Cutaneous wounds in teleosts are commonly encountered in both public and home aquaria. The aqueous environment is particularly conducive for opportunistic infections, making rapid wound healing especially important. Previous studies in teleosts have demonstrated that topical use of medical-grade honey produces positive effects on wound healing, whereas topical silver sulfadiazine and misoprostol/phenytoin gel delay or worsen the wound-healing process. Given the paucity of research on wound healing and topical treatments in teleosts, further investigation is necessary to enhance the management of cutaneous wounds in these species.

Similar to other vertebrates, teleost integument includes an epidermis, dermis, and hypodermis; however, the structure can vary depending on the species, life stage, season, nutritional state, anatomic location, water quality, and overall health status. Given the variability in integument and understudied nature of dermatologic conditions in teleosts, topical

**OBJECTIVE**
To evaluate the effects of topical naltrexone on wound healing in freshwater fish.

**ANIMALS**
25 blackbelt cichlids (*Vieja maculicauda*).

**METHODS**
A randomized, controlled, experimental trial was performed, with each individual serving as its own control. Bilateral 6-mm periepaxial cutaneous wounds were created in the body-wall skin of each fish under anesthesia. Three treatment groups were as follows: topical 0.04% naltrexone in administration vehicle (iLEX ointment; iLEX Health Products) at day 0 only (n = 10), topical 0.04% naltrexone in iLEX every 72 to 96 hours (n = 10), or iLEX only every 72 to 96 hours (n = 5) for 10 total treatments. The contralateral wound was left untreated as a control. Fish were maintained in a common enclosure at 24.7 to 25.4°C for 35 days. Macroscopic wound assessment and image collection were performed every 72 to 96 hours. On day 35, fish were humanely euthanized, and skin samples were collected for histopathology.

**RESULTS**
Time to complete visual resolution of wound healing was faster (P = .002) in wounds treated every 72 to 96 hours with topical 0.04% naltrexone in iLEX (day 19.4) compared to untreated wounds (day 23.3). An interaction between treatment and day was observed (P = .002), with fish treated with 0.04% naltrexone in iLEX every 72 to 96 hours having reduced (P < .05) wound area compared to both controls and fish treated with topical 0.04% naltrexone in iLEX once. No significant differences were noted in histologic sections of wound sites examined at day 35.

**CLINICAL RELEVANCE**
Fish improved earlier postsurgery and time to complete wound resolution was faster in wounds treated with topical 0.04% naltrexone in iLEX every 72 to 96 hours.

**Keywords:** cichlid (*Vieja maculicauda*), fish, naltrexone, skin, wound healing

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therapeutic treatment regimens are typically empirical or extrapolated from human or domestic species. Recent studies have demonstrated the utility of topical naltrexone, an opioid receptor antagonist, in the treatment of lateral line depigmentation (LLD), a chronic integumentary condition in teleosts that results in depigmented to ulcerated skin lesions along the lateral line of the head and flank of fish.

In addition to the beneficial effects of topical naltrexone on chronic integumentary conditions, such as LLD, in teleosts, naltrexone has been used to assuage dermatologic conditions in humans and other mammals. Topical application of the opioid antagonist naltrexone functions via selective blockade of the opioid growth factor (OGF) receptor (OGFr) regulatory pathway. The role of this pathway in wound healing is exemplified in diabetic humans and animals in which the inhibitory peptide, OGF, is overexpressed, leading to the downregulation of cell proliferation and thus a delay in injury resolution. Opioid growth factor receptor blockade by an antagonist such as naltrexone restores the proliferative homoeostasis required for tissue repair, with resultant increases in angiogenesis and granulation in epithelial tissues essential for effective wound healing in both diabetic and nondiabetic patients.

One of the challenges in treating aquatic animals is ensuring that topical treatments have sufficient contact time with the wound to be effective. In the present study, a hydrophobic skin protectant paste, iLEX (ILEX Health Products), composed of proprietary concentrations of petroleum jelly, calcium/sodium copolymer, cornstarch, zinc oxide, sodium carboxymethyl cellulose, and lipicar preservative, was mixed with naltrexone to create a waterproof and protective barrier. iLEX ointment was chosen as the administration vehicle for naltrexone based on its hydrophobic properties, safety, low cost, and successful results in the treatment of LLD in fish.

The objective of this study was to determine the effects of topical naltrexone and the naltrexone administration vehicle (iLEX ointment) on wound healing in cichlids. We hypothesized that topical naltrexone would promote wound healing compared to both iLEX ointment alone and untreated wounds.

Methods

Animals

The experimental population consisted of 25 captive-bred, adult, sex-undetermined blackbelt cichlids (Vieja maculicauda) (total length, 18.9 to 22.5 cm; weight, 252 to 495 g). This species was selected because of its size and frequent representation in both public and home aquariums. Fish were housed together in a 600-gallon system with biologic and physiochemical filtration and continuous temperature monitoring (24.7 to 25.4°C). Water quality (ammonia, nitrite, nitrate, phosphate, alkalinity, pH) was checked weekly, and water changes were performed bimonthly. This project was approved by the research oversight and approval committee at the John G. Shedd Aquarium.

Experimental wounds

Fish were anesthetized with 100 mg/L tricaine methanesulfonate buffered with sodium bicarbonate, and a passive integrated transponder (PIT) was injected SC in the right epaxial region (at least 3 cm from experimental wound sites) to identify individual fish. Left and right lateral body-wall cutaneous wound sites (just dorsal to the lateral line, between the operculum and dorsal fin) were infiltrated with lidocaine (1 mg/kg, SC), and fish received a single dose of meloxicam (0.5 mg/kg, IM). A sterile biopsy punch device (6-mm diameter biopsy punch; Integra Life Sciences) was used to create bilateral periepaxial cutaneous wounds with exposure of the underlying muscle. Prior to cutaneous wound creation, scales were removed from the 6-mm-diameter wound area (AREA) and 1 row adjacent to the external perimeter manually with hemostats. Treatments were applied according to the study group, and fish were allowed to recover from anesthesia before transfer back to the common enclosure for the remainder of the study period. Fish were monitored daily during the study period for changes in activity, appetite, opercular rate, and posture in the habitat.

Wound treatments

A 0.04%-concentration naltrexone ointment was formulated by mixing 4 mg naltrexone hydrochloride injectable solution (ZooPharm Inc) and 10 g iLEX ointment to form a topical compound. Three treatment groups were evaluated: group 1 (n = 10), naltrexone in iLEX once on day 0 (ONCE); group 2 (n = 5), iLEX only every 72 to 96 hours for 10 total treatments; group 3 (n = 10), naltrexone in iLEX every 72 to 96 hours for 10 total treatments. Fish were randomly divided into each group, and the side of the fish selected for treatment was randomized via random number generator. The contralateral wound was left untreated to serve as a control. Wounds were dried, and topical treatments were applied using a curve-tipped syringe and tongue depressor in a 1- to 3-mm-thick layer. Wounds were held out of water for 30 seconds following treatment application before being reimmersed in system water.

Clinical wound assessment and analysis

Immediately after wounds were made and every 72 to 96 hours thereafter, visual wound assessment and image collection were performed in anesthetized (100 mg/L tricaine methanesulfonate buffered with sodium bicarbonate) fish. Images were captured of wounds out of water using an iPhone 14 (Apple Inc) at a standardized distance from the lesion with a 3-cm scale placed next to each wound. Macroscopic wound assessment was conducted at each timepoint for all groups using a previously published protocol with modifications to account for the rapid wound healing seen in this species. Each wound was assigned a grade (0 to 5), defined as the visual estimate (VE), at each time point based on the following characteristics: wound contraction, tissue reaction, signs of necrosis, or signs of inflammation (hyperemia and swelling; Table 1). The wound scoring was
Histologic wound assessment

On day 35 postsurgery, fish were humanely euthanized via overdose of buffered tricaine methanesulfonate (500 mg/L) followed by exsanguination according to AVMA guidelines.21 Each wound was excised with 1-cm lateral cutaneous margins and a 1-cm-deep skeletal muscle margin to preserve specimen integrity and prevent any artifactual distortion during fixation and processing. Specimens were fixed in 10% neutral-buffered formalin. Tissues were routinely processed and sectioned at 5 µm for light microscopic evaluation. Histologic sections were evaluated by 1 of the investigators (JAL), who was blinded to the treatment received and date of collection. Total AREA was measured using ImageJ software (version 1.54f). Lesions were measured in centimeters squared using the polygon measurement feature, with the scale based on the in-photograph scale placed next to each wound. Calibration was performed prior to AREA evaluation for each photograph using the in-photograph scale. Wound imaging and macroscopic evaluation were performed on days 3, 7, 10, 14, 17, 21, 24, 28, 31, and 35 postsurgery.

Statistical analysis

Statistical analyses were performed using STATA (StataCorp LLC). A two-level linear mixed-effects restricted maximum likelihood regression model was used to analyze the effects of independent variables on either dependent variable (VE or AREA). For both VE and AREA models, the random portion of the model (level 2) was set at individual fish identification (ID) with random intercepts. A random slopes model, allowing slopes to vary within trial or ID, was also compared to a model with only random intercepts with ID using a likelihood ratio (LR) test to determine if either of these additions significantly improved the model. Once the random portion of the model was determined, the fixed portion of the model was added, which included the dependent variable (VE or AREA) and independent variables. Independent variables included the day postsurgery (day, as a categorical variable), treatment (coded 0, control; 1, ONCE; 2, iLEX only all days [iLEX only]; and 3, naltrexone in iLEX all days [NALTX]), and treatment by day interaction. Interaction variables were removed if nonsignificant (P > .1), and the model was rerun for results.

To evaluate time to healing postsurgery (HDAY, dependent variable), the day at which no lesions were observed macroscopically was recorded for each treatment and control wound. A two-level linear mixed-effects restricted maximum likelihood regression model with ID as the random variable, HDAY as the dependent variable, and scoring method (VE, 0; AREA, 1), treatment (0, control; 1 any treatment), or TRIAL and the interaction between scoring method and treatment (or TRIAL) as the fixed variables was used to assess the effects of treatment on HDAY. Interaction variables were removed if nonsignificant (P > .1), and the model was rerun for results.

For histologic evaluation, each scored microscopic feature (n = 6) was evaluated individually to compare treatment versus control groups using a generalized linear model with log link and Poisson family. The dependent variable was the histologic score for each separate microscopic feature, and thus the model was run 6 times against the various treatments to represent each different experimental trial and the control group combination. Finally, a model was run by combining all wounds that had received any treatment into 1 group versus nontreated controls within each histological score to determine if any treatment effect on histologic wound healing was detectable.

All final mixed-effects models were checked for normality using quantile plots of the standard residuals. If quantile-quantile plots of standardized residuals exhibited non-normal distributions, then data was transformed as predicted by the Shapiro-Wilk test until residuals were normalized if appropriate. The significance of all final models was determined
using a Wald χ² test. Marginal (predicted) means within each of the categorical variables were determined, and multiple-comparisons tests were performed with Sidak corrections (P < .05) to look for differences between fixed variables as appropriate.

**Results**

**Clinical wound assessment**

Median and 95% CI values for AREA and macroscopic wound assessment (VE) for each treatment over time are presented in Tables 2 and 3, respectively. For AREA model development, the random effects portion of the model was improved (LR test χ² = 22.6; P < .0001). Both day (χ² = 2324; P < .0001) and day by treatment (χ² = 53.3; P = .0018) were significant; treatment was not. Within day by treatment, significant differences between treatments were detected by days 10, 14, and 17, with only NALTX being significantly improved compared to control and ONCE (Figure 1). In addition, NALTX was significantly improved compared to iLEX only on days 10 and 14. By day 21, no significant intragroup differences were detected across the treatments (Figure 1).

For wound VE model development, the random effects portion of the model was improved (LR test χ² = 19.4; P = .0001) by allowing random slopes for the treatments and random intercepts with animal ID. The random effects portion of the model significantly accounted for model variance (LR test χ² = 7.1; P = .03) by allowing random slopes for the treatments and random intercepts with animal ID. The random effects portion of the model was further improved (LR test χ² = 22.6; P < .0001). Both day (χ² = 7.1; P = .003) and day by treatment (χ² = 22.6; P < .0001) were significant; treatment was not. Within day by treatment, significant differences between treatments were detected by days 10, 14, and 17, with only NALTX being significantly improved compared to control and ONCE (Figure 1). In addition, NALTX was significantly improved compared to iLEX only on days 10 and 14. By day 21, no significant intragroup differences were detected across the treatments (Figure 1).

**Table 1**—Median (95% CI) of the clinical wound assessment results. Wounds were graded at each timepoint based on the macroscopic grading criteria in Table 1 on a scale from 0 to 5. The results for control were taken as the average result for the untreated side of fish from all 3 treatment groups.

**Table 2**—Wound area.

<table>
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</thead>
<tbody>
<tr>
<td>Naltrexone (single treatment)</td>
<td>0.29 (0.28–0.31)</td>
<td>0.29 (0.28–0.31)</td>
<td>0.27 (0.22–0.28)</td>
<td>0.20 (0.17–0.23)</td>
<td>0.16 (0.13–0.18)</td>
<td>0.02 (0.01–0.05)</td>
<td>0 (0.00–0.00)</td>
<td>0 (0.00–0.01)</td>
<td>0 (0.00–0.00)</td>
<td>0 (0.00–0.00)</td>
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<tr>
<td>Naltrexone (treated q 72–96 h)</td>
<td>0.26 (0.24–0.28)</td>
<td>0.26 (0.24–0.28)</td>
<td>0.21 (0.17–0.22)</td>
<td>0.16 (0.12–0.18)</td>
<td>0.10 (0.07–0.11)</td>
<td>0 (0.00–0.03)</td>
<td>0 (0.00–0.01)</td>
<td>0 (0.00–0.00)</td>
<td>0 (0.00–0.00)</td>
<td>0 (0.00–0.00)</td>
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<tr>
<td>iLEX only (treated q 72–96 h)</td>
<td>0.28 (0.25–0.31)</td>
<td>0.28 (0.27–0.31)</td>
<td>0.26 (0.22–0.30)</td>
<td>0.26 (0.24–0.29)</td>
<td>0.16 (0.12–0.17)</td>
<td>0.01 (0.00–0.01)</td>
<td>0 (0.00–0.01)</td>
<td>0 (0.00–0.01)</td>
<td>0 (0.00–0.00)</td>
<td>0 (0.00–0.00)</td>
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<tr>
<td>Control</td>
<td>0.28 (0.26–0.29)</td>
<td>0.27 (0.26–0.28)</td>
<td>0.26 (0.24–0.27)</td>
<td>0.23 (0.21–0.25)</td>
<td>0.14 (0.1–0.25)</td>
<td>0.05 (0.02–0.05)</td>
<td>0 (0.00–0.03)</td>
<td>0 (0.00–0.02)</td>
<td>0 (0.00–0.00)</td>
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**Table 3**—Macroscopic scoring results.

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</thead>
<tbody>
<tr>
<td>Naltrexone</td>
<td>4.0 (3.7–4.1)</td>
<td>4.0 (3.6–4.0)</td>
<td>3.3 (3.0–3.4)</td>
<td>1.8 (1.5–2.1)</td>
<td>0.5 (0.4–0.9)</td>
<td>0 (0–0.2)</td>
<td>0 (0–0.1)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
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<tr>
<td>(single treatment)</td>
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<tr>
<td>Naltrexone</td>
<td>4.0 (3.7–4.2)</td>
<td>3.8 (3.6–4.0)</td>
<td>3.0 (2.5–3.3)</td>
<td>1.6 (1.4–2.0)</td>
<td>0.3 (0.1–0.5)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
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<tr>
<td>(treated q 72–96 h)</td>
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<td></td>
<td></td>
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<tr>
<td>iLEX only</td>
<td>4.0 (3.4–4.2)</td>
<td>4.0 (3.4–4.1)</td>
<td>3.0 (2.4–3.3)</td>
<td>2.3 (1.6–2.5)</td>
<td>0.5 (0.2–0.6)</td>
<td>0 (0–0.3)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>(treated q 72–96 h)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.0 (3.5–4.0)</td>
<td>4.0 (3.5–3.9)</td>
<td>3.3 (2.9–3.4)</td>
<td>2.0 (1.7–2.2)</td>
<td>0.5 (0.2–0.9)</td>
<td>0 (0–0.1)</td>
<td>0 (0–0.3)</td>
<td>0 (0–0.2)</td>
<td>0 (0–0.2)</td>
<td>0 (0–0)</td>
</tr>
</tbody>
</table>

Median (95% CI) of the clinical wound assessment results. Wounds were graded at each timepoint based on the macroscopic grading criteria in Table 1 on a scale from 0 to 5. The results for control were taken as the average result for the untreated side of fish from all 3 treatment groups.
Complete resolution of surgical wounds via macroscopic assessment was achieved in all fish by day 35. Across all treatments, random effects indicated that individual animals had significant effects in wound healing ($\chi^2 = 10.1; P = .0008$), and within fixed effects and across all treatments, treated wounds healed faster (21.9 ± 0.58 days; $\chi^2 = 11.6; P = .0006$) than controls (24.0 ± 0.58 days). However, an effect ($\chi^2 = 11.2; P = .0008$) of scoring method (for both control and treatment groups) was detected with VE, estimating that healing occurred by 22.0 days (95% CI, 20.8 to 23.1 days) versus 24 days (95% CI, 22.9 to 25.2 days) for AREA. Because scoring methods had significant differences in HDAY, we repeated the analysis for each scoring method separately and compared individual treatment protocols. For VE scoring, HDAY was only different ($P < .05$) for NALTX (19.4 ± 1.2 days) when compared to CONTROL (23.3 ± 1.2 days). For AREA, no differences were detected in HDAY between combined treatment and control groups, but direct comparisons of AREA for individual treatments groups and the control group control (24.8 ± 0.6 days) indicated that, similar to VE, NALTX (22.2 ± 1.0 days) was different ($P < .05$; Table 4).

### Discussion

This study documented faster wound healing ($P = .002$) in fish treated with topical 0.04% naltrexone in iLEX ointment every 72 to 96 hours (day 19.4) compared to untreated wounds (day 23.3). Additionally, fish treated with 0.04% naltrexone in iLEX every 72 to 96 hours had reduced ($P < .05$) AREA compared to both controls and fish treated with topical 0.04% naltrexone in iLEX once.

In a previous study that used topical naltrexone in iLEX at the same concentration for the treatment of LLD, a chronic and often erosive dermatologic condition of teleosts, a reduction in lesion size and improvement in pigmentation was noted 33 days after a single application of topical naltrexone. Although naltrexone in iLEX ointment–treated wounds had a significant reduction in AREA at days 10, 14, and 17 postsurgery, no significant intragroup differences were detected across the treatments from days 21 to 35. The difference in the timing of treatment response with topical naltrexone in the current study as compared with the previous LLD investigation may be attributable to discrepancies in lesion pathogenesis. The current study evaluated experimentally induced acute mechanical wounds. LLD represents manifestations of a more chronic process with variable underlying etiologies and histologic features that may include epidermal loss without inflammation, epithelial hyperplasia with or without hyperplasia of mucus-producing cells and inflammation, and loss of melanocytes.

The mechanism by which topical naltrexone in iLEX led to a reduction in wound size and an improvement in lesion pigmentation in fish with LLD was unknown. Similarly, underlying mechanisms contributing to the observed treatment effects in the current study were not identified. The cellular

**Table 4**—Time to healing.

| Group                          | Macroscopic scoring | $P$ values (macroscopic scoring) | Wound area | $P$ values
|-------------------------------|----------------------|----------------------------------|------------|---------------------|
| Naltrexone (single treatment) | 21.7 (19.3–24.1)     | .198                             | 23.8 (21.8–25.7) | .035
| Naltrexone (treated q 72–96 h) | 19.4 (17.0–21.8)     | .002                             | 22.2 (20.2–24.1) | .016
| iLEX only                     | 21.0 (17.7–24.3)     | .179                             | 24.5 (21.8–27.3) | .883
| Control                       | 23.3 (21.7–24.9)     | —                                | 24.8 (23.5–26.0) | —

Marginal mean (95% CI) of the time (days) to complete resolution of wound healing as assessed by macroscopic evaluation and wound area measurements. $P$ values depict comparison of treatment group to control group for the macroscopic evaluation and wound area measurements.

**Table 5**—Histopathologic scoring.

<table>
<thead>
<tr>
<th>Group</th>
<th>Epithelization</th>
<th>Scales</th>
<th>Dermal inflammation</th>
<th>Dermal neovascularization</th>
<th>Muscle inflammation</th>
<th>Muscle fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naltrexone (single treatment)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (0–1.0)</td>
<td>1.0 (0–2.0)</td>
<td>1.5 (0–3.0)</td>
<td>1.0 (0–2.0)</td>
<td>1.0 (0–2.0)</td>
</tr>
<tr>
<td>Naltrexone (treated q 72–96 h)</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
<td>0 (0–1.0)</td>
<td>0.5 (0–2.0)</td>
<td>0.5 (0–1.0)</td>
<td>0.8 (0–2.0)</td>
</tr>
<tr>
<td>iLEX only</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (1.0–1.0)</td>
<td>0 (0–1.0)</td>
<td>0 (0–3.0)</td>
<td>0 (0–1.0)</td>
<td>0 (0–2.0)</td>
</tr>
<tr>
<td>Control</td>
<td>1.0 (1.0–1.0)</td>
<td>1.0 (0–1.0)</td>
<td>1.0 (0–1.0)</td>
<td>2.0 (0–3.0)</td>
<td>1.0 (0–2.0)</td>
<td>1.0 (0–3.0)</td>
</tr>
</tbody>
</table>

Distribution of histological scores median (range) for each parameter assessed in naltrexone and iLEX wounds and untreated wounds. Results for control were taken as the average result for the untreated side of fish from all 3 treatment groups.
mechanisms that participate in wound healing in fish have yet to be fully elucidated. The results of these studies utilizing topical naltrexone in teledonts suggest that an OGF-OGFr or similar cell proliferation regulatory pathway may exist in fish. Future studies to identify and characterize cell proliferation regulatory pathways in fish would be applicable toward a better understanding of healing mechanisms and identification of possible novel treatment modalities.

This study found that fish treated with topical 0.04% naltrexone in iLEX every 72 to 96 hours healed faster than untreated wounds. In a previous study that assessed the efficacy of misoprostol/phenytoin on wound healing in brook trout (Salvelinus fontinalis), wound healing was variable after 120 days. Possibly, other factors (eg, environmental parameters, secondary infection) impacted variable or prolonged healing in other studies as compared with the results of the current investigation. Histologic evaluation of wounds was performed on day 35 in this study, when macroscopic lesions were no longer present. Histologic findings were compatible with chronic reactive changes indicative of normal healing. The lack of differences in histologic scores between treatment and control groups was a likely consequence of wound chronicity and due to the timing of evaluation. There were no adverse effects seen during routine procedural health monitoring following repeated treatment (every 72 to 96 hours) with 0.04% naltrexone in iLEX or iLEX alone. In fish, wound healing speed decreases with time; thus, by assessing a single point histologically at the end of the study, temporal and dynamic histologic differences between the treatments could not be assessed.

ILEX ointment is a hydrophobic ointment used on excoriated skin in humans to protect wounds from moisture. Wounds treated with either 0.04% naltrexone in iLEX or iLEX alone healed faster than untreated wounds; however, AREA was significantly reduced in fish treated every 72 to 96 hours with 0.04% naltrexone in iLEX compared to fish treated with iLEX alone or 0.04% naltrexone in iLEX once. Furthermore, the time to complete resolution of the wound was significantly improved with naltrexone. The results indicate that although iLEX treatment alone provides a positive effect on wound healing, the addition of 0.04% naltrexone speeds the process and elicits earlier resolution. The efficacy of iLEX ointment alone was attributed to the hydrophobic properties, which provide a barrier from the aqueous environment, or presence of zinc oxide, which promotes wound healing in rodents and humans.

The use of iLEX ointment in wound healing is also reported in aquatic chelonian shell fracture repair, where it is applied in cases of closed shell fractures to keep water out of the wounds. Although a reduction in AREA compared to control was noted 10, 14, and 17 days postsurgery in fish treated every 72 to 96 hours with 0.04% naltrexone in iLEX, the interval of evaluation (every 3 to 4 days) may have precluded detection of additional temporal differences in healing amongst treatment groups.

Study findings should be evaluated in light of possible confounding factors that could have influenced outcomes. Fish were maintained at relatively warm temperatures (24.7 to 25.4°C). Previous studies have documented an improvement in inflammation and AREA in common carp (Cyprinus carpio) kept at 18 to 21°C. Water temperatures in the current study were also warmer than those associated with prolonged (greater than 120 days) experimental wound healing in brook trout. Previous studies have determined the positive correlation of water temperature and wound closure due to increased motility of keratocytes at warmer temperatures. Although water temperature could have influenced wound healing in the current study, this variable fails to account for differences among study groups given each fish served as its own negative/untreated control and all fish were maintained under the same environmental conditions. Another factor that may have influenced outcomes was that wound evaluations were conducted at multiple time points under anesthesia; the effect of stress and exposure to buffered tricaine methanesulfonate water every 72 to 96 hours on wound healing is unknown. Given the aforementioned specifics of the study design, however, possible anesthesia-related stress would have been equivalent across control and treatment groups.

In conclusion, wound healing was faster in fish treated with 0.04% naltrexone in iLEX ointment every 72 to 96 hours for 10 total treatments compared to untreated wounds. Significant reductions in AREA postsurgery were observed with the application of 0.04% naltrexone in iLEX ointment every 72 to 96 hours compared to controls and fish treated with 0.04% naltrexone in iLEX ointment once. Future studies on teledont wound healing may benefit from more frequent intervals of evaluation and data collection to assess possible earlier temporal differences in treatment effects. Additionally, study design to accommodate prospective (as opposed to only end point) histologic examination of wound tissues could provide further insight into the processes of wound resolution. Continued investigation to determine the role of OGF-OGFr pathway dysregulation in acute wound healing and other dermatologic conditions in fish, potential systemic absorption of the topical naltrexone, and the effects of topical naltrexone on chronic nonhealing wounds in teledonts are warranted. The study findings suggest that OGFr inhibition may influence cutaneous wound healing in teledonts.

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