

Pediatric Burn Treatment Using Tilapia Skin as a Xenograft for Superficial Partial-Thickness Wounds: A Pilot Study

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This study aims to evaluate the efficacy of Nile tilapia skin as a xenograft for the treatment of partial-thickness burn wounds in children. This is an open-label, monocentric, randomized phase II pilot study conducted in Fortaleza, Brazil. The study population consisted of 30 children between the ages of 2 and 12 years with superficial “partial-thickness” burns admitted less than 72 hours from the thermal injury. In the test group, the tilapia skin was applied. In the control group, a thin layer of silver sulfadiazine cream 1% was applied. Tilapia skin showed good adherence to the wound bed, reducing the number of dressing changes required, the amount of anesthetics used, and providing benefits for the patients and also for healthcare professionals, by reducing the overall work load. The number of days to complete burn wound healing, the total amount of analgesics required throughout the treatment, burn improvement on the day of dressing removal, and pain throughout the treatment were similar to the conventional treatment with silver sulfadiazine. Thus, tilapia skin can be considered an effective and low-cost extra resource in the therapeutic arsenal of pediatric superficial partial thickness burns.

Burns constitute the fifth most common cause of nonfatal childhood injuries in the world, with the majority of the lesions corresponding to partial thickness burns.¹ Scalds correspond to the most common etiologic factor, with flame burns following as the next most common cause.² Improper adult supervision, child maltreatment, poverty, crowding, lack of education, and not being the son or daughter of the household head, are additional risk factors that may contribute to burns in pediatric patients.^{1,3}

Infection prevention and promotion of a moist wound environment form the basis of the burn treatment in children.⁴ Complete healing is expected within 7 to 14 days for superficial partial thickness wounds and can take up to 4 to 6 weeks in deep dermal burns.⁵ Treatment modalities include silver-containing creams, such as silver sulfadiazine, and biological dressings, such as amnion membrane, human allograft skin, and xenografts. Silver-containing dressings, semisynthetic and

synthetic dressings, enzymatic debridement, and surgery are other possibilities.⁶

The Nile tilapia (*Oreochromis niloticus*) belongs to the *Cichlidae* family, originates from the Nile River basin in East Africa and is widely distributed in tropical and subtropical regions. Today, it is Brazil's most cultivated fish and ranks fourth worldwide, according to the United Nations Food and Agriculture Organization (FAO).⁷ Our team of researchers showed tilapia skin had a noninfectious microbiota and morphological structure similar to that of human skin, with even a larger composition of type I collagen.^{8,9} This combination of factors supported its application as a biomaterial for burn treatment. The same researchers also performed in vivo studies using rats (*Rattus norvegicus*, Wistar lineage) to demonstrate the use of tilapia skin as a xenograft for the treatment of experimental burns.¹⁰

Based on the promising results of our single case report with a pediatric patient¹¹ and of our still unpublished phase II randomized controlled trial with adults, this phase II pilot study aims to evaluate the efficacy of Nile tilapia skin as a xenograft for the treatment of partial-thickness burn wounds in children from 2 to 12 years of age.

METHODS

Participants

This was an open-label, monocentric, randomized phase II pilot study conducted at a burn treatment center in Fortaleza, Ceará, Brazil, from May 2017 to March 2018. The local Institutional Review Board (IRB) approved the study protocol and informed consent. The latter was obtained from each participant's legal caregiver before any study procedure

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was performed and after detailed explanation of the study conditions. The research was conducted in accordance with the 1975 Declaration of Helsinki and its amendments. The study population consisted of 30 children between the ages of 2 and 12 years admitted to our institution with superficial partial thickness burns less than 72 hours from the thermal injury. Patients were excluded for a burn greater than 20% total BSA (TBSA), the presence of a previous treatment for the current burn, the presence of a chemical or electrical burn, the presence of other significant diseases that could impact the volunteer's participation in the study, use of medications that could have an impact on wound healing (eg, steroids) and presence of hypersensitivity to materials used in the study or to related compounds.

Randomization

The study was randomized with each research subject being assigned to one of two groups (test or control) by drawing of lots, after determination of the children's burn wound depth and TBSA on clinical grounds. To assign the groups, 15 folded papers with the word "TEST" and 15 folded papers with the word "CONTROL" were placed in the same envelope, which was not accessible to the treating physician. The folded papers were removed from the envelope, one at a time, and opened to reveal the assigned group. The name of the research subject, as well as the group to which he/she was randomized, was noted on a specific form, recorded in the order in which they were included in the study. Also, patients were blinded to the hypothesized effects of either treatment.

Tilapia Skin Preparation

The Nile tilapia skin patent is registered at National Institute of Industrial Property (INPI) under number BR 10 2015 021435 9. A detailed description of the process of preparing, decontaminating, and sterilizing the biomaterial is present in our recently published case report.¹¹ A single 10.0 cm × 5.0 cm piece of tilapia skin is able to cover around 2% of TBSA.

Interventions

After enrollment in the study, the patients went through standard procedures depending on the treatment group to which they were allocated. In both of the groups, the first dressing was done with anesthesia (ketamine with or without midazolam, depending on the anesthetist's preference) to remove loose skin and debris. In the test group, after cleaning the lesion with tap water and 2% chlorhexidine gluconate, the tilapia skin was applied and covered with gauze and bandage. These dressings were changed only if the tilapia skin did not adhere properly to the wound bed. In the control group, after cleaning the lesion with tap water and 2% chlorhexidine gluconate, a thin layer of silver sulfadiazine cream 1% was applied and covered with gauze and bandage. In these patients, the dressings were changed daily. The patients were evaluated by the research team every 6 hours for vital signs and clinical conditions and every 24 to 72 hours for the study parameters. Significant detachment of the biomaterial from wound borders, revealing healed underlying patient skin, was the sign for researchers that complete re-epithelialization had occurred and tilapia skin could be removed. The dressing removal

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.

Outcome Assessment

The primary outcome variables were the number of days to complete burn wound healing ($\geq 95\%$ re-epithelialization), calculated via clinical judgment from the consultant, and the number of dressings performed. In the control group, a dressing change was defined as the daily act of cleaning the wound and reapplying the silver sulfadiazine cream 1%, which is then covered with new gauze and bandage. In the test group, a dressing change was defined as the act of replacing the tilapia skin which did not adhere properly and/or replacing gauze and bandage that is full of exudate. Differently from the control group, the time between dressing changes was not fixed, but instead dependent on the necessity for change, checked during clinical evaluation of the dressing, regularly performed every 24 hours. In both groups, the dressing change can be performed under anesthesia or under no anesthesia, according to the physician's judgment. Tilapia skin observed to have adhered to the burned area was left in the wound bed until completion of re-epithelialization. If tilapia skin did not adhere, it was removed, the area was cleaned, and then the biomaterial was applied again, with the child under anesthesia. The following secondary outcome variables were also defined: the total amount of anesthetics and analgesics required throughout the treatment, assessment of pain via the Faces *Pain* Scale-Revised (*FPS-R*) and assessment of illness improvement on the day of dressing removal via the Clinical Global Impression Scale-Improvement (*CGI-I*). To audit anesthetics and analgesics intake, nurses were trained to register on the patient's clinical record all analgesic and anesthetic medications used. The pain evaluation via the *FPS-R* was performed by the patient himself or herself if aged 5 years or more, or by their caregiver in patients under 5 years of age. The scale consists of six faces, presented horizontally, depicting different degrees of pain, from "no pain" to "most pain possible." A numerical value from 0 to 10 is assigned to each face.¹² 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."¹³

Statistical Methods

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 median, interquartile range, and minimum and maximum values (non-parametric data) were calculated. Comparisons between the groups Silver Sulfadiazine and Tilapia Skin were made using the unpaired t test (parametric data) or the Mann-Whitney

U test (nonparametric data). Regarding the parametric data, in addition to statistical significance, there were also determined the difference of means and its respective 95% confidence interval. Categorical variables, in turn, were expressed as absolute and relative frequency, while ordinal variables were expressed as median, interquartile range and minimum and maximum values. Comparisons between the two treatments in relation to the categorical variables were carried out using the chi-square test. The Mann–Whitney *U* test was used to compare the two groups in relation to ordinal variables. In addition, time until complete wound re-epithelialization curves for the two treatments were determined according to the Kaplan–Meier method and compared using the log-rank test. In all analyzes, two-tailed tests were applied and the significance level was set at 0.05 (5%) so that *P* values lower than .05 were considered statistically significant. GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, 2016) and IBM SPSS Statistics version 23.0 (IBM Corp., Armonk, NY, 2015) were used to perform statistical procedures. The first software was also used to plot the graphics.

RESULTS

Demographic Characteristics

Thirty children were randomized into the study and included for analysis, with half allocated in the tilapia skin group and the other half allocated in the silver sulfadiazine group. Groups were similar with respect to the following baseline variables: age, gender, body mass index, TBSA involved, number of body segments affected, score on the Clinical Global Impression–Severity scale (CGI-S) before initiation of treatment, score on the Faces Pain Scale–Revised (FPS-R) before initiation of treatment, and mechanism of burn (Table 1).

Time to Re-epithelialization

The mean number of days to complete re-epithelialization was 10.47 ± 0.74 in the silver sulfadiazine group and 10.07 ± 0.46 in the tilapia skin group. The difference between the two groups was not statistically significant ($P = .0868$). The rate of re-epithelialization (defined as the ratio between the TBSA involved and the number of days until complete re-epithelialization) of each group was also calculated, but the difference between the two groups was not statistically significant ($P = .3889$), even though the mean rate of re-epithelialization was slightly higher in the tilapia skin group (Table 2). Figure 1 shows the time curve until complete burn wound healing, created according to the Kaplan–Meier method. On each day, the data correspond to the probability of complete re-epithelialization of the lesion until that day. Comparisons between the curves were made using the log-rank test, which showed no statistically significant, although marginally significant, difference between them ($P = .0689$). In the Silver Sulfadiazine group and Tilapia Skin group, the complete re-epithelialization of the lesions on the 10th day of treatment was 53.33% and 86.67%, respectively, while on the 11th day of treatment, the proportion of complete re-epithelialization was 93.33% and 100%, respectively. Figure 2 shows the evolution of two research participants treated with tilapia skin.

Evaluation of Burn Improvement

The attending physician performed the evaluation of burn improvement on the day of dressing removal, using the Clinical Global Impression Scale–Improvement (CGI-I). The median score was found to be equal to 1 (very much improved since the initiation of treatment) in both the silver sulfadiazine group and the tilapia skin group; therefore, there was no statistically significant difference between treatment groups ($P = .9999$).

Table 1. Baseline demographic and clinical characteristics of the study participants

Characteristic	Silver Sulfadiazine	Tilapia Skin	Significance
Sample size	15	15	–
Age (years) – mean \pm SD	5.20 ± 2.70	5.67 ± 3.66	$P = .6942^a$
Gender			
Male	8 (53.33%)	10 (66.67%)	$P = .4561^b$
Female	7 (46.67%)	5 (33.33%)	
BMI (kg/m ²) – mean \pm SD	18.16 ± 2.74	17.75 ± 2.56	$P = .6682^a$
Total body surface area burned (%) – mean \pm SD	10.13 ± 4.16	11.13 ± 4.94	$P = .5534^a$
Burn site (corporal segments)			$P = .4921^b$
1 segment	2 (13.33%)	2 (13.33%)	
2 segments	4 (26.67%)	7 (46.67%)	
3 segments	9 (60.00%)	6 (40.00%)	
Global clinical impression (burn severity) – median (IQR)	4 (4–4)	4 (4–4)	$P = .8276^c$
Pain intensity (FPS-R scores) – mean \pm SD	8.00 ± 3.21	9.20 ± 1.47	$P = .1986^a$
Mechanism of burn			
Scald	12 (80.00%)	14 (93.33%)	$P = .2827^b$
Flame	3 (20.00%)	1 (6.67%)	

BMI, body mass index; FPS-R, Faces Pain Scale – Revised; IQR, interquartile range; SD, standard deviation.

^aunpaired *t* test; ^bchi-square test; ^cMann–Whitney test.

Table 2. Evaluation of the re-epithelialization process in the two study arms, according to the number of days until complete wound re-epithelialization and the re-epithelialization rate, defined as the ratio between TBSA-burned (%) and the number of days until complete re-epithelialization

Re-epithelialization Assessment	Silver Sulfadiazine Mean \pm SD	Tilapia Skin Mean \pm SD	Significance (unpaired <i>t</i> test)	Difference of Means	95% CI
Number of days until complete re-epithelialization	10.47 \pm 0.74	10.07 \pm 0.46	<i>P</i> = .0868	0.40	-0.06 to 0.86
Re-epithelialization rate	0.96 \pm 0.39	1.11 \pm 0.49	<i>P</i> = .3889	-0.15	-0.47 to 0.19

95% CI, 95% confidence interval of the difference of means; SD, standard deviation.

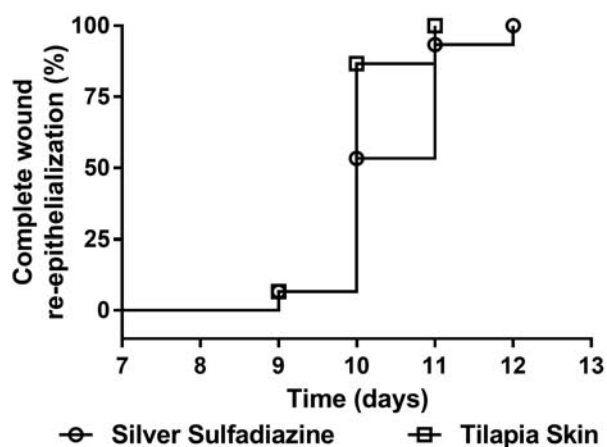


Figure 1. Time until complete wound re-epithelialization curves for the two treatments. On each day, the data correspond to the probability of complete re-epithelialization of the lesion until that day. The curves were plotted according to the Kaplan–Meier method and compared using the log-rank test, which showed no statistically significant difference between them (*P* = .0689).

Pain Assessment

Figure 3 shows the overall pain assessment of the participant children by determining the area under the curve (AUC) of pain intensity (measured by Faces Pain Scale-Revised - FPS-R) versus time (days). In A, a graphical representation of the average AUC is seen. In B, the data correspond to the mean and standard deviation of the measurements performed in 15 patients of each group. The unpaired *t* test was used to compare the two treatment groups, but no statistically significant difference (*P* = .1020) between AUC for the Tilapia Skin group (21.73 \pm 8.51) and AUC for the Silver Sulfadiazine group (27.47 \pm 10.00) was found (difference of means of -5.74 with a 95% confidence interval of -12.68 to 1.21).

Anesthetics and Analgesics Intake

No statistically significant difference was found for the amount (in mg) of dipyron required for oral analgesia of children in either treatment group (*P* = .6969). Otherwise, there was a statistically significant difference (*P* = .0014) for the amount (in mg) of intravenous ketamine required during the anesthetic procedures of the patients treated with silver sulfadiazine (150.07 \pm 70.14) or tilapia skin (76.73 \pm 39.12) according to the anesthesiologist's judgment (**Table 3**). There

was no statistically significant difference in the amounts of tramadol (*P* = .4049), fentanyl (*P* = .3488), or midazolam (*P* = .3677) administered intravenously during the anesthetic procedures of the research patients.

Number of Dressings Performed

The number of dressings under anesthesia performed in patients treated with Tilapia Skin was significantly lower (*P* = .0251) than the number of dressings under anesthesia performed on the volunteers treated with Silver Sulfadiazine. A statistically significant difference was also found for the number of dressings without anesthetics (*P* < .0001). Finally, the total number of dressings was significantly reduced (*P* < 0.0001) in the Tilapia Skin group (3.00 \pm 0.76) when compared with the Silver Sulfadiazine group (9.27 \pm 1.39). All of these data can be seen in **Table 4**.

DISCUSSION

In the current study, the number of dressings under anesthesia, dressings without anesthetics and the total number of dressings were significantly reduced in the Tilapia Skin group when compared with the Silver Sulfadiazine group. Since dressing changes can cause patients to experience pain, stress, and itching, which have all been linked with delayed wound healing,¹⁴ a decreased number of dressings may increase the child's well-being in the already anxiety-provoking hospital setting. A reduction in the time spent by healthcare professionals on dressing changes may be also obtained. Another important finding was the reduced requirement for the anesthetic medication ketamine during the children's stay in the hospital, reducing costs and the potential side effects of this medication in patients treated with tilapia skin.

The abundance of dressing materials and topical treatment modalities for pediatric patients makes it a daily challenge to determine which materials should be preferred for a specific wound type.⁵ Providing comfort to enhance functional recovery while protecting from contamination, dryness and additional trauma, are essential considerations for the appropriate choice.¹⁵ One of the few current consensuses is that membranous dressings perform better on various wound-healing parameters when compared with cream based topical antiseptics treatments.⁵ Unfortunately, in the Brazilian public healthcare system, almost all burn centers still use the silver



Figure 2. Evolution of two research participants treated with tilapia skin, both with partial-thickness burn wounds caused by scalds. First picture of each patient corresponds to burn wound before application of the tilapia skin. Second picture of each patient corresponds to burn wound after application of the tilapia skin. Third picture of each patient corresponds to the last day of treatment. 2A had complete re-epithelialization within 10 days with two dressings performed. 2B had complete re-epithelialization within 9 days with three dressings performed.

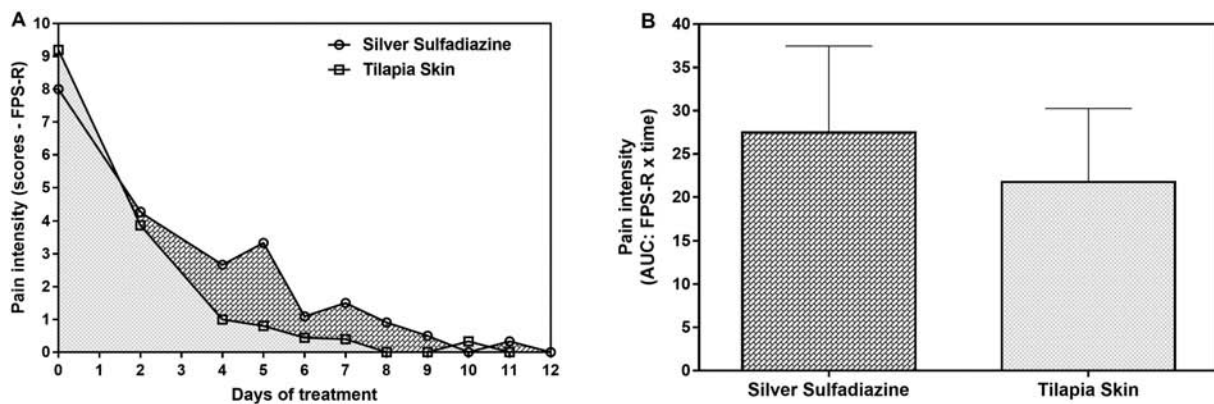


Figure 3. Overall assessment of pain in children treated with Silver Sulfadiazine and Tilapia Skin by determining the area under the curve (AUC) of pain intensity (measured by the FPS-R) versus time (days): (A) graphical representation of the average AUC; (B) mean and standard deviation values of AUC. Unpaired *t* test was used to compare the two treatments, which did not show a statistically significant difference between them ($P = .1020$).

sulfadiazine cream as standard treatment, demonstrating an urgent need to seek alternative treatments which fit our poor-resource reality.

Bioengineered approaches, such as modern products incorporating dermal scaffolds, stem cells, and growth factors, have largely been tested for use in patients with partial thickness

Table 3. Amount of dipyron and ketamine required for oral analgesia and anesthetic procedures of the children treated with Silver Sulfadiazine or Tilapia Skin

Analgesic/Anesthetic	Silver Sulfadiazine Mean ± SD	Tilapia Skin Mean ± SD	Significance (unpaired <i>t</i> test)	Difference of Means	95% CI
Dipyron (mg)	3246.67 ± 2247.83	3561.67 ± 2135.14	<i>P</i> = .6969	-315.00	-1954.71 to 1324.71
Ketamine (mg)	150.07 ± 70.14	76.73 ± 39.12	<i>P</i> = 0.0014	73.34	30.86 to 115.81

95% CI, 95% confidence interval of the difference of means; SD, standard deviation.

A significant decrease in the used of ketamine was seen in the Tilapia Skin group (*P* = .0014).

Table 4. Number of dressings, with and without anesthesia, performed throughout the trial in patients treated with Silver Sulfadiazine and Tilapia Skin

Number of Dressings	Silver Sulfadiazine Mean ± SD	Tilapia Skin Mean ± SD	Significance (unpaired <i>t</i> test)	Difference of Means	95% CI
Number of dressings under anesthesia	3.20 ± 1.08	2.40 ± 0.74	<i>P</i> = .0251	0.80	0.11 to 1.49
Number of dressings without anesthesia	6.07 ± 1.39	0.60 ± 0.74	<i>P</i> < .0001	5.47	4.64 to 6.30
Total number of dressings	9.27 ± 1.39	3.00 ± 0.76	<i>P</i> < .0001	6.27	5.43 to 7.10

95% CI, 95% confidence interval of the difference of means; SD, standard deviation.

Significantly decreased number of dressings without anesthesia and total number of dressings were seen in the Tilapia Skin group (*P* < .0001).

burns.^{6,15} Although highly promising, those approaches are far from feasible in low- and middle-income countries. In this scenario, biological alternatives, such as xenografts, rise as cost-effective possibilities to reduce pain and the need for dressing changes.¹⁶ A variety of species (eg, cat, chicken, cow, dog, frog, lizard, pig, pigeon, rabbit, sheep) have shown clinical potential for use as a biological dressing in humans.¹⁷⁻¹⁹ These xenografts have many preparation methods (eg, cryopreservation, lyophilization, chemical dehydration with glycerol), which differ in the way they address the compromise between the effectiveness of fresh skin and convenience of preserved skin.¹⁷

Cadaveric allograft skin and allogenic amniotic membranes are additional options associated with good results but, despite strict adherence to protocols, potential pathogenic microbial and viral contamination does not reach zero.¹⁹ The difficulty with these materials in our country is the presence of only four skin banks (based in the cities of São Paulo, Porto Alegre, Recife and Curitiba), which, although opened to donate skin for government insured patients (about two-thirds of our burns patients), do not have enough skin to distribute for the more than 30 registered tertiary care burns centers in Brazil.^{20,21} Also, in a porcine skin focused meta-analysis, Hermans et al (2013) suggested that, since allografts and xenografts appear to be equally effective, xenografts might be a superior choice for their increased safety and reduced price.²²

Although frog skin was previously used as a burn treatment in Brazil,²³ it was never registered by the National Sanitary Surveillance Agency (ANVISA). Porcine skin was neither registered nationally for the use in burn wounds and has very low availability in the specialized centers. Therefore, tilapia skin carries the promise of an innovative, easy to apply, highly available, pioneering product, which can turn out to be the first nationally studied animal skin registered by ANVISA for the use in burn treatment.

The morphology of Nile tilapia skin presented similarities with human skin, with a deep dermis formed by thick

organized collagen fibers, on parallel/horizontal and transversal/vertical arrangement. The tilapia skin also presented a larger composition of type I collagen, compared with human skin, and high resistance and tensile extension at the break. When subjected to the processes of chemical sterilization and irradiation, tilapia skin did not present variations in its microscopic and tensiometric structure and recovered its natural consistency after the rehydration process.^{9,24} It has been reported in the literature that glycerolization in biological dressings, at moderate doses, can fix tissues by reducing interstitial fluid without, however, causing degeneration.²⁵ Also, our researchers concluded the colony-forming units found in samples of tilapia skin indicated the presence of normal, noninfectious microbiota.¹⁰ A recent study showed tilapia collagen significantly induces epidermal growth factor and fibroblast growth factor expression, which can promote proliferation and differentiation of fibroblasts and keratinocytes, thus hastening the wound healing process.²⁶

Several previous studies have proven enclosed silver dressings of different types to be more cost-effective than the silver sulfadiazine cream for partial thickness burn treatment.²⁷⁻³⁰ The high availability of the Nile tilapia in Brazil and the inexpensive methods of preparing it for human use,^{9,13} suggest the costs could be additionally reduced with the use of tilapia skin, but further studies need to be made to confirm this assertive. Additionally, side effects from the use of the silver sulfadiazine cream (eg, allergic reactions to its sulfadiazine moiety, silver staining of the treated burn wound, hyperosmolality, methemoglobinemia, and hemolysis), although uncommon and generally mild, are a possibility,³¹ while no side effects were yet recorded with the tilapia skin use, but again, further studies are needed.

There are a few limitations to this study. Since this is a pilot study, only a relatively small number of children were included from a single medical center, resulting in undetermined external validity. We recognize the use of a substandard randomization method; thus, an improvement with computer-based

randomization sequences is going to be attained in the next studies. Also, it was not possible for the consultant to be blind for the study group allocation due to the necessity of visualizing the treatments in order to conduct them properly (ie, both types of dressing need to be seen to be applied, evaluated, replaced and removed adequately). The application of tilapia skin was found to be difficult in some anatomical locations compared to the silver sulfadiazine cream (eg, face, genitals, neck, axillae, antecubital fossa, inguinal area). In addition, even though some studies indicate the lower number of dressing changes often correlates with superior rates of wound healing, increased patient's satisfaction and reduction of pain,³² those correlations were not found in our study, probably due to the low number of patients included. However, at this stage of clinical development, this small sample of patients provided preliminary data for sample size calculation for larger phase II and phase III randomized clinical trials, which in turn will be multicentric. Furthermore, we compared the efficacy of the use of a xenograft with silver sulfadiazine cream topical application. Future studies are warranted, comparing tilapia skin with other occlusive dressings, including other xenografts. Finally, the reductions of cost associated with the treatment with tilapia skin are only hypothesized based on the wide availability of a material which used to be discarded. A cost-benefit analysis is the next logical step in proving our assumptions.

In conclusion, in this pilot study, tilapia skin showed good adherence to the wound bed, reducing the number of dressing changes required, and, consequently, the amount of anesthetics used, helping the healing process, reducing fluid loss, providing benefits for the patients and also for healthcare professionals, by reducing the overall work load. The number of days to complete burn wound healing, the total amount of analgesics required throughout the treatment, burn improvement on the day of dressing removal, and pain throughout the treatment were similar to the conventional treatment with silver sulfadiazine, establishing the tilapia skin as an extra low-cost-effective resource in the therapeutic arsenal of pediatric superficial partial thickness burns.

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