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Short communication

Does UV disinfection compromise sutures? An evaluation of tissue response and suture retention in salmon surgically implanted with transmitters

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ABSTRACT

Ultraviolet radiation (UVR) can be used as a tool to disinfect surgery tools used for implanting transmitters into fish. However, the use of UVR could possibly degrade monofilament suture material used to close surgical incisions. This research examined the effect of UVR on monofilament sutures to determine if they were compromised and negatively influenced tag and suture retention, incision openness, or tissue reaction. Eighty juvenile Chinook salmon *Oncorhynchus tshawytscha* were surgically implanted with an acoustic transmitter and a passive integrated transponder. The incision was closed with a single stitch of either a suture exposed to 20 doses of UV radiation (5 min duration per dose) or a new, sterile suture. Fish were then held for 28 days and examined under a microscope at day 7, 14, 21 and 28 for incision openness, ulceration, redness, and the presence of water mold. There was no significant difference between treatments for incision openness, redness, ulceration or the presence of water mold on any examination day. On day 28 post-surgery, there were no lost sutures; however, 2 fish lost their transmitters (one from each treatment). The results of this study do not show any differences in negative influences such as tissue response, suture retention or tag retention between a new sterile suture and a suture disinfected with UVR.

1. Introduction

In human medicine and veterinary practice, aseptic technique is required, which includes sterilization of tools or use of new disposable sterile tools (e.g., Kumar, 1996; Kirk, 2010). However, a completely aseptic surgery is not possible when using aquatic animals such as fish (Mulcahy, 2003; AFS, 2004). Their natural environment is not pathogen free prior to or post-surgery, so there is not an adequate way to keep the wound aseptic once the fish is returned to the water. Therefore, most institutional animal care and use committees (IACUC) require that fish surgeries be as aseptic as possible, something advocated by veterinarians with aquatic animal health training (Harms and Lewbart, 2011). Since there are few guidelines directly related to fish surgery, there is a need for the development of science-based methods that provide researchers and IACUCs with a variety of disinfection techniques, including those relevant to field situations.

Biotelemetry is a tool that is commonly used around the world to study the spatial ecology and survival of fish in marine and freshwaters (Lucas and Baras, 2000). Biotelemetry studies can have sample sizes that vary from relatively small (e.g., under 20 as in Dunlop et al., 2010; Hahn et al., 2007) to very large (e.g., thousands as in McMichael et al., 2010; Harnish et al., 2012). It is therefore not unreasonable to use all sterile tools for surgically implanting transmitters when the sample sizes are small by simply having multiple sets of tools and using new supplies (e.g., sutures) on each animal (Mulcahy, 2003). However, when hundreds of fish need to be tagged daily during a field season, it is important to have a variety of options for disinfection of tools between surgeries (e.g., ethanol, benzalkonium chloride, and chlorhexidine; Wagner et al., 2011), including methods that can rapidly disinfect tools such as ultraviolet radiation (Walker et al., 2013).

Ultraviolet radiation is a quick and effective method for disinfecting surgery tools (Walker et al., 2013). It has several practical advantages over other techniques including that it can be done in

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the field (requires a power source such as a car or deep cycle battery and power converter) due to the UV systems size and weight, does not involve hazardous chemicals, and does not involve use of extreme temperatures (i.e., autoclaves and hot bead sterilizers; NRC, 2011) that could melt (heat) or shatter (cold) sutures which can be used in the field with an adequate power source. However, some researchers and veterinarians are concerned that UVR could potentially degrade sutures, leading to lower tensile strength or an increased rate of absorption (R. Brown, personal communication) as seen in a variety of high strength fibers (Said et al., 2006) and other manufactured materials (Andrady et al., 1998). Compromised sutures could lead to an increase in negative tissue reaction, premature structural failure or a lack of incision closure during healing. This could reduce tag retention in fish with surgically implanted transmitters.

We hypothesized that using a UVR-exposed suture to close an incision made to implant a biotelemetry transmitter would not increase tissue response or lead to premature incision opening or tag expulsion when compared to a new certified sterile suture. To examine this, water mold, tissue response (redness and ulceration) around the incision, openness of the incision, suture retention, and tag retention were examined over a 28-day period in fish with incisions closed using a UVR-exposed monofilament (poliglecaprone 25) suture and fish with non-exposed sutures.

2. Methods

2.1. Fish acquisition and handling

Juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*) were transported from Leavenworth Fish Hatchery (Leavenworth, WA) to the Aquatic Research Laboratory (ARL) at Pacific Northwest National Laboratory (PNNL, Richland, WA) as eyed eggs and were reared to the juvenile stage at the ARL. Test fish for the control group (incision closed with a new certified sterile suture) ranged in fork length from 95 to 118 mm (mean 107 mm) and 12.0 to 22.9 g (mean 17.8 g) in weight. Test fish having a UVR-exposed suture ranged in fork length from 100 to 129 mm (mean 110 mm) and 14.3 to 31.5 g (mean 19.2 g) in weight. Fish were held in a 490 L circular tank at 17 °C non-recirculating river water and were not feed 1 day prior to surgery.

2.2. Surgery

Acoustic transmitters (Juvenile Salmon Acoustic Telemetry System transmitters; ATS, Isanti, MN) and passive integrated transponders (Destron Technologies, St. Paul, MN) weighed 0.3 g and 0.1 g in air respectively. Tags were surgically implanted into 80 fish using methods similar to Deters et al. (2012) and a single suture with a reinforced square knot ($1 \times 1 \times 1 \times 1$) was used to close the incision. Incision lengths ranged from 5.5 to 9.0 mm (mean 6.8 mm). Tag burdens (ratio of transmitter mass to fish mass) ranged from 1.7% to 3.3% (mean 2.2%) and 1.3% to 2.8% (mean 2.1%) for fish with a new sterile suture and fish with a UVR-exposed suture, respectively. To compare the effects of UV radiation on the suture material and fish, the incisions were closed with either a UVR-exposed suture ($n = 40$) or a new sterile suture (control; $n = 40$). Sutures were only used once before being discarded.

Sutures used for the holding study were sterile 5-0 absorbable monofilament (poliglecaprone 25) sutures (brand name Monocryl; Ethicon, San Angelo, TX). Absorbable monofilament sutures were chosen because they have been shown to have higher retention and lower inflammation than other sutures (Deters et al., 2010; Ivasauskas et al., 2012). In addition, these sutures are non-porous and single-stranded, making it a viable option for UVR disinfection because a porous suture, like a silk braided suture may have areas where pathogens could be shaded from UVR and thus not effectively disinfected. Prior to surgery, sutures were cut to 114 mm using sterile scissors. Half of the sutures were exposed to UVR in the UV system using methods described by Walker et al. (2013). The remaining non-UVR exposed sutures were reserved in a sterile container until surgery. Exposure to UV radiation consisted of 20 5-min exposures with doses ranging from 181 to 339 mJ/cm^2 (mean 320 mJ/cm^2) per exposure. This simulated the initial use on a single fish and then repeated use to close incisions on 19 other fish (i.e., 20 is the number of fish that could potentially be sutured using a single 450 mm suture). To simulate the reuse of sutures in multiple fish, which would result in exposure to moisture, the UVR exposed sutures were passed through a ~2 mm piece of wet non-sterile neoprene foam prior to each 5-min exposure.

2.3. Post-surgery

Following surgery, a stereomicroscope (0.65× magnification; Stemi 2000–CS, Zeiss AG, Jena, Germany) connected to a computer and monitor was used for viewing and taking images of the fish incision area. Fish were allowed to recover from anesthesia in a 20-L bucket for approximately 15 min. Then fish were placed in a 490 L circular tank inside the ARL where they were held at approximately 17 °C for 28 days post-surgery. Lights inside the ARL were controlled to follow the natural photoperiod and fish were fed daily an ad libitum ration of Bio Vita Fry (Bio-Oregon, Longview, WA).

2.4. Evaluation criteria

At 7, 14, 21, and 28-day post-surgery, all fish were anesthetized in an 80 mg/L solution of MS-222 buffered with an equal amount of sodium bicarbonate until they lost equilibrium. Before fish were evaluated, a ruler at a fixed height under the microscope was calibrated with image analysis software (Image-Pro Plus and Image-Pro Analyzer, version 7.0.1, Media Cybernetics, Bethesda, Maryland). The fish was then elevated so that the incision was in the same plane as the ruler. The area of incision openness, ulceration, redness, and water mold (*Saprolegnia* spp.) were then outlined on examination photographs with the imaging software; area was calculated in square millimeters. Incision openness was examined by measuring any areas in which wound edges were gaping similar to Deters et al. (2012). Fish were then allowed to recover from anesthesia and returned to the holding tank. Mortalities and tag loss were monitored daily throughout the 28-day holding period.

2.5. Analysis

The area of openness, ulceration, redness, and water mold were compared to determine if there was a difference between the two treatments. Since measurements of redness, ulceration, openness, and water mold are continuous variables, but failed to meet the assumptions of a parametric test, they were analyzed for each observation day (days 7, 14, 21, and 28) individually using a Mann–Whitney *U*-test. A repeated-measures analysis of variance was not used because many values approached or remained at zero on days 7–28, thus obscuring or eliminating the correlations between weeks that repeated measures would determine (similar to Panther et al., 2011). An α of 0.05 was used for all statistical tests.

3. Results

3.1. Mortality, tag retention, and suture retention

There was no mortality and 100% of sutures were retained over the 28-day holding period. Although all PIT tags were retained in

fish, 2 (3%) of the acoustic transmitters were expelled. The first acoustic transmitter (from the control group) was found on day 14; this fish had a rather large incision (8.25 mm). The second acoustic transmitter (from the UVR-exposure group) was not found during the day 28 necropsy and therefore was assumed to have been expelled. Images taken of the fish on days 14 and 21 provided evidence that the missing tag was actively expelled from the 7 mm incision.

3.2. Openness

Openness was observed in 3 fish (3.8%; range 0.15–1.08 mm^2) throughout the 28-day holding period (Fig. 1). On day 7 all incision edges remained 100% approximated, and thus no incision openness, for all fish. Openness at days 14, 21 and 28 did not vary significantly ($P = 0.289$, $P = 0.937$, and $P = 0.289$, respectively) between treatments. The largest amount of incision openness was observed in the fish mentioned above that expelled its transmitter.

3.3. Ulceration and redness

Ulceration of the wound was observed in 2 fish (2.5%; range 0.1–3.7 mm^2) throughout the 28-day holding period (Fig. 1). Ulceration was not observed on any fish at day 7 or day 21. Ulceration at days 14 and 28 did not vary significantly ($P = 0.372$ for both comparisons) between treatments.

Redness of the incision was observed in 10 fish (8.0%; range 0.03–1.25 mm^2) throughout the 28-day holding periods (Fig. 1). Redness at days 7, 14, 21, and 28 did not vary significantly ($P = 0.393$, $P = 0.110$, $P = 0.372$ and $P = 0.259$, respectively) between treatments.

3.4. Water mold

Water mold was observed in 4 fish (5.0%; range 0.37–9.33 mm^2) and one fish having more extensive presence of water mold (29.63 mm^2). Water mold was not observed on any fish at day 7. Water mold at days 14, 21, and 28 did not vary significantly ($P = 0.372$, $P = 0.937$, and $P = 0.409$, respectively) between treatments.

4. Discussion

This research indicates that the exposure of sutures to a UVR disinfection process does not negatively influence juvenile salmonids surgically implanted with transmitters. Although a variety of other means are used to disinfect or sterilize surgical tools, such as the use of chemicals (e.g. ethanol, benzalkonium chloride, chlorhexidine; see Mulcahy, 2003; Wagner et al., 2011), there have not been evaluations to determine if these processes may compromise sutures. In fact, there is generally a lack of reporting on how sutures are treated during the process of surgically implanting fish. Several researchers have noted that much of the scientific literature related to the use of telemetry lacks detailed information on methods and materials used for surgical implantations of transmitters (Brown et al., 2011; Thiem et al., 2011). So it should not be a surprise that although many fisheries researchers may disinfect and reuse sutures, disinfection techniques are generally not included in reports or publications.

It is important when reusing sutures that they not prematurely fail and not increase tissue irritation or increase the presence of water mold. Testing the degradation of fibers is commonly done in the textile engineering field using tensile strength measuring devices; this kind of testing is not likely to address all the issues associated with fish surgery applications. While tensile strength could be tested at a point that could be relevant to structural integrity before use on fish, this would not provide information relative to the long term effects on the incision. Therefore the testing method needs to be all encompassing of the effects a potentially degraded suture could have on the fish during the incision healing period. This was accomplished in this research by examining the ability to properly tighten suture knots during surgery and examining suture retention and tissue reaction at four time points (7, 14, 21 and 28 days after surgery).

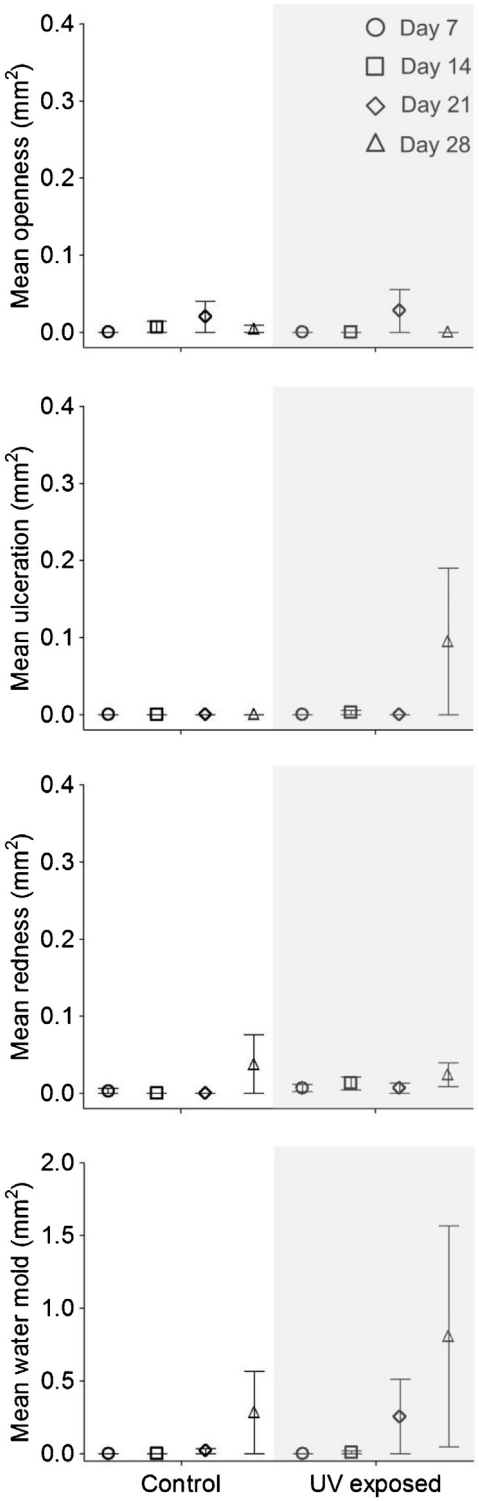

Fig. 1. Mean (whiskers represent ±standard error) area in mm^2 of incision openness, ulceration, redness, and water mold by treatment (control and UV exposed) for observations made on days 7, 14, 21, and 28.

The purpose of this research was to provide scientific justification that can be used by fisheries researchers in general and by IACUC bodies when recommending guidelines for surgical procedures on fish. While fisheries researchers may commonly disinfect and reuse sutures, our experience has been that this can be a concern to an IACUC. These committees generally include veterinarians, which would not consider the reuse of a suture to be acceptable when conducting surgery on mammals. Thus, this research is very relevant and with research dollars scarce, reusing UV sterilized sutures on multiple fish provides a cost-effective strategy which does not appear to compromise suture performance and thus fish welfare or study objectives.

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