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Evaluation of the Gross and Histologic Reactions to Five Commonly Used Suture Materials in the Skin of the African Clawed Frog (*Xenopus laevis*)

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Surgical harvest of *Xenopus laevis* oocytes for developmental research is a common procedure that requires closure of a 0.5- to 2.0-cm incision with suture material. Although such harvests are a frequent practice, little published information exists to provide guidance regarding the most appropriate suture material for wound closure in laboratory amphibians. To determine which suture material elicits the least response in amphibian skin, we used *Xenopus laevis* as a model to investigate the gross and histologic tissue reactions to 5 commonly used suture materials—3-0 silk, monofilament nylon, polydioxanone, polyglactin 910, and chromic gut. The skin reacted in 3 ways to suture material, showing edema, epidermal changes, and inflammation. Although the gross reactions to monofilament nylon, polydioxanone, and polyglactin 910 were clinically indistinguishable and were associated with lowest gross reaction scores, monofilament nylon elicited the least histologic reaction and therefore seems to be the most appropriate choice for use in amphibian skin.

Amphibian medicine and surgery is becoming commonplace in research, zoological park and aquarium settings, and in the exotic animal companion medicine industry. The African clawed frog (*Xenopus laevis*) is used extensively as a nonmammalian model for basic research in vertebrate developmental biology, physiology, and biochemistry laboratories.[10,17] Much of the research using *Xenopus laevis* requires repeated surgical oocyte extractions in a laboratory setting. Typically, oocytes are harvested from anesthetized animals through a 0.5 to 2.0-cm incision through the coelomic skin and muscular body wall. After harvest, the site is sutured closed.

A brief Internet-based review of institutional animal care and use committee policies for *X. laevis* oocyte harvest procedures revealed no consensus regarding what is considered the most appropriate material for wound closure in this species.[1,5,22] In light of clinical experience, Wright subjectively recommended the use of nonabsorbable monofilament suture material for aquatic amphibians because absorbable sutures tend to dissolve in damp or aquatic environments.[25] For a variety of species, severe tissue reactions and dehiscence of the suture line have been reported to cause inflammation, infection, and loss of tissue integrity,[19,23] ultimately rendering animals unsuitable for future research and debilitating collection and pet animals. The tissue reactions of koi to various suture types[11] and the effect of suture material on surgical wound healing in sea turtles[9] have been reported, and studies of suture reactions have been performed in human, canine, feline, rodent, and avian subjects,[3,4,7,10,14-16,24] but published information regarding preferred suture material(s) for use in *X. laevis* or other aquatic amphibians is unavailable. To this end, we investigated the tissue reactions of *X. laevis* to 5 commonly used suture materials: silk, monofilament nylon, polydioxanone, polyglactin 910, and chromic gut. To our knowledge, this investigation is the first to address suture reactions in an amphibian species.

Materials and Methods

We obtained 12 adult *Xenopus laevis* (6 males [body weight, 50 to 65 g; mean, 56 g] and 6 females [body weight, 78 to 91 g; mean, 84 g) from a commercial vendor (Xenopus I, Dexter, MI). They were housed individually in standard-sized transparent plastic mouse microisolation cages that were half covered with dark plastic. Frogs were maintained in approximately 8 l of static well water maintained at 65 to 71 °F (18.3 to 21.7 °C) and a pH of 6.8 to 7.1, with no detectable ammonia, nitrate, and nitrite. The full volume of water was changed every other day, and a steam-sterilized cage was provided at each water change. Subjects were fed a commercially available maintenance diet for *X. laevis* (Adult Frog Brittle, Nasco, Ann Arbor, MI) ad libitum 3 times weekly and kept on a 14:10-h light:dark cycle. They were acclimated for 2 wk and received a physical examination to exclude overt health problems prior to the start of the study. All handling of subjects was approved by the North Carolina State University College of Veterinary Medicine Institutional Animal Care and Use Committee. The college's laboratory animal care program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

On day 1, each frog was anesthetized by immersion in a tricaine methanesulfonate solution (1 g/l of MS-222 buffered with 2 g/l sodium bicarbonate; Tricaine-S, Aquatic Eco-Systems, Apopka, FL) until they no longer responded to tactile stimulation. Frogs then were removed from the anesthesia bath and placed in dorsal recumbency on gauze pads that had been soaked in anesthetic-free well water. The frogs' respirations and heart rates were monitored visually throughout anesthesia. The mucus layer was removed from the ventral surface of each frog by gentle wiping with a sterile gauze sponge, and surgery was performed with aseptic technique. Six stab incisions (3 mm long and full thickness through the skin) were made on the

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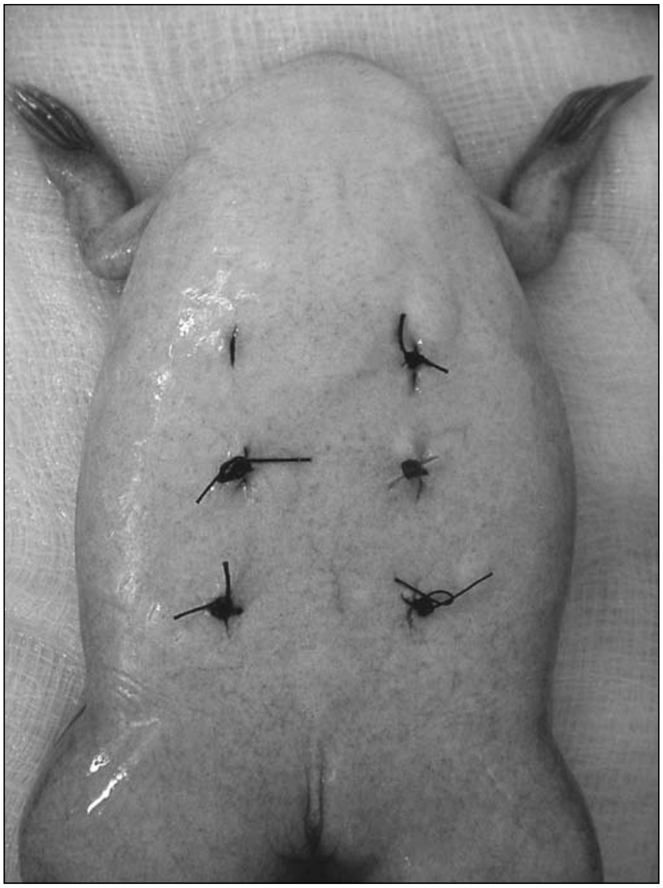

Figure 1. Diagram of suture placement on ventral surface of *X. laevis*.

animals' ventrums with a sterile #15 scalpel blade in a pattern of 2 columns with 3 stab incisions each. A single simple interrupted suture was placed in each of 5 of the 6 stab incisions with 3-0 silk (Ethicon, Ethicon, Irvine, CA), monofilament nylon (Ethilon, Ethicon), chromic gut (Ethicon), polyglactin 910 (Vicryl, Ethicon), and polydioxanone (PDS II, Ethicon) such that 1 suture material was used at each site and all 5 suture materials were used in each frog (Figure 1). The last stab incision was not closed with any suture material and served as the control. Suture position was rotated among frogs so that each suture material occupied all 6 placement sites twice. The same surgeon (ADT) performed all procedures.

After surgery, each frog received butorphanol tartrate (0.2 mg/kg; Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA) intramuscularly for analgesia, because butorphanol has been reported to have analgesic action in an experimental pain assay in amphibians.[18,21] Frogs then were recovered from anesthesia in a bath of clean well water. When each frog had recovered fully from anesthesia, it was returned to its original holding container. Sutures were examined visually daily for gross signs of dehiscence and inflammation and were graded on a scale of 0 to 5 as indicated in Table 1.

On day 7, 6 of the subjects were again anesthetized as described. The sutures were removed, and a 5-mm full-thickness biopsy of the skin surrounding and including each incision site was obtained. Biopsies were immediately fixed in 10% neutral-buffered formalin. This procedure was repeated in the remaining 6 subjects on day 14. Subjects recovered from anesthesia, and biopsy sites were left to heal by second intention.

The biopsies were processed routinely, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for

Table 1. System for scoring gross and histologic tissue reaction to suture in the skin of *X. laevis*

Score	Description
0	No deviation from incised but nonsutured site
1	Minimal edema, inflammation, and epidermal changes
2	Mild edema, inflammation, and epidermal changes
3	Moderate edema and inflammation, ± scattered areas of necrosis
4	Moderately severe edema and inflammation, with many areas of necrosis
5	Marked edema, inflammation, and epidermal changes

examination with light microscopy. The tissue biopsies were coded (ADT) and evaluated by a pathologist (JML) blinded to suture type. The pathologist used a 6-point scale previously used to interpret suture reactions in koi[11] and graded each of the biopsies in terms of degree of inflammation, epidermal changes, and edema (Table 1). Scores for each of these changes were recorded individually for the incision-only control and each suture material. The score for the control was subtracted from each suture score and then the average of all 3 tissue reaction components was calculated to obtain the overall reaction score. Data points for which suture was associated with dehiscence prior to the date of intended suture removal and biopsy were excluded.

Data were analyzed statistically using Statistix 8.0 software (Tallahassee, FL). Suture reaction data were analyzed by Friedman 2-way analysis of variance. Where significant differences were found, multiple comparison procedures were used to determine which suture reactions were different. The Wilcoxon matched-pairs signed-rank test was applied to data for this purpose. A Wilcoxon rank-sum test was applied to determine whether significant differences between days 7 and 14 and male and female frogs were present. A P value of <0.05 was regarded as statistically significant.

Results

The tissue changes seen histologically after placement of sutures in *Xenopus laevis* can be grouped into 3 main categories: edema, epidermal changes, and inflammation. Photomicrographs of these changes and a control biopsy for comparison are shown in Figure 2 A to C. The edema seen in response to suture placement was predominantly dermal but in many biopsies also was present in subcutaneous tissues and occasionally extended into the epidermis, especially with severe reactions. The increased clear space due to edema in the dermis gave a jumbled appearance to the mucus and granular glands in the skin and made the glands appear less dense. Epidermal changes noted included epidermal hyperplasia, apoptotic cells, remodeling caused by wound healing, and necrosis at the base of epidermal pegs. Although parasites such as *Pseudocapillaroides xenopodis* can cause epidermal hyperplasia,[20] we saw no clinical evidence of these parasites in any of the frogs or in the histologic sections reviewed. Furthermore, epidermal hyperplasia was noted in all suture biopsies but not in any control biopsies, lending support to the conclusion that the suture material was the inciting cause.

The inflammation seen in response to the suture materials was predominantly mixed (heterophils, lymphocytes, and macrophages) perivascular dermatitis in the deep dermis but often extended into the superficial dermis in severe reactions and included the presence of fibrin and hemorrhage. Heterophils were the most numerous of cells present in severe reactions.

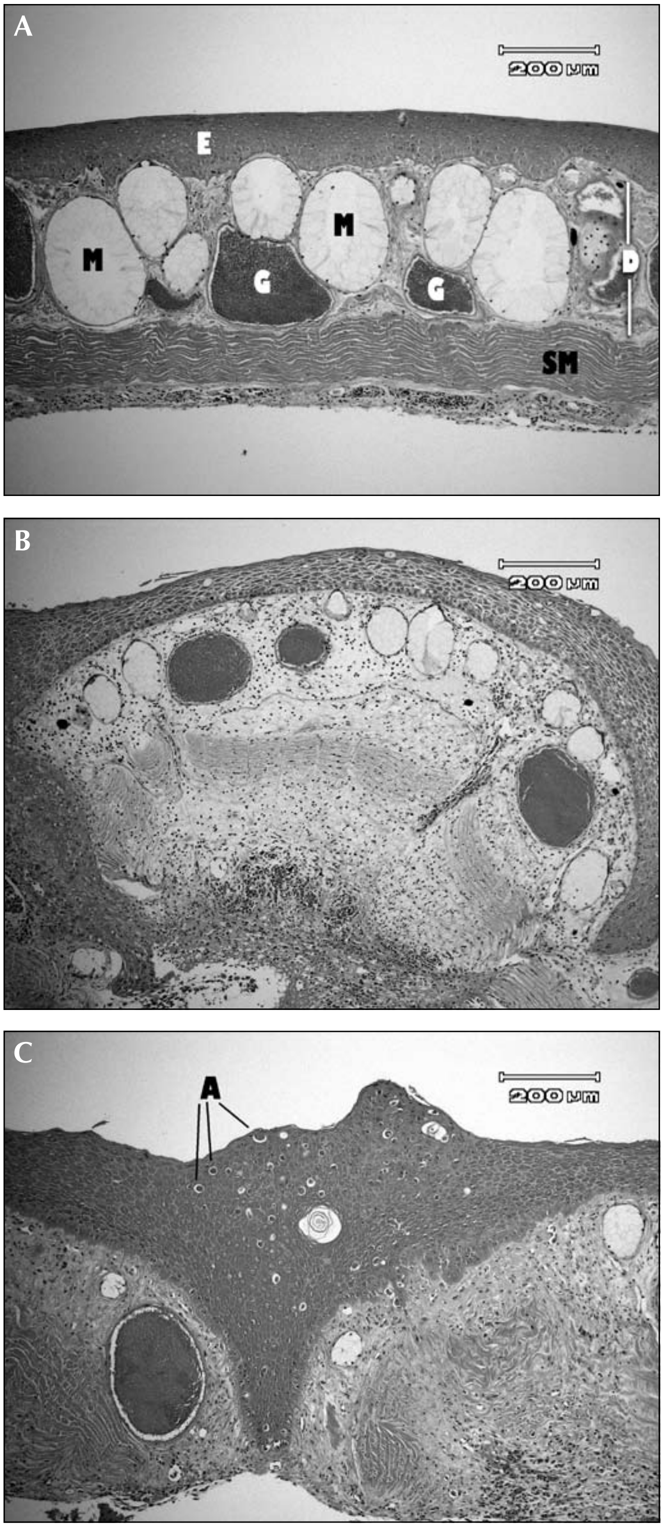

Figure 2. (A) Normal ventral coelomic skin of *X. laevis*. E, epidermis; D, dermis; M, mucus gland; G, granular gland; SM, subcutaneous muscle layer. (B) Skin reaction of *X. laevis* to silk suture: edema and inflammation. Severe edema extends from the dermis to the epidemis and subcutaneous muscle layer causing compression and jumbling of the skin glands and the appearance of increased clear space throughout these tissues. Moderate inflammation is also present, predominantly in the subcutis but extending to the dermis. (C) Skin reaction of *Xenopus laevis* to polydioxanone suture: epidermal changes. Severe epidermal hyperplasia and an increased incidence of apoptotic cells (A) and remodeling are present. There is also mild necrosis at the base of the epidermal peg.

Foreign-body granulomas comprising mixed-cell population containing epithelioid macrophages and giant cells and organized around pieces of suture material were visible in many of the sections taken on day 14 and included increased fibrosis and scarring.

All suture materials elicited significantly ($P < 0.05$) stronger tissue reactions, both grossly and histologically, than did the control stab incision. The histologic tissue reactions to suture materials overall were mild: the overall median reaction score for all suture types was 1.8 (10th percentile, 1.3; 90th percentile, 2.7). The suture to elicit the least histologic suture reaction was monofilament nylon (median, 1.7; 1.3, 2.0). Polydioxanone and polyglactin 910 caused significantly ($P < 0.05$) more histologic reaction in the skin than did the monofilament nylon and significantly ($P < 0.05$) less reaction than did chromic gut and silk but were not significantly different from each other ([1.8; 1.4, 2.0] and [1.8; 1.4, 2.6], respectively). Chromic gut elicited the next higher skin reaction score (2.0; 1.3, 2.7), and silk (2.3; 1.7, 2.6) elicited the most severe histologic reaction. Reaction scores did not differ significantly between male and female frogs or between days 7 and 14, although the epidermal changes were more severe on day 14 than day 7 and approached significance ($P = 0.06$).

Regardless of suture material, the overall reaction score for edema (2.5; 1.0, 4.0) was significantly ($P < 0.05$) higher than for epidermal changes (2.0; 1.0, 3.0), which was significantly ($P < 0.05$) higher than for inflammatory changes (1.0; 0.0, 2.0). The degree of edema and inflammation varied significantly by suture type. Because epidermal change did not differ significantly between suture types, edema and inflammatory scores were the primary determinants in the tissue reaction and overall score. Silk had the highest reaction score for both edema (3.0; 2.0, 4.0) and inflammation (2.0; 1.0, 2.9). Compared with all other suture materials except silk, chromic gut had a significantly ($P < 0.05$) higher edema reaction score (2.0; 2.0, 4.0), but its inflammation reaction score (1.0; 0.0, 2.0) did not differ significantly from that of any other suture material except silk. The inflammation score of monofilament nylon (1.0; 0.1, 2.0) was significantly ($P < 0.05$) different from only that of silk (2.0; 1.0, 2.9), and monofilament nylon caused the significantly ($P < 0.05$) lowest edema reaction score (1.0; 0.0, 2.0). The edema and inflammation scores for polydioxanone were 2.0 (1.1, 3.0) and 2.0 (1.0, 2.5), respectively, and for polyglactin 910 were 2.0 (1.1, 3.0) and 2.0 (0.0, 2.0), respectively.

The histologic grade of severity by suture type correlated well with mean gross scores where the least severe gross reaction scores were assigned when nylon, polyglactin 910, and polydioxanone (1.0 [1.0, 2.0], 1.5 [1.0, 2.0], and 1.0 [1.0, 2.0], respectively) were used. These sutures elicited a small area (1 to 4 mm) of redness with no visible swelling beginning at 2 to 3 d after suture placement, whereas chromic gut and silk sutures (histologic reaction score, 3.0 [2.0, 3.5] and 3.5 [2.0, 3.9], respectively) elicited larger areas (>4 mm) of redness and visible swelling at 1 and 2 d after suture placement, respectively.

Suture dehiscence was a common complication of suture placement during this study. Polyglactin 910 had the least dehiscence (8%), whereas 17%, 17%, 25%, and 67% of monofilament nylon, polydioxanone, silk, and chromic gut sutures dehisced, respectively. No dehiscence was noted prior to day 7, and most cases of dehiscence occurred 7 to 9 d after suture placement. Dehisced sutures retained intact knots, indicating that knot security was good but that dehiscence occurred from within as suture material was broken down or expelled. Biopsies of tissue from which suture dehisced had more retained suture material than did those from which the suture material

Table 2. Summary of tissue reaction to suture in the skin of *X. laevis*

Suture type	Gross reaction score	Histologic reaction score	Dehiscence
Monofilament nylon	1.0 (1.0, 2.0)	1.7 (1.3, 2.0)	17%
Polydioxanone	1.0 (1.0, 2.0)	1.8 (1.4, 2.0)	17%
Polyglactin 910	1.5 (1.0, 2.0)	1.8 (1.4, 2.6)	8%
Chromic gut	3.0 (2.0, 3.5)	2.0 (1.3, 2.7)	25%
Braided silk	3.5 (2.0, 3.9)	2.3 (1.7, 2.6)	67%

Data for gross and histologic reaction scores are presented as: median score (10th percentile, 90th percentile).

was removed prior to biopsy. Day 14 biopsies from sites where chromic gut was used showed higher incidence of giant cell formation than did those from other suture sites. Gross and histologic data scores and percentage dehiscence by suture type are displayed in Table 2.

Discussion

Pronounced tissue reaction to silk suture material has been reported to occur in other species,[4,11,12,23,24] and the skin reactions we noted in the *X. laevis* biopsies taken from incision sites closed with silk suture were consistent with these prior investigations. Chromic gut also elicited strong tissue reactions in *X. laevis* similar to what has been reported[2-4,6-8,12,16,19,23] but elicited stronger tissue reactions in *X. laevis* than those reported in sea turtles.[9] The high percentage of dehiscence noted with chromic gut suture use in *X. laevis* is consistent with findings in koi (*Cyprinus carpio*)[11] and may be due to these animals' aquatic environments or increased degradation of suture material by their proteolytic enzymes, macrophages, and giant cells. This hypothesis is supported by our finding of greater numbers of giant cells in biopsies from areas where chromic gut suture had been placed and then dehisced compared to biopsies in which sutures had remained intact and were removed manually (data not shown).

Other studies in poikilothermic animals[8,9,11] showed the least histologic reaction when polyglyconate suture was used. In our study, gross reactions to polydioxanone, polyglactin 910, and monofilament nylon could not be distinguished; however, the least histologic reaction was noted with the use of monofilament nylon, followed by polydioxanone and polyglactin 910. Polyglactin 910 has been shown to cause severe reactions in other species[2-4, 7-9] that did not occur in our study. This may be because polyglactin 910, a multifilament suture material, is associated with wicking of bacteria into skin.[6] In our study, we noted few or no bacteria in any of the skin biopsies of any of the suture types investigated. This absence may be due to the secretion of magainins—peptides which inhibit the growth of gram-positive and gram-negative bacteria, several fungi, and some protozoal species—from the granular glands in *X. laevis* skin.[13] The lack of bacterial colonization of the incisions may have averted strong tissue reactions to polyglactin 910 in *X. laevis*.

Reported tissue reactions to monofilament nylon were mild[2,3] to moderate.[4,11] In fish,[11] gross and histologic reactions to monofilament nylon were slightly greater than those for polyglyconate and polyglactin 910, whereas *X. laevis* in this study showed slightly milder reactions to monofilament nylon than to polydioxanone and polyglactin 910, which also elicited mild reactions.

All suture materials elicited a greater tissue reaction than did the stab incision alone suggesting that, when suturing aquatic amphibian skin, the minimum effective amount of suture material for achieving closure should be used. Furthermore, sutures of any type should be removed as soon as sufficient wound healing has occurred to maintain wound closure. In our study, subjects were kept at the upper end of the recommended temperature range for housing *X. laevis*[17] such that their metabolism and rate of wound healing would be greater than those of frogs housed at the lower end of the range, which might require a longer time for equivalent wound healing to occur. That said, overall the suture reactions we noted were milder and included less fibrosis and granulation tissue and fewer giant cells than those reported for other poikilothermic animals.[9,11] These results suggest that aquatic amphibians may not mount as intense a response to wounds and foreign material as do fish and reptiles and that whether suture material is present or absent may have a greater effect on the magnitude of tissue response than does the suture material used, except for those that have been shown to elicit moderate to severe reactions.

Because chromic gut and silk elicited the strongest gross and histologic reactions and due to the high rate of dehiscence associated with their use, we suggest that use of these suture materials in amphibians should be avoided. Although the gross skin reactions to polyglactin 910 and polydioxanone were clinically indistinguishable from that of the monofilament nylon, the histologic data showed that monofilament nylon caused the least histologic tissue reaction and a low incidence of dehiscence. Therefore we conclude that, of the 5 suture types we investigated in this study, monofilament nylon is the preferred suture material to place in *X. laevis* skin. This recommendation is consistent with clinical observations, in which nonabsorbable monofilament sutures caused the least problems in amphibians.[25] Our study on *X. laevis* can be used as a model upon which to base choices regarding suture material in other species of aquatic amphibians while species-specific data are unavailable.

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