

Effect of Four Different Suture Materials on the Surgical Wound Healing of Loggerhead Sea Turtles, *Caretta caretta*

Pamela D. Govett^{1,2,4}, DVM, Craig A. Harms^{1,2,5}, DVM, PhD, DACZM, K. E. Linder³, DVM, PhD, DACVP, J. C. Marsh⁶, MEM, Jeanette Wyneken⁷, PhD

1. Environmental Medicine Consortium, North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606, USA

2. Department of Clinical Sciences, North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606, USA

3. Department of Population Health and Pathobiology, North Carolina State University, College of Veterinary Medicine, 4700 Hillsborough Street, Raleigh, NC 27606, USA

4. North Carolina Zoological Park, 4401 Zoo Parkway, Asheboro, NC 27205, USA

5. Center for Marine Sciences and Technology, 303 College Circle, Morehead City, NC 28557, USA

6. Duke University Marine Laboratory, 135 Duke Marine Lab Road, Beaufort, NC 28516, USA

7. Department of Biological Sciences, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33231, USA

ABSTRACT: The tissue reaction to four suture materials placed in the skin of juvenile loggerhead sea turtles, *Caretta caretta*, was evaluated both grossly and histologically. Chromic gut, polyglyconate, polyglactin 910, and poliglecaprone 25 were used in 258 turtles to close a wound produced at the time of laparoscopic sex determination. Gross tissue reactions were graded in 68 turtles at one week, and in the remaining 190 turtles at two weeks following surgery. Gross observations (eversion formation, holding of sutures, epibiont [organisms growing on suture site] present and crusts) were graded from one to three with one being mild and three being most severe. Gross observation scores did not differ among suture types. Crust scores were significantly greater for chromic gut and for polyglactin than for poliglecaprone 25 and polyglyconate. At the suture site, 32% of the turtles had an eversion in the incision ranging in size from 0.25 to 10 mm² [mean 2.02 (+/- 1.95) mm²]. Eversion size did not vary significantly among suture types. Change in the body weight of the turtles at 16-18 days following surgery ranged from -12.6 g to 77.9 g [mean 23.1 (+/- 13.8) g]. The amount of weight change did not vary significantly among suture types used. One week after surgery, 20 additional turtles (five from each suture group), were selected for skin biopsies. Suture tract, dermal, and panniculus inflammation, along with crust formation were graded histologically on a zero to three scale, with zero being none and three being most severe. Histologically, polyglactin 910 was assigned more grade three scores than any other suture type, however this was only statistically significant for panniculus inflammation. Poliglecaprone 25 and polyglyconate caused the least tissue reaction of the four suture types examined in sea turtle skin.

KEY WORDS: Loggerhead sea turtle, *Caretta caretta*, reptile, sea turtle, surgery, suture, suture reaction.

INTRODUCTION

Post-hatchling loggerhead sea turtles, *Caretta caretta*, are epipelagic inhabitants of tropical to subtropical waters worldwide. They are listed as threatened under the United States Department of the Interior's Endangered Species Act and as endangered with the World Conservation Union (Pritchard, 1997). It is therefore important to provide individuals of this species with the best possible chance to

contribute to the gene pool following surgical procedures. An increasing amount of soft tissue surgery is being performed in sea turtles for research and rehabilitation purposes including fat biopsies, laparoscopic sex determination, fishing hook removal, and amputation of traumatized limbs. The animals are often placed back in water soon after surgery to minimize stress and to facilitate feeding, but wounds are not yet healed. Healing in reptiles is slow and is influenced by environmental temperature,

wound orientation, and nutrition and health status (Bennett, 1996). The aquatic environment is rich with potential pathogens that could penetrate compromised epidermis during surgical wound healing. Previous surgical wound closures performed in sea turtles even under optimal conditions by board certified veterinary surgeons have resulted in skin irritation, sloughing of buried sutures, infection, and wound dehiscence (CAH personal observation). It is important to use a suture material that will cause the least amount of tissue reaction and provide the most secure tissue closure (Varma, 1981). The objective of this study was to examine the gross and histopathologic reactions to four suture materials used to close laparoscopic surgical skin incisions in loggerhead sea turtles.

MATERIALS AND METHODS

Loggerhead sea turtle hatchlings ($n = 480$) were collected from various nests on east coast beaches from Georgia to North Carolina, to be captive-reared at Duke University Marine Laboratory (Beaufort, North Carolina), as part of a study intended to track temporal and spatial differences in sex ratios and gonad development (Wyneken, *et al*, 2003). Of these 480 turtles, 258 were included in this suture study. The animals were housed in baskets of 18 x 18 x 12 cm inner dimensions (water height in baskets varied between 6 and 10 cm), placed in 123-357 L tanks of natural seawater (salinity 30 – 35 ppt), maintained at 26 – 30° C (78.8 – 86° F) with a pH of 7.5 – 8.5. Full spectrum lighting was provided on a 12 hr light cycle. The animals were provided with chopped shrimp or a gel food diet (pureed fish, Mazuri® turtle pellets, spinach, carrots, and Reocal® calcium supplement in Knox® gelatin) daily, and mysid shrimp once weekly. When they reached at least 11 weeks of age and weighed greater than 120 g, they were considered eligible for laparoscopic sex determination. All of the surgical procedures were performed over three different four-day periods, between October 2002 and January 2003, with closures supervised by the same veterinarian (PDG).

Before laparoscopic sex determination, the animals were fasted 24 hr, bathed in dilute povidone-iodine (Betadine® 7.5% surgical scrub, 15 ml/L H₂O, Purdue Frederick Co, Stamford, CT) and received a povidone-iodine followed by a chlorhexidine diacetate (Nolvasan® 2% solution, 20 ml/L H₂O, Fort Dodge, IA) scrub of the right inguinal fossa. Local anesthesia was attained by infiltrating lidocaine hydrochloride (2% Phoenix Pharmaceutical Inc., St. Joseph, MO, diluted to 1 mg/ml with sterile water, 1.3 – 2.1 mg/kg ID/SQ) into the skin and subcutis. This was performed 10 – 45 min prior to surgery using two to three injections around the proposed incision site. If circumstances occurred in which the animal had received the lidocaine greater than 45 min prior to surgery, it was given a supplementary 0.25 mg dose of lidocaine. For potentially additional analgesic purposes, each turtle was given butorphanol tartrate (1 mg/ml, Bedford Laboratories, Bedford, OH, 0.1 mg/kg SQ) between 2 and 112 minutes [mean 23.6 (+/- 19.6) min] prior to surgery. Preoperative cefazidime (Fortaz, Glaxo Wellcome Inc. Research Triangle Park, NC, 20 mg/kg SQ/IM) was administered between 2 and 315 min [mean 48.9 (+/- 54.7) min] prior to surgery.

For restraint purposes during laparoscopic sex determination, each turtle was hand-held. A 1.5-cm incision was made through the skin of the right inguinal fossa with a #11 scalpel blade, and Mayo scissors were used to bluntly dissect through the subcutis and body wall. A rigid endoscope (Medical Diagnostic Systems, PS100/330, 2.7 mm x 60 mm, 30°), cleansed with chlorhexidine diacetate (Nolvasan® 2% solution, 20 ml/L H₂O Fort Dodge, IA) in between turtles, was placed through the wound into the coelom and the characteristics of the animal's gonads were determined. The animal was then transferred to a foam pad with a "V" shaped trough cut into it, which was covered with a clean Huck towel followed by a sheet of clean crystal clear polyethylene cling wrap. The Huck towel was changed when it became soiled or wet, and new cling wrap was placed for every patient in order to provide a clean surface for each individual. The incision in the skin was closed with two simple interrupted sutures rotating among the four different suture types: chromic gut (Surgigut®, 4-0 FS-2 cutting, United States Surgical, Norwalk, CT), polyglyconate (Maxon®, 4-0 CV-23 taper, United States Surgical, Norwalk, CT), polyglactin 910 (Vicryl®, 4-0 RB-1 taper, Ethicon, Somerville, NJ), and poliglecaprone 25 (Monocryl®, 4-0 RB-1 taper, Ethicon, Somerville, NJ). Sutures were equally spaced and placed full-thickness through the skin. A gauze sponge containing povidone-iodine ointment (Betadine® 10% ointment, Purdue Frederick Co, Stamford, CT), was placed on the incision. The animal's bodies were coated with water-soluble lubricating gel (K-Y Jelly®, Johnson & Johnson, Arlington, Texas) to help prevent the skin from drying as they were kept out of water for 24 hr following surgery. Animals were recovered in 18 x 18 x 12 cm plastic baskets lined with a wet paper towel maintained under a heat lamp, prior to being placed back in their previously described housing.

Incision sites were grossly evaluated using a three point scale at seven (68 turtles) or 14 (190 turtles) days following surgery. Weights were measured both before and 7 (20 turtles) or 14 d (238 turtles) after surgery. A single observer (CAH) scored all gross observations. Weight change, crust formation, eversion of wound edges, and the holding of sutures were used as gross indicators of wound healing. Overall gross scores were assigned as follows: grade 1: no eversion, clean with minimal epibiota, no crust, sutures present and holding on both sides; Grade 2: between grades 1 and 3; Grade 3: noticeable deep eversion, abundant epibiota or crust, suture(s) either missing, or pulled through to one side or the other. Crust formation was scored separately as minimal or negligible (grade 1), moderate (grade 2), or marked and obscuring the sutures (grade 3). Wound edge eversions were counted and measured with a ruler. The two greatest dimensions were then multiplied to obtain each eversion's size.

Seven days after surgery, 5 mm diameter skin biopsies were obtained from the surgical site of 20 of the animals (five from each suture group). The same veterinarian that collected the biopsies (PDG) had also closed the incisions seven days previously. The animals used were prospectively chosen based on the availability to maintain them all under the same time period and conditions. The biopsies were closed with one simple interrupted suture using 4-0 Maxon, chosen based on the results of a previous suture

inflammation study involving an aquatic species (Hurty, *et al*, 2002). Tissue samples were immediately fixed in 10% neutral-buffered formalin, and later embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin for examination by light microscopy. In a blinded review of the tissue sections, a relative grading scheme was developed to compare significant histological changes. Inflammation of the suture tract, dermis and panniculus, were each graded separately with a scale of 0-3. Grade 0 had no apparent changes from normal skin. Grade 1 lesions had mild inflammatory cell infiltrates and subtle edema (Figure 1.) while grade 3 lesions had the most

date with superficial epidermal and dermal coagulation necrosis) at the wound site was also graded with a relative 0-3 scale. The tissue sections were randomized and evaluated blindly in concert by two investigators (PDG, KEL). Biopsies of normal skin from recently dead juvenile sea turtles preserved in formalin were used for comparison.

Statistical analyses were performed using a commercial statistics program (JMP®, SAS Institute, Cary, NC). Suture scores and percent weight change were compared across all suture materials by chi square analysis followed, where appropriate, by correspondence analysis and subdivision of the contingency tables to determine which suture reactions differed (Glanz 1991). Statistical significance was set at $p < 0.05$.

RESULTS

At the time of surgery, turtles weighed 164.3 (+/- 22.1) g. General gross suture reaction observations, and changes in weight did not vary appreciably among suture types. At two weeks, the majority of the animals scored a grade 1 or 2 grossly (43%, and 55% respectively), and most (97%) of the turtles examined gained weight (mean 23.1 (+/- 13.8) g, range - 12.6 to 77.9 g). Results for gross variables were qualitatively identical at 1 week (data not shown). A few of the animals (32%) had incisional eversions, with some having more than two; however, no suture type was associated with more eversions than the others. Eversion sizes, when present, ranged from 0.25 to 10 mm² with the mean (+/- SD) being 1.71 (+/- 1.07) mm² for poliglecaprone 25, 1.85 (+/- 2.51) mm² for polyglactin 910, 1.98 (+/- 1.48) mm² for chromic gut, and 2.50 (+/- 2.26) mm² for polyglyconate. Most of the animals' crusts scored a grade 1 or 2 (39% and 40% respectively).

Crust formation at two weeks following surgery was found to differ significantly between suture types [N = 190, $X^2 = 15.7$, $p = 0.0155$ (Table 1)]. By correspondence analysis polyglactin 910 grouped with chromic gut and polyglyconate grouped with poliglecaprone 25. Subdividing the contingency table accordingly, polyglactin 910 and chromic gut had higher crust scores than the two monofilament suture materials examined (N = 190, $X^2 = 11.8$, $p = 0.0027$).

Histologic evaluation was performed at seven days following surgery as the authors had observed that some of the previously laparoscoped turtles' incisions were bridged with scar tissue and epithelium, with sutures missing at 14 d (regardless of suture type). Histologic crust formation, as well as suture tract and dermal inflammation, did not vary appreciably among suture types. The majority of the animals had a noticeable amount of histologic crust formation, with 45% and 35% scoring grades 3 and 2 respectively. Histologically, inflammation consisted of varying degrees of infiltration with heterophils, lymphocytes, and macrophages. The majority of the biopsied animals exhibited considerable inflammation surrounding the suture tract (47% grade 3, n = 17; no suture tract was identified in the examined sections from 3 of the biopsied turtles), but less severe inflammation in the surrounding dermis (60% grade 2, n = 20). Panniculus inflammation was noted to be more severe than dermal inflammation

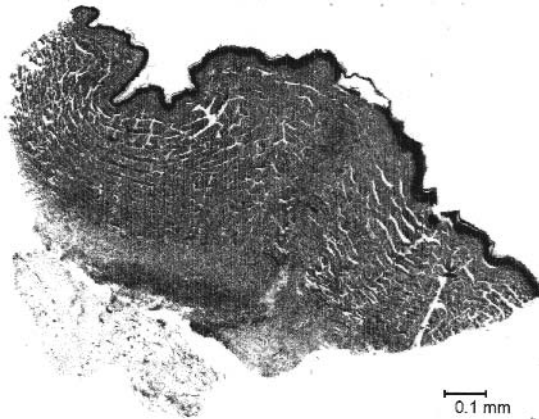
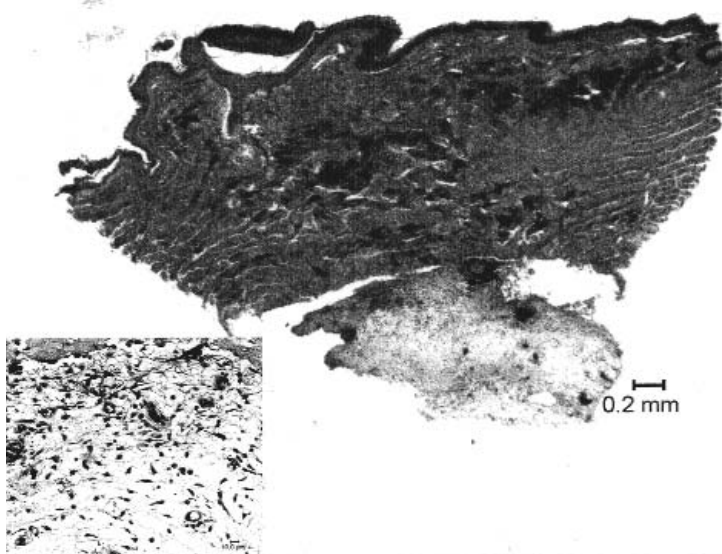


Figure 1. 10x. Representative photomicrographs demonstrate the variation of inflammation in the panniculus (P) and the dermis (D) at sutured wound sites in loggerhead sea turtles, *Caretta caretta*. Above. Grade 1 lesions had mild inflammatory cell infiltrates and subtle edema. Below. Grade 3 lesions had the most pronounced interstitial and perivascular inflammatory cell infiltrates and edema (insert 40X). (Hematoxylin and eosin).



severe perivascular to interstitial cell inflammatory infiltrates, hypertrophic vessels, and/or edema (Figure 1b.). Surface crust formation (inspissated inflammatory cell exu-

regardless of the suture type used. Polyglactin 910 was assigned more grade 3 scores than any other suture type for general, suture tract, and panniculus inflammation, though the score was significantly greater than that of other suture types only for panniculus inflammation [$N = 14$, $X^2 = 12.7$, $p = 0.0486$ (Table 2)].

DISCUSSION

Analysis of the tissue reaction to suture materials has been performed in many animals including dogs (Homsy, *et al*, 1968, Varma, *et al*, 1981, Wood, *et al*, 1984), cats (Freeman, *et al*, 1987, DeNardo, *et al*, 1996), rodents (Sharp, *et al*, 1982, Sanz, *et al*, 1988), birds (Bennett, *et al*, 1997), and fish (Nemetz and MacMillan, 1988, Gilliland, 1994, Lowartz, *et al*, 1999, Hurty, *et al*, 2002). Sea turtles and fish are both poikilothermic and share an aquatic environment. They also have similarities in their inflammatory response. It is not surprising that previous studies in fish reported results similar to our findings. In one study (Hurty, *et al*, 2002), out of five suture materials examined, polyglyconate induced the mildest tissue reaction in koi, *Cyprinus carpio*, and as is a characteristic of monofilament suture materials, was less likely to wick bacteria into the suture tract. In our study, polyglyconate was chosen because of these findings, and was found to have similar results when used in sea turtles. Polyglyconate is an

Table 1. Gross crust formation ($n = 190$, 14 d following surgery). Crust formation was scored as minimal or negligible (grade 1), moderate (grade 2), or marked and obscuring the sutures (grade 3). Polyglactin 910 and chromic gut had more grossly visible crust formation than the other two suture materials examined ($X^2 = 15.7$, $p = 0.0155$).

	Grade 1 (% turtles)	Grade 2 (% turtles)	Grade 3 (% turtles)
Chromic gut	22	45	33
Polyglyconate	48	44	8
Poliglecaprone 25	52	31	17
Polyglactin 910	35	40	25

Table 2. Histologic panniculus inflammation ($n = 14$). Inflammation of the panniculus, was graded on a scale of 0 – 3 with zero having no apparent changes from normal skin and underlying subcutis and 3 being most severe. The proportion of grade 3 scores for Polyglactin 910 was significantly greater than that of other suture types ($X^2 = 12.7$, $p = 0.0486$). NA = not available; deep cuts were not present on all biopsies examined.

	Grade 0 (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	NA
Chromic Gut	0	0	40	20	40
Polyglyconate	0	40	40	0	20
Poliglecaprone 25	0	20	60	0	20
Polyglactin 910	0	0	0	60	40

absorbable monofilament synthetic suture, with good knot security and prolonged tensile strength (Fossum, 2002). Polyglyconate sutures have also been shown to be more resistant to infection in contaminated wounds than fifteen other types of absorbable and nonabsorbable suture materials (Sharp, *et al*, 1982).

Poliglecaprone 25 is similar to polyglyconate, being a monofilament and a polyglycolic acid derivative. It has high initial strength, but is degraded more quickly than polyglyconate. In rats, only 60% of the initial suture strength remains after seven days, and 30% after 14 d, compared to 80% and 75% with polyglyconate (Monocryl® and Maxon® package inserts). Synthetic sutures may degrade more slowly in reptiles than in mammals due to temperature differences (Craig, *et al*, 1975), and variances in hydrolytic enzyme activity (Montali, 1998). Most of our turtles showed substantial healing by seven days, and in some, the surgical wound was completely bridged by scar tissue and epithelium, and the sutures were extruded by 14 d. Such a quick healing response may be attributed to their young age and growing status, and maintenance under warm temperature conditions with good nutrition; however, such conditions may not prevail in all situations.

Polyglactin 910 is a synthetic multifilament suture material and although it has good knot security, inherent characteristics of a multifilament suture are tissue drag and bacterial wicking (Fossum, 2002). Polyglactin 910 caused minimal tissue reaction in one study done in rats (Craig, *et al*, 1975). In an aquatic environment subjected to periodic contamination with food residues and feces, despite water changes and filtration, increased bacterial wicking is an undesirable quality, and potentially may have contributed to the high inflammation scores and larger crust formation seen in this study. Bacterial culture was not performed as part of this study to compare among suture types. Polyglactin 910 was associated with more undesirable results, including higher grade crusts and panniculus inflammation than any of the other suture materials investigated.

That chromic gut scores were similar to those of the synthetic monofilament materials by several measures was surprising, as studies done in other animals have shown chromic gut to cause a strong inflammatory response (Sharp, *et al*, 1982, Freeman, *et al*, 1987, Sanz, *et al*, 1988, Greenwald, *et al*, 1994, Bennett, *et al*, 1997). An inflammatory response to sutures similar to those in our study were shown in largemouth bass, *Micropterus salmoides*, (Gilliland, 1994), in which absorbable, synthetic, monofilament, suture material was the least reactive, followed by chromic gut, and then by polyglactin 910. Made from bovine and ovine intestine, chromic gut is treated with for-

malin and chromic salts to delay absorption, decrease tissue reactivity, and increase tensile strength (Bellenger, 1982). Unlike polyglyconate, poliglecaprone 25, and polyglactin 910, which are degraded by hydrolysis, chromic gut is degraded by proteolytic enzymes that may be lacking in reptile heterophils (Montali, 1988). Contrary to findings in fish, where chromic gut is quickly absorbed (Gilliland, 1994) or presumably expelled (Hurty, *et al*, 2002), Bennett and Mader (1996) suggest that chromic gut may be inappropriate for use in snakes, as it does not tend to be degraded. This suture material was found to be intact 12 wk after peritoneal and subcutaneous placement in a rhinoceros viper (Jacobson, *et al*, 1985). In animals maintained in sea water, one would expect suture degradation to occur more rapidly since chromic gut, along with polyglactin 910 and polyglycolic acid derivatives, tend to degrade more rapidly in alkaline environments (DeNardo, 1996). Although the turtles in the present study were not evaluated past two weeks, our findings were more similar to the findings in snakes, as in most of the animals, suture was still present regardless of suture type. Although not systematically evaluated, of the 28 times when sutures were noted to be sloughing, no one suture type appeared to slough more than another. Based on previous findings, chromic gut is not a suggested suture material for use in reptile skin, as it could lead to a chronic foreign body reaction and act as a nidus for infection.

CONCLUSION

Four different suture materials were used to close surgical wounds in juvenile sea turtles. Although cutting needles are generally recommended for the suturing of skin, and other difficult to penetrate tissues (Fossum, 2002), the skin of these small juvenile turtles was easily penetrable, and cutting needles were not necessary. Gross and histopathologic results indicate that poliglecaprone 25 and polyglyconate cause the least tissue reaction of the four suture types examined. These synthetic, monofilament, absorbable suture materials caused significantly less crust formation and panniculus inflammation than chromic gut and polyglactin 910. They promote minimal bacterial wicking, and are absorbed by hydrolysis. Sutures that cause the least tissue reaction lead to the most secure closure (Jacobson, *et al*, 1985), indicating that poliglecaprone 25 and polyglyconate are good choices for use in sea turtle skin.

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