a lack of global consensus concerning the rules and regulations governing veterinary clinical trials. The establishment of such rules and regulations could possibly make the translational process from veterinary subjects to man more flexible for some medical devices by providing substantial data and thereby allow a faster route to regulatory approval. It is suggested that the authors add a couple of sentences in the discussion section on the potential prospect of the proposed wound healing product for human applications and the long-term objectives or plans of the authors for the clinical translation of the product.
2. There is not sufficient information on the characteristics of the collagen biomembrane to fully differentiate and appreciate the current approach. Please provide more information concerning the main attributes of the collagen biomembrane and a comparison with similar marketed products or products in development. How does the presented collagen membrane differ from other biomembranes including cell-incorporating and synthetic alternatives?
3. It is somewhat unclear with the current status of the animal studies. Please provide additional information regarding the current status with the number of completed animal subjects and information and timeline for the remaining studies and subjects
4. If there were any adverse effects noticed such as infection and necrosis after application of the biomembrane, what measures are there to investigate this further? Did this occur in this current study? If yes, please provide some insight as to why you think this was the case.
5. Regarding photographing the patients, how do the authors explain the method of 'Semi-quantitative assessment'? More detail is required about this method. Going forward, the method of photographing animals should be standardised in order to lessen the chance of errors in the study.
6. There are no clear controls for each model presented. We appreciate that this is a very challenging task to achieve at this stage, however going forward a 'quasi-experimental control group' will not suffice. We would advise the researcher to have solid controls in place when recruiting subjects for the next studies.
7. The histological evidence provided here is very limited. This needs to be improved upon significantly. The histological data presented in this paper is only for one patient and only in a pre-surgical stage (A and C). The researchers need to elaborate on the reasons why they were unable to provide small histological samples from patients after treatment. The local elevations present on the biomembrane and pores are highlighted in Figure 7, however, the biological significance of each characteristic is missing. It would also be great if the researchers made the scales more visible on the H&E images.
- 8- The authors refer to an apparent fur growth on one of the patients, however, this is not backed by supporting evidence as the numbers are very low. Please refrain from using terms such as 'benefit' and 'stimulation' in these instances and just state what has been observed and report fully when enough evidence is available.

9. Please provide an indication of the time generally required to get a veterinary medical device on the market.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

We have read this submission. We believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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