Innovative Burn Treatment Using Tilapia Skin as a Xenograft: A Phase II Randomized Controlled Trial

Edmar Maciel Lima Júnior, MD, MS,* Manoel Odorico de Moraes Filho, MD, PhD,† Bruno Almeida Costa, MBBS,† Andréa Vieira Pontes Rohleder, BSPharm, PhD,† Marina Becker Sales Rocha, BSNut, PhD,† Francisco Vagnaldo Fechine, MD, PhD,† Antonio Jorge Forte, MD, PhD,‡ Ana Paula Negreiros Nunes Alves, BDent, PhD,‖ Francisco Raimundo Silva Júnior, BSN,* Camila Barroso Martins, BSN,† Mônica Beatriz Mathor, BSPharm, PhD,§ and Maria Elisabete Amaral de Moraes, MD, PhD†

Skin substitutes are considered a useful alternative for occlusive dressings in the treatment of superficial burns as they reduce the frequency of dressing replacement. This phase II randomized controlled trial aimed to evaluate the efficacy of Nile tilapia (*Oreochromis niloticus*) skin as an occlusive xenograft dressing for the treatment of burn wounds in humans. In order to assess the use of tilapia skin, the following variables were evaluated: number of days for wound healing, the number of times the occlusive dressing was changed, use of anesthetics or analgesics, pain assessment using the Visual Analogue Scale, and evaluation of burn improvement on the day of dressing removal. In total, 62 participants completed the study. It was found that in participants treated with tilapia skin, complete reepithelialization occurred in significantly fewer days; reported pain intensity was lower (study arms B and C), the amount of anesthetics/analgesics required was lower (study arms B and C), and the necessity of dressing changes was significantly reduced in comparison with volunteers treated with silver sulfadiazine. In our study, the tilapia skin xenograft showed good efficacy as an occlusive biological dressing for burn wound treatment in humans.

According to the World Health Organization, burns are responsible for 180,000 deaths annually. Moreover, nonfatal burns account for prolonged hospitalization, disfigurement, and disability, coupled with stigma and rejection.[1] The time required for wound healing is one of the main determinants for the development of complications,[2] and infection is one of the leading causes of death in burn patients.[3]

Numerous studies have been carried out to identify dressings capable of reducing wound contamination, enabling the healing process, and providing better aesthetic results. As a result, biological materials have been sought as cost-effective alternatives for the local treatment of burn wounds.[4] Given these facts, the skin of the Nile tilapia (*Oreochromis niloticus*) appears as a new high-quality biomaterial with a peculiar, leather-like resistance[5] and several potential clinical applications, with case reports in the fields of plastic surgery[6,7] and gynecology.[8]

Before considering Nile Tilapia Fish Skin (NTFS) as a therapeutic option for burns in humans, preclinical studies were conducted, with NTFS showing a noninfectious microbiota,[9] morphological structure similar to that of human skin[10,11] and good outcomes when used for the treatment of experimental burns in animal models.[12] Based on our preliminary preclinical and phase I studies, and on the promising results of our recently published pilot study with pediatric patients,[13] this phase II randomized controlled trial (RCT) aims to evaluate the efficacy of NTFS as a xenograft for the treatment of burn wounds in humans.

MATERIAL AND METHODS

Participants

This is a randomized, monocentric, open-label, phase II clinical study conducted in Fortaleza, Brazil, from October 2016 to September 2017. The local Institutional Review Board (IRB) approved the study protocol and informed consent. The research was conducted in accordance with the 1975 Declaration of Helsinki and its amendments and is registered at ClinicalTrials.gov with the identification number NCT03592498. Informed consent was obtained from all participants.

The study participants were recruited from a local burn treatment center. Both female and male participants were included. Inclusion criteria were: age ≥18 and ≤50 years; the presence of dermatological wounds caused by superficial partial-thickness burns (SPTB) affecting up to 20% of the total body surface area (TBSA), or deep partial-thickness burns (DPTB) affecting 5% to 15% of the TBSA; and the absence of previous treatment for

*From the *Burn Treatment Center, Dr. José Frota Institute, Fortaleza, CE, Brazil; †Clinical Pharmacology Unit, Drug Research and Development Center, Federal University of Ceará (UFC), Fortaleza, CE, Brazil; ‡Division of Plastic and Reconstructive Surgery, Mayo Clinic, Jacksonville, Florida; ‖Nursing, Dentistry and Pharmacy School of the Federal University of Ceará (UFC), Fortaleza, CE, Brazil; §Institute of Nuclear Energy Research—IPEN-CNEN/SP, São Paulo, SP, Brazil*

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Address correspondence to Bruno Almeida Costa, MBBS, Clinical Pharmacology Unit, Drug Research and Development Center, Federal University of Ceará (UFC), Fortaleza, Ceará, Brazil. Email: bruno_almeida_costa@hotmail.com

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the current burn and an absence of other significant diseases that could affect the volunteer's participation in the study (coronary artery disease, peripheral vascular disease, cancer, diabetes mellitus, among others). Exclusion criteria included hypersensitivity to materials used in the study or to related compounds; history of severe adverse reactions; drug addiction, including alcohol; use of medications that could have an impact on wound healing (eg, steroids); and pregnancy, labor, or miscarriage in the 12 weeks before the scheduled start of treatment. Finally, patients with burns in the face, neck, genitalia, perineum, axillae, groin, and buttocks were not included due to the difficulty associated with NTFS adherence in these areas.

Study Arms

The study was divided into three arms. Patients were allocated in each of the arms according to burn depth and TBSA affected: Arm A—outpatients with SPTB involving <10% of TBSA; Arm B—inpatients with SPTB involving 10% to 20% of TBSA; Arm C—inpatients with DPTB involving 5% to 15% of TBSA. Participants from each study arm were randomly assigned to the following treatment groups: Test Group—NTFS used as a xenograft; Control Group: conventional treatment with silver sulfadiazine cream 1% (SSDC). A diagram explaining the study design can be seen in Figure 1.

Interventions

In the test group, the treatment was NTFS (*Oreochromis niloticus*) (Patent registered with INPI under number BR 10 2015 021435 9). NTFS was subjected to a rigorous process of chemical sterilization, glycerolization, and irradiation, followed by microbiological tests for bacteria and fungi, before storage in sterile-refrigerated packaging. Costa et al described this process in more details.[6] Lima Junior et al showed that the biomaterial did not present variations in its microscopic and tensiometric structure after chemical sterilization and irradiation and recovered its natural consistency after the rehydration process.[11]

In the control group, silver sulfadiazine (dermatological cream: 10 mg/g) was used. The choice of this cream-based topical antiseptic, instead of a different xenograft, cadaver allograft, or other membranous dressing, resides in the fact that, in the Brazilian public healthcare system, almost all burn centers still use the SSDC as a standard treatment.[14]

Following clinical evaluation (physical examination, vital signs, and anthropometric data), study participants were allocated to their respective study arms (A, B, or C). Then, patients in each of the study arms were assigned to one of the two treatment groups (test or control).

Randomization was performed using a predefined computer-generated list for each arm of the study and patients were blinded to the hypothesized effects of either treatment. After enrollment in the study, the patients went through standard procedures depending on the treatment group to which they were allocated.

In the test group of each study arm, after adequate removal of loose skin and debris of the lesion using tap water and 2% chlorhexidine gluconate, NTFS was applied and covered with gauze and bandage. Throughout the treatment, dressings with NTFS were only changed if the biomaterial was not properly adhered to the wound bed. In order to check such adherence, bandages were removed every 48 hours; if the biological dressing had adhered adequately, bandages were replaced. In study arms B and C, the first dressing of the hospitalized patient was prepared under anesthesia with ketamine and midazolam, allowing painless removal of loose skin and debris. NTFS dressing removal in the last day of treatment consisted of a quick and simple process, with no analgesia or anesthesia needed. Patients were placed under a shower and the wounds were soaked with water. The hydration process led to weakening, breaking, and slippage of the tilapia skin, with exposure of the underlying healed skin. NTFS could also be removed by digital separation of the biomaterial from the wound bed with the aid of petrolatum jelly.

In the control group of each study arm, after cleaning the lesion with tap water and 2% chlorhexidine gluconate, a thin layer of SSDC was applied and covered with gauze and bandage. The dressing changes occurred every 48 hours for patients in study arm A (outpatients) and daily for patients in

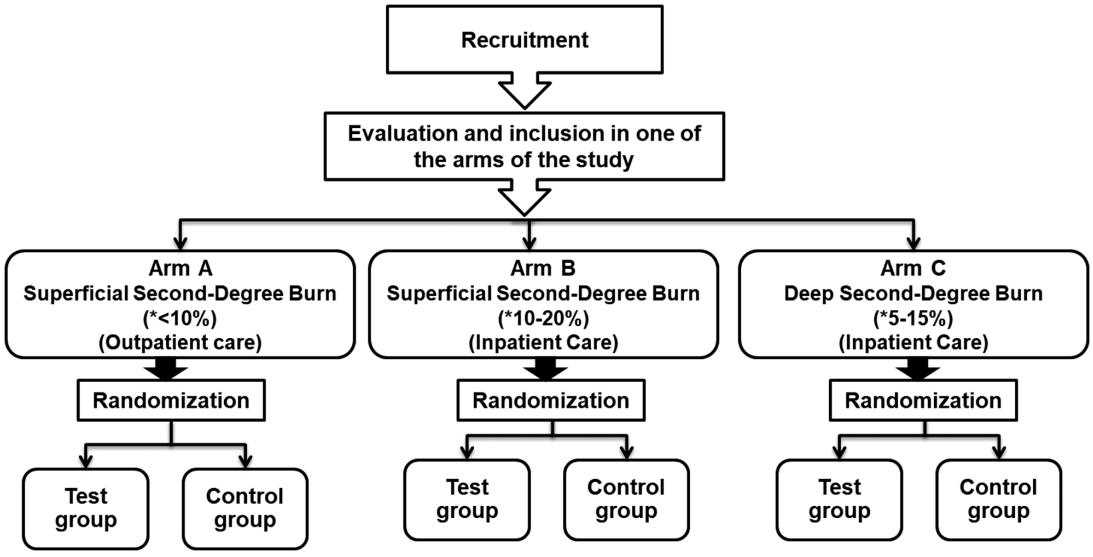

Figure 1. Flowchart of the inclusion and allocation of the research participants in the study groups.

study arms B and C (inpatients). Also, in study arms B and C, the first dressing of the hospitalized patient was prepared under anesthesia with ketamine and midazolam, allowing proper removal of loose skin and debris.

Outcome Assessment

Patients in the study arm A were evaluated clinically and for the study parameters every 48 hours until the wound had healed. Patients in the study arms B and C were evaluated every 6 hours for vital signs and clinical conditions, every 24 hours for dressing change necessity, and every 48 hours for the other study parameters until the wound had healed. The same research team performed all evaluations.

The primary outcome variables were the number of days until complete burn wound healing (≥95% reepithelialization calculated via clinical judgment from the consultant), the number of dressings performed, and the amount of anesthetics or analgesics required throughout the treatment. The following secondary outcome variables were also defined: degree of pain throughout the treatment, as assessed via the Visual Analogue Scale (VAS), and evaluation of burn improvement on the day of dressing removal, as assessed via the Clinical Global Impression Scale-Improvement (CGI-I).

In the control group, a dressing change was defined as the act of cleaning the wound and reapplying the SSDC, which was performed daily (study arms B and C) or every 48 hours (study arm A). In the test group, a dressing change was defined as the act of replacing the NTFS which did not adhere properly and/or replacing gauze and bandage that is full of exudate. Differently from the control group, the time between test group dressing changes was not fixed, but instead dependent on the necessity for change. NTFS observed to have adhered to the burned area was left in the wound bed until completion of reepithelialization. If NTFS did not adhere, it was removed, the area was cleaned, and then the biomaterial was applied again, usually with the patient under anesthesia.

To audit analgesic and anesthetic medication intake in arm A subjects (outpatients), study participants received an analgesic medication prescription and a daily record card and were instructed to take the medication only if they felt pain and register the amount of medication taken and the date on the daily record card. Upon returning for medical evaluation, the remaining medication was checked and compared with the daily record card. In study arms B and C (inpatients), nurses were trained to record all analgesic and anesthetic medication used on the patient's clinical record, allowing calculation of the amount of medication used.

Regarding pain evaluation, after application of the dressing, participants were submitted to the Visual Analogue Scale (VAS). The VAS was assessed on a 10 cm horizontal line. The patients were informed that the left end of the scale (with a "0") represented "no pain" and that the right end of the scale (with a "10") represented the "most severe pain imaginable." Study participants were asked to make a mark on the line that represented their current pain intensity, and the VAS pain intensity level was scored by measurement of the distance from the mark to the "no pain" end of the line.[15] Pain measurement was performed immediately after dressing changes in Arm A subjects (in an outpatient setting). In hospitalized subjects (groups B and C), the pain scale application was performed when they were awake, 1 hour after dressing changes. When assessing the pain level during the subsequent visits, the patients were not informed of the previous VAS results.

The CGI-I is evaluated by the physician responsible and answers the following question: "Compared to the patient's condition at admission to the project, this patient's condition is: 1 - very much improved since the initiation of treatment; 2 - much improved; 3 - minimally improved; 4 - no change from baseline; 5 - minimally worse; 6 - much worse; 7 - very much worse since the initiation of treatment."[16]

Statistical Analysis

The analysis was based on the intention-to-treat principle. The quantitative variables (continuous and discrete) were initially analyzed by the Shapiro–Wilk test to verify the normality of the distribution. For descriptive statistics, the mean and standard deviation (parametric data) or the median, interquartile range, and minimum and maximum values (nonparametric data) were calculated. Point-to-point comparisons between the SSDC and NTFS groups were made using the *t* test for unpaired variables (parametric data) or the Mann–Whitney *U* test (nonparametric variables). A type of variance analysis for two classification factors with repeated-measures (two-way repeated-measures ANOVA) was used to compare the effect of the two treatments on pain intensity (factor 1: silver sulfadiazine and tilapia skin, factor 2: visit number), complemented by the Bonferroni multiple comparisons test (comparisons between treatments at each visit) and Dunnett's test (comparisons between follow-up visits and V1 within the same treatment group).

Regarding parametric data, in addition to statistical significance, we also determined the mean difference and its respective 95% confidence interval. Categorical variables, in turn, were expressed as absolute and relative frequency, whereas ordinal variables were expressed as median, interquartile range, and minimum and maximum values. Comparisons of the categorical variables between the two treatments were performed by the chi-square test. To compare the two groups in relation to ordinal variables, the Mann–Whitney *U* test was used. In all analyses, two-tailed tests were used, considering a *P*-value less than .05 as statistically significant. GraphPad Prism software version 7.00 (GraphPad Software, La Jolla, CA, 2016) was used for both statistical procedures and development of the graphs.

RESULTS

Demographic and Clinical Data

A total of 62 participants completed the study: 23 were allocated to arm A (10 in the control group and 13 in the test group), 19 were allocated to arm B (10 in the control group and 09 in the test group), and 20 were allocated to arm C (10 in the control group and 10 in the test group). The demographic and clinical characteristics of the participants of all arms of the study (A, B, and C) can be observed in Table 1.

Reepithelialization

Table 2 shows the number of days until complete reepithelialization in all study arms. The number of days for

Table 1. Demographic and clinical characteristics of the research participants

Patient and burn characteristics	Study A			Study B			Study C		
	Silver sulfadiazine	Tilapia skin	*P*	Silver sulfadiazine	Tilapia skin	*P*	Silver sulfadiazine	Tilapia skin	*P*
N	10	13	–	10	09	–	10	10	–
Age (yr) – Mean ± SD	32.50 ± 8.15	37.77 ± 12.75	.2683*	37.40 ± 7.82	33.00 ± 7.77	.2231*	36.60 ± 10.00	31.20 ± 11.22	.2709*
Sex			.8548†			1.0000†			.6392†
Male	5 (50.00%)	7 (53.85%)		4 (40.00%)	4 (40.00%)		7 (70.00%)	6 (60.00%)	
Female	5 (50.00%)	6 (46.15%)		6 (60.00%)	6 (60.00%)		3 (30.00%)	4 (40.00%)	
BMI (kg/m^2) – Mean ± SD	26.54 ± 2.79	26.40 ± 2.73	.9023*	25.71 ± 3.43	26.68 ± 2.14	.4560*	26.92 ± 5.30	25.02 ± 2.45	.3168*
Wound area (% body surface area) – Mean ± SD	5.20 ± 3.29	4.54 ± 1.85	.5473*	15.40 ± 3.84	16.40 ± 3.41	.5453*	20.20 ± 8.07	13.40 ± 2.12	.0189*
Burn location			.4142†			.5150†			.0523†
1 segment	6 (60.00%)	10 (76.92%)		0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
2 segments	2 (20.00%)	3 (23.08%)		1 (10.00%)	1 (11.11%)		0 (0.00%)	4 (40.00%)	
3 segments	1 (10.00%)	0 (0.00%)		7 (70.00%)	5 (55.56%)		3 (30.00%)	4 (40.00%)	
4 segments	1 (10.00%)	0 (0.00%)		1 (10.00%)	3 (33.33%)		6 (60.00%)	1 (10.00%)	
5 segments	0 (0.00%)	0 (0.00%)		1 (10.00%)	9 (00.00%)		1 (10.00%)	1 (10.00%)	
Global clinical impression (severity of burn) – medium (IQ)	4 (3–4)	4 (3–4)	.4636‡	4 (4–5)	4 (4–5)	.8296‡	5.00 (4.00–5.00)	4.00 (4.00–4.25)	.0318‡
Pain intensity (AVS) – Mean ± SD	8.40 ± 1.78	6.69 ± 2.98	.1245*	9.10 ± 1.73	8.40 ± 1.84	.3919*	8.30 ± 2.26	8.30 ± 2.45	1.0000*
Causative agent			.548†			.3679†			.5268†
Hot liquid	8 (80.00%)	11 (84.62%)		5 (50.00%)	7 (77.78%)		0 (0.00%)	0 (0.00%)	
Fire	1 (10.00%)	1 (7.69%)		1 (10.00%)	0 (0.00%)		1 (10.00%)	1 (10.00%)	
Hot oil	1 (10.00%)	0 (0.00%)		0 (0.00%)	1 (11.11%)		2 (20.00%)	1 (10.00%)	
Butane gas	0 (0.00%)	1 (7.69%)		3 (30.00%)	1 (11.11%)		4 (40.00%)	1 (10.00%)	
Hot steam	0 (0.00%)	0 (0.00%)		1 (10.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)	
Gasoline	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)		2 (20.00%)	2 (20.00%)	
Alcohol	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)		1 (10.00%)	3 (30.00%)	
Electricity	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)		0 (0.00%)	1 (10.00%)	
Gunpowder	0 (0.00%)	0 (0.00%)		0 (0.00%)	0 (0.00%)		0 (0.00%)	1 (10.00%)	

SD, standard deviation; *BMI*, Body mass index; *IR*, interquartile range; *AVS*, analog visual scale.
*t test for unpaired data.
†chi-square test.
‡Mann–Whitney test.

Table 2. Number of days for complete reepithelialization in all study arms

Reepithelialization (number of days)	Silver sulfadiazine Mean ± SD	Tilapia skin Mean ± SD	*P*	Means differences	CI 95%
ARM A	11.20 ± 0.63	9.77 ± 0.83	.0002	1.43	0.77–2.09
ARM B	11.70 ± 0.67	10.56 ± 1.13	.0147	1.14	0.25–2.03
ARM C	21.30 ± 1.42	18.10 ± 0.99	.0001	3.20	2.05–4.35

SD, standard deviaton; *CI* 95%, confidence interval of 95% out of the means differences.

complete reepithelialization was significantly lower in the groups treated with NTFS than in the groups treated with SSDC in study arms A, B, and C ($P = .0002$, $P = .0147$, and $P < .0001$, respectively). Wound healing progression of three patients treated with NTFS, each one from a different study arm, can be seen in Figure 2.

Pain Measurement

In all three study arms, both in the SSDC and NTFS treatment groups, there was a significant reduction in pain intensity from the first (V1) to the second visit (V2), considering measures inside the groups. The exception was the SSDC group of study arm C, in which such a decrease was observed only in the third visit (V3).

In study arm A, no statistically significant difference was found in the temporal evaluation of pain intensity between patients treated with SSDC or NTFS (Figure 3A). In study arm B, it was found that reported pain intensity was significantly lower in patients treated with NTFS than in those treated with SSDC in both the second ($P = .0393$), third ($P < .0001$), fourth ($P < .0001$), and fifth ($P < .0001$) evaluation visits (Figure 3B). In study arm C, it was also found that reported pain intensity was significantly lower in patients treated with NTFS than in those treated with SSDC at the second ($P = .0111$), third ($P = .0013$), fourth ($P < .0001$), fifth ($P < .0001$), sixth ($P < .0001$), seventh ($P < .0001$), and eighth ($P = .0180$) evaluation visits (Figure 3C).

Burn Improvement Assessment by Attending Physician

In all three study arms, the attending physician evaluated burn improvement on the day of dressing removal, using CGI-I. It was verified that the median score was equal to 1 (much better) in both the SSDC group and the NTFS group; therefore, there was no statistically significant difference between the treatments in any of the study arms.

Anesthetic/Analgesic Intake

In study arm A, no statistically significant difference between treatment groups was found for the amount (in mg) of orally administered dipyrone ($P = .1776$) or tramadol ($P = .4357$). In study arm B, there was also no statistically significant difference between treatment groups regarding the amount of intravenous dipyrone ($P = .1299$) and tramadol ($P = .7802$) used. Likewise, there was no statistically significant difference in the amounts of propofol ($P = .5075$), fentanyl ($P = 0.4244$), or midazolam ($P = .5009$) administered intravenously during the anesthetic procedures of the patients treated with SSDC or NTFS. However, patients treated with NTFS required significantly fewer milligrams of ketamine ($P = .0035$) than patients in the SSDC treatment group. In study arm C, patients treated with NTFS required a significantly lower amount of intravenous dipyrone ($P = .0337$) than those treated with SSDC, but there was no statistically significant difference between the treatment groups in the amount of intravenous tramadol required ($P = .3739$). As for the intravenous drugs used in anesthetic procedures, patients treated with NTFS required significantly fewer milligrams of fentanyl ($P = .0173$) and ketamine ($P = .0037$), when compared with those treated with SSDC, although there was no statistically significant difference in the amount of propofol ($P = .7905$) or midazolam ($P = .0666$) required between the two treatment groups.

Number of Dressing Changes

In study arm A, the number of dressing changes performed on patients treated with NTFS was significantly lower than in the patients receiving the SSDC treatment ($P < .0001$). In study arm B, a significant reduction in the number of dressing changes was also found in the patients treated with NTFS compared with those treated with SSDC ($P < .0001$). Similar results were also found in study arm C, where the number of dressing changes was again significantly lower in patients receiving the NTFS treatment ($P < .0001$). All data cited above can be seen in Table 3.

DISCUSSION

Complete healing is expected within 2 weeks for SPTB and usually takes longer than 3 weeks for DPTB.[17] In this RCT, with application of NTFS as a xenograft, the mean time for reepithelialization of outpatient and inpatient SPTB and inpatient DPTB was, respectively, 11.20, 11.70, and 21.30 days, similarly to the expected with many of the current available treatment options. In all arms of the study, when the mean time for reepithelialization in the NTFS group was compared with mean time for reepithelialization in the SSDC group, a statistically significant reduction was seen. Although the mean differences were modest for SPTB (1.43 days for outpatients and 1.12 days for inpatients), a more clinically relevant healing time reduction was seen for DPTB. Also, CGI-I scores were equivalent in both treatment groups, suggesting similarity regarding burn improvement on the day of dressing removal.

In all arms of the study, the total number of dressings was significantly reduced in the NTFS group when compared with the SSDC group. The need for fewer dressing changes during burn treatment provides large benefits to the health service and the patient, reducing the time spent by health professionals on

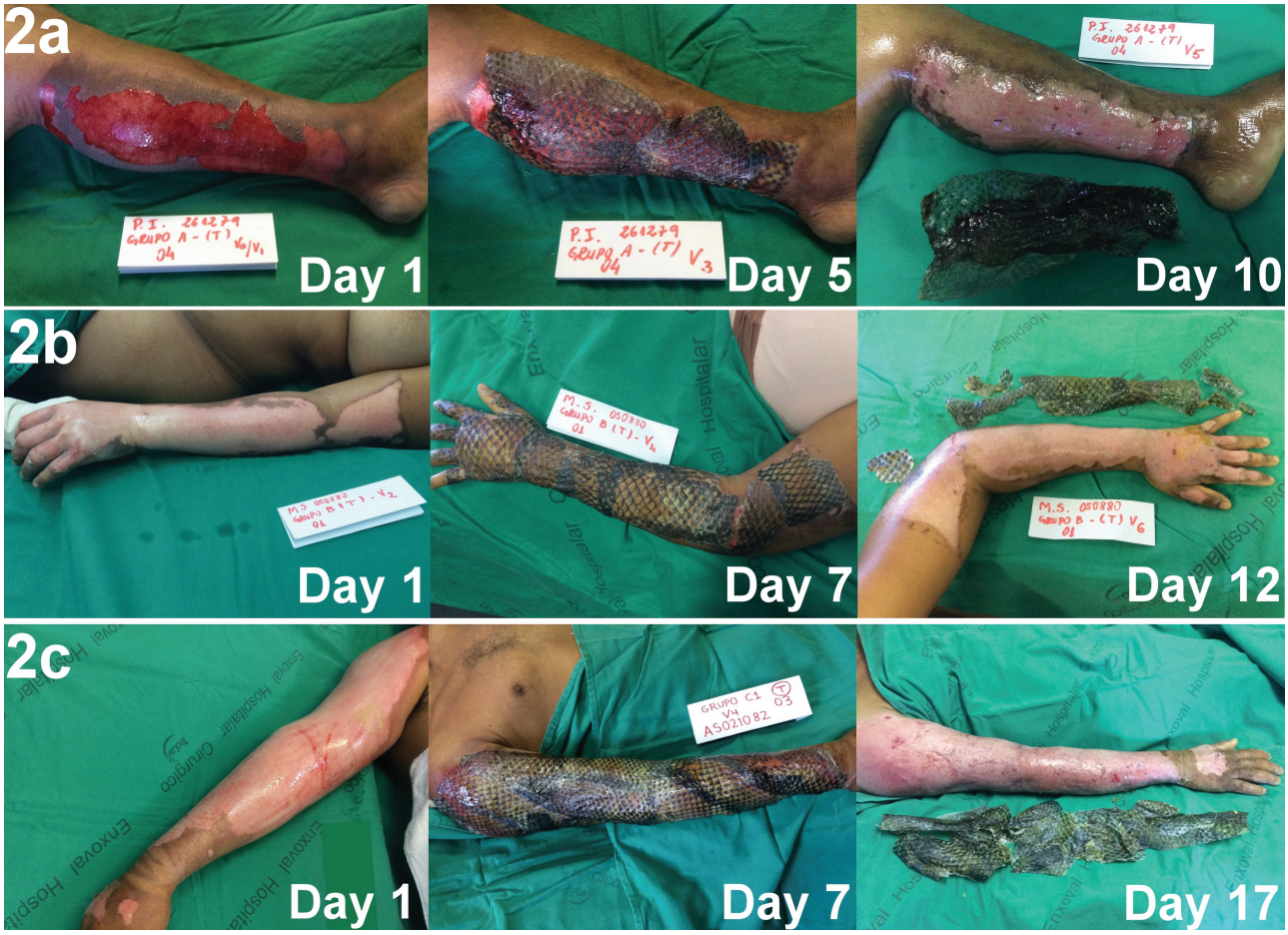

Figure 2. Participants of each arm of study A (a), B (b), and C (c), treated with tilapia skin before, during, and after treatment. Day of treatment in which each photo was taken is indicated.

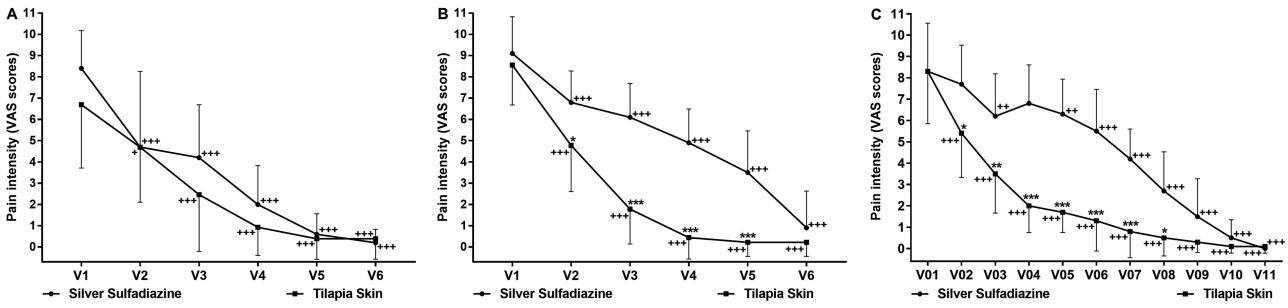

Figure 3. Temporal evaluation of pain intensity in patients treated with Silver Sulfadiazine and Tilapia Skin, considering the A (A), B (B) and C (C) arms of the study. Data are expressed as mean and standard deviation of measurements performed on at least 10 patients in both groups. *(P <.05), ** (P <.01) and *** (P <.001) mean the Tilapia Skin group showed statistically significant differences in relation to the Silver Sulfadiazine group at the same visit (two-way ANOVA followed by Bonferroni multiple comparisons test). +(P <.05), ++(P <.01) and +++(P <.001) mean statistically significant differences in relation to visit 1 (V1) within the same group (two-way ANOVA followed by Dunnett's multiple comparisons test). * V = Patient evaluation visit.

dressing changes and the amount of unpleasant sensations and psychological stress felt by the patient.

Additionally, for inpatients treated with NTFS (study arms B and C), a statistically significant reduction in pain intensity was observed in comparison to the control group, as measured by the VAS. Such a reduction was seen from second through fifth evaluation visits for inpatients with SPTB and from second through eight evaluation visits for inpatients with DPTB. We hypothesize that the presence of low to no pain when the wounds are near to complete reepithelialization, independently of the treatment used, has resulted in no difference in pain intensity in the last study visits for patients managed with NTFS or SSDC.

Even though burn injury-associated pain can be regarded as part of tissue-protecting and tissue regeneration mechanisms,[18] prolonged acute pain may lead to pain

Table 3. Number of dressings throughout treatment in patients treated with silver sulfadiazine and Tilapia skin in all arms of the study

Number of dressings	Silver Sulfadiazine Average	Tilapia Skin Average ± SD	*P*	Averages differences	CI 95%
ARM A	5.80 ± 0.42	2.08 ± 0.28	.0001	3.72	3.42–4.03
ARM B	11.00 ± 0.47	2.33 ± 0.71	.0001	8.67	8.09–9.24
ARM C	20.20 ± 1.69	6.10 ± 2.02	.0001	14.10	12.35–15.85

SD, standard deviation; *CI* 95%, confidence interval of 95% out of the means differences.

centralization, increased incidence of persistent pain, development of depression or posttraumatic stress disorder,[19] and a diminished trust in the medical team, negatively affecting the wound treatment outcome and decreasing compliance with rehabilitation therapies.[20] Thus, the healthcare team must provide appropriate burn pain management, offering, in association with analgesic pharmacological interventions, dressing options able to reduce procedural and background pain as much as possible. In hospitalized patients with DPTB, decreased pain intensity was, in part, corroborated by the statistically significant reductions in the amount of dipyrone and fentanyl required throughout treatment. In addition, inpatients with SPTB and DPTB treated with NTFS required less ketamine than the SSDC group..

Biological tissues of animal origin, such as dog, pig, and frog skin, have been used as xenografts in burn injuries.[21] However, to obtain approval for routine medical use, these materials must be subjected to rigorous protocols, in order to identify their real contribution, safety, efficacy, and biocompatibility. Cadaveric allograft skin is an additional option associated with good results.[14] However, in our country, there are only four human skin banks, which do not have enough skin to distribute for the more than 30 registered tertiary care burns centers in Brazil.[13]

The Nile tilapia (*Oreochromis niloticus*) is Brazil's most cultivated fish and ranks fourth worldwide, according to the United Nations Food and Agriculture Organization (FAO).[22] In search of new therapeutic alternatives for burns, biocompatible or biological dressings have been highlighted.[4] In Brazil, although frog skin was previously used as a treatment for burn wounds,[23] it was never registered by the National Sanitary Surveillance Agency (ANVISA). In this context, the possibility of taking advantage of NTFS (which used to be a waste product), as a treatment for these highly symptomatic wounds, provides an affordable novel option of biomaterial for burn treatment centers in low- to middle-income countries. NTFS is currently on its final steps for register as an approved medical graft by ANVISA.

In preclinical studies, the morphology of NTFS presented similarities with human skin, with a deep dermis formed by thick organized collagen fibers, on parallel/horizontal and transversal/vertical arrangement. A larger composition of type I collagen, compared with human skin, and high resistance and tensile strength were also detected.[10] Interestingly, when subjected to the processes of chemical sterilization and complementary irradiation, NTFS did not present variations in its microscopic and tensiometric structure and that recovered its natural consistency after the rehydration process.[11] It has also been reported in the literature that glycerolization and irradiation in biological dressings at moderate doses can fix tissues by reducing interstitial fluid without, however, causing degeneration.[24]

Glycerolized NTFS and tilapia collagen nano-fibers were found to enhance skin wound healing speed in rats.[12,25] Also, promising results were obtained with the application of marine collagen peptides from tilapia skin on DPTB in rabbits.[26] Another study showed that tilapia collagen significantly induces epidermal growth factor and fibroblast growth factor expression, which can promote proliferation and differentiation of fibroblasts and keratinocytes.[27] In a video article, neovaginoplasty using NTFS offered an anatomical and functional neovagina to a patient with Mayer–Rokitansky–Küster–Hauser syndrome, with histological analysis of the canal revealing the presence of stratified squamous epithelium.[8]

In our recently published pilot study, NTFS was used as a xenograft for the treatment of pediatric SPTB, reducing the number of dressing changes and the amount of anesthetics used. Conversely, pain intensity scores, number of days for complete burn wound healing, and total amount of analgesics required were similar to the SSDC group.[13] The present study, thus, adds further data supporting the use of NTFS for burn treatment, considering a reduction of pain intensity in hospitalized patients and of the number of days for reepithelialization in all study arms were consistently seen. Also, NTFS was effective for DPTB, which were not evaluated in the previous study. At this point, no adverse effects have ever occurred with use of NTFS in humans.

There are several limitations to this study. Since this is a pilot phase II RCT, only a relatively small number of patients were included from a single medical center. However, at this stage of clinical development, this small sample of participants provided preliminary data for sample size calculation for phase III clinical trials, which are currently being developed and will include a more significant number of participants and a cost–benefit analysis. Also, it was not possible for the consultant to be blind for the study group allocation because both types of dressing need to be seen to be applied, evaluated, replaced, and removed adequately. Furthermore, a xenograft was compared with a topical antibiotic cream; future studies, comparing NTFS with other occlusive dressings, including other xenografts, are warranted. Finally, only a subjective tool for pain assessment (ie, the VAS) was used. In future studies, we intend to perform objective assessment of pain using quantitative sensory testing modalities.

In conclusion, in this phase II RCT including 62 patients, NTFS showed good adherence to the wound bed, helping the healing process, reducing fluid loss, and decreasing the number of dressing changes required, with a potential for lowering the

overall work-load of healthcare professionals in burn treatment centers. Most importantly, its use decreased the number of days until complete burn wound reepithelialization, especially for hospitalized patients with DPTB, who also showed decreased analgesic use. Besides, lower pain intensity, as evaluated by the VAS, was seen for hospitalized patients with SPTB or DPTB treated with NTFS. Considering the exposed, we expect NTFS to become soon established as a safe, effective, and low-cost option for burn treatment, especially in middle- to low-income countries.

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