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Contraction of Abdominal Wall Muscles Influences Incisional Hernia Occurrence and Size

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Abstract

Background—Incisional hernias are a complication in 10% of all open abdominal operations and can result in significant morbidity. The purpose of this study is to determine if inhibiting abdominal muscle contraction influences incisional hernia formation during laparotomy healing. We hypothesize that reducing abdominal musculature deformation reduces incisional hernia occurrence and size.

Study Design—Using an established rat model for incisional hernia, a laparotomy through the linea alba was closed with one mid-incision, fast-absorbing suture. Three groups were compared: a SHAM group (SHAM; n = 6) received no laparotomies while the Saline Hernia (SH; n = 6) and Botox Hernia (BH; n = 6) groups were treated once with equal volume saline or Botulinum Toxin (Botox®, Allergan) before the incomplete laparotomy closure. On post-operative day 14, the abdominal wall was examined for herniation and adhesions and contractile forces were measured for abdominal wall muscles.

Results—No hernias developed in SHAM rats. Rostral hernias developed in all SH and BH rats. Caudal hernias developed in all SH rats, but in only 50% of the BH rats. Rostral hernias in the BH group were 35% shorter and 43% narrower compared to those in the SH group ($p < 0.05$). The BH group had weaker abdominal muscles compared to the SHAM and SH groups ($p < 0.05$).

Conclusions—In our rat model, partial paralysis of abdominal muscles reduces the number and size of incisional hernias. These results confirm abdominal wall muscle contractions play a significant role in the pathophysiology of incisional hernia formation.

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Keywords

Incisional Hernia; Abdominal Hernia; Herniorrhaphy; Botulinum Toxin; Muscle Paralysis

Introduction

An incisional hernia occurs when the myofascial tissue surrounding a laparotomy abdominal incision fails to heal and permanently support the load-bearing muscle and tendon layers of the abdominal wall. While surgical intervention may be carried out in a technically correct manner, clinical studies find that incisional hernias are a complication in at least 10% of all open abdominal cases.^{1–3} In the United States, this translates to approximately 200,000 incisional hernia repairs performed annually.⁴ Mesh repair of these defects results in recurrence rates of 2–36%.^{5–14} Suture repair recurrence rates are between 12–54%.^{5–7, 15–18} As most studies provide only short-term follow-up, these recurrence rates may even be underestimated. Serious complications, such as enterocutaneous fistulas, bowel obstructions, and infection can also result from incisional hernia repairs and can lead to further deterioration of a patient's condition.¹⁹ The basic biological mechanism that results in the formation of an incisional hernia remains unknown. As such, incisional hernias present an unresolved surgical challenge.

Surgeons consider laparotomy surgery a tenorrhaphy of the linea alba central tendon, which traverses the abdominal wall. The oblique, transversus abdominis, and rectus abdominis muscles connect to the linea alba and aid with posture and respiration, flex and twist the trunk, maintain compression on abdominal compartment organs, and act as antagonists to trunk extensors. Normal rectus abdominis muscle force moments are directed laterally through the tendinous interstices as well as longitudinally through the serial rectus abdominis muscle bellies. Contractions of these muscles normally stabilize the external obliques at the midline while simultaneously shortening and stabilizing the soft tissue area between the anterior end of the sternum and the pubis. When the linea alba is cut and repaired, force moments through the central tendon are focused at the suture sites leading to early wound failure^{20, 21}. Incisional hernias are partially attributed to increasing loads on the abdominal wall that cannot be supported due to the debilitation of the linea alba following surgical intervention.^{21, 22} Previous studies have shown that incisional hernia formation is related to the distance between the muscles lying on both sides of the suture site.^{23, 24} During the early stages of recovery, wounds depend exclusively on suture integrity to maintain abdominal wall closure until there is adequate supporting tissue strength to offset loads placed on the wound.²² As recovering patients increase activity and intensify loads across the wound, contractions of the abdominal wall may lead to shearing or distractive forces at the wound edge during this critical, vulnerable phase of healing.^{25, 26}

Most studies aimed at improving laparotomy outcome and preventing hernia formation focused on surgical technique or the properties and utility of tools such as suture material and mesh. Nevertheless, purely surgical methods have proven unreliable.²²

In an alternative approach, we suggest that manipulation of local muscle contraction could affect the formation of incisional hernias. Partial, temporary paralysis of select abdominal

musculature during laparotomy surgery alleviates stress and strain from the linea alba, providing a mechanism to determine whether force moments along the tendon influence the development and size of an incisional hernia. We hypothesize that incomplete, bilateral paralysis of the abdominal musculature in an incisional hernia rat model will reduce the formation and size of incisional hernias by alleviating the local muscle deformation that leads to stress and strain along the linea alba.

Botulinum neurotoxin serotype A (BoNT-A) is used medically for partial, reversible chemical denervation of muscle. It works by preventing the release of neurotransmitters from excitatory axon terminals, thus inhibiting muscular contraction. The FDA first approved a preparation of BoNT-A called Botox® (Allergan, Inc., Irvine, CA) in 1989 for the treatment of blepharospasm and strabismus. Due to its paralytic effects, Botox® has since been applied to numerous muscle-related pathologies²⁷ such as cervical dystonia,²⁸ head tremors,²⁹ ear clicking due to palatal tremors,³⁰ palmar and axillary hyperhidrosis,³¹ detrusor hyperreflexia,³² gastrointestinal smooth muscle and sphincter spasms,³³ and esophageal achalasia.³⁴ Whether the paralytic effects of Botox® on a laparotomized abdominal wall can prophylactically affect incisional hernia formation is a novel approach to studying wound healing and hernia formation.

Methods

All procedures involving rats were approved by the Institutional Animal Care and Use Committee of the University of Michigan. Rats were handled in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals, 1996.³⁵ Rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) and were all of the F344 strain, male, retired as breeders, and between 8 and 10 months old.

Rat Model

An established incisional hernia model in the rat (explained below) uses two fast-dissolving sutures to partially close the peritoneal cavity after laparotomy incision. Using this model, Dubay *et al.* (2005) cite an incisional hernia incidence of 40% at post-operative day 28.¹ We modified this model for our study, placing one suture at the midpoint of the incision through the linea alba (Fig. 1).

Pilot Study on Injection Technique and Botox® Dosing

A single rat was studied to determine the correct level of Botox® dilution and to plan the spacing of drug injection sites. The rat abdominal wall was injected with 1% Evan's blue in aqueous solution and then excised for microscopic analysis. A single bolus of 0.03 ml was found to successfully spread the dye across the muscle fibers while avoiding leakage through the epimysium (Fig. 2). The study also found that 14 injection sites into the abdominal wall (seven per side) provided optimal infiltration.

Previous studies have shown that the potency of Botox® is dose-dependent.³⁶ By consulting dose-response curves from studies involving Botox® injections into the rat gastrocnemius^{37–39} and the average masses of the gastrocnemius, rectus abdominis, and

external oblique muscles, it was determined that a total dose of 10 units would achieve significant abdominal wall paralysis.

Surgical Procedure

A total of 18 rats were randomly assigned to one of three groups: SHAM, Saline Hernia (SH), or Botox Hernia (BH). Each rat was anesthetized with a dose of sodium pentobarbital at a concentration of 65 mg/kg of body mass injected into the peritoneal space. A 3.0 × 5.0 cm skin flap opening laterally was incised over the rat abdomen and undermined by 1.0 cm.

All rats received injections of 0.5 mls of carrier (saline) or Botox solution. A 25 gauge needle was inserted at the linea alba and advanced approximately 1.5 cm laterally through the rectus abdominis into the abdominal wall musculature. Fourteen equally spaced injections of 0.03 mls were made bilaterally along the rectus abdominis. Botox was injected in the external oblique and rectus abdominis muscles while the needle was withdrawn. Rats in the SHAM group were injected with a total of 0.5 ml saline but received no laparotomy incision. SH group and BH group rats received a 60 mm midline laparotomy incision through the linea alba. Those in the SH group then received injections into the abdominal wall totaling 0.5 ml of saline. BH group rats then received 0.5 ml of Botox® solution (10 units). The laparotomy incisions were closed with a single interrupted suture (5-0 Fast Absorbing Chromic Gut, PC-1) placed at the midpoint of the incision (Fig. 1). For all groups, skin flaps were closed with 4-0 monofilament nylon stitches. All rats were left to recover for 14 days before hernia and muscle analysis.

Hernia and Muscle Analysis

On post-operative day 14, each rat was anesthetized and measurements were completed in an order prescribed to maintain muscle viability and hernia integrity. In order, the measurements were: hernia assessment and measurement, maximal force testing for two identical external oblique (EO) muscle strips [9 mm wide × 15 mm long × variable thickness (0.6 to 1.4 mm)], adhesion rating, and maximal force testing for two rectus abdominis (RA) muscle strips [variable width (9.2 to 12.2 mm) × 15 mm long × variable thickness (1.7 to 4.9 mm)]. An *incisional hernia* was defined as ‘any abdominal wall gap with or without bulge in the area of a postoperative scar perceptible or palpable by clinical examination or imaging’.⁴⁰ Muscle sections were excised specifically to ensure that muscle fibers lay parallel to the vector along which force would be measured. Outlines of external oblique muscle fibers were landmarks to assure parallel cuts with the fibers. Also the left or right RA muscle was removed from the clavicle to just below the umbilicus.

Contractile testing was performed in a manner described previously.⁴¹ Muscle strips were placed in a custom muscle bath containing physiological Tyrode’s solution that was maintained at 25°C and aerated with a gas containing 95% oxygen and 5% carbon dioxide. Custom software (LabView Version 5.1, National Instruments, Austin, TX) was used to control stimulation voltage and record and store force data. Stimulation voltage was increased until strip twitch force was maximized; then optimal sarcomere overlap was estimated by adjusting strip length until twitch force was maximized a second time. Using this “optimal strip length” and stimulation voltage, a force frequency curve was generated

by applying 300 ms stimulation trains which ultimately produced tetanic muscle contraction. Stimulation frequency was incrementally raised from 50 Hz to 200 Hz until the maximal isometric strip force was recorded. Two minutes of rest were allowed between each force trial. Muscle testing was completed within 10 minutes from the time the strip was fully undermined and excised.

After force testing, EO and RA muscle strips were frozen and stored for histological analysis. The frozen muscle samples were sectioned longitudinally at a thickness of 10 μm , stained with hematoxylin and eosin, and mounted for microscopic evaluation.⁴² Sections were evaluated for a) large bluish, centrally located nuclei which could indicate fiber regeneration, b) average fiber width with more smaller fibers indicating fiber atrophy, c) the quality of extra cellular matrix around the fibers with greater ECM to fiber area indicating stiffening of the fiber, and d) the presence of any lymphocytes or macrophages which could indicate infection.

The appearance of adhesions *in vivo* was assessed using a scale of 0 to 5, where 0 indicated no adhesions and 5 indicated dense adhesions with incarcerated and strangulated bowel.^{43–45} The complete scoring criteria can be found in Appendix 1. Hernia analysis was performed by noting the appearance of a variably dense fibrous bridge between the two edges of the abdominal wall at the point where the suture had been placed. The presence of incisional hernias either rostral or caudal to the fibrous bridge was noted and, if present, was measured for length (craniocaudally) and width (transversely) at their widest point.

Statistical Analysis

Statistical computations were performed using a general linear model (GLM) with software from the Statistical Analysis System (SAS Institute, Inc., Cary, NC). A Bonferroni correction was used for multiple comparisons if the GLM was significant. In addition, χ^2 tests with equal expected hernia occurrence were applied to hernia occurrence data using SPSS 16.0 (SPSS Inc., Chicago, IL). For this study, α was set a priori at 0.05 for all statistical tests. Calculations to test the strength of no differences alternative hypotheses indicated power values of 0.98, 0.90 and 0.78 for rostral hernia formation, caudal hernia formation and total caudal and rostral hernias respectively.

Results

Data are presented for all 18 rats unless otherwise noted. No rats died before the evaluation date. Hernia size and adhesion rating data were not included for the first rat tested (one rat in the SH group) due to a rating scale error. Additionally, one rat in the BH group expired from a presumed anesthesia overdose during final evaluation. Although hernia size and adhesion rating data were measured for this rat, muscle force data were not collected. Two EO sections from another BH rat could not be tested for contractile force due to equipment malfunction.

The appearance of the abdominal walls following removal of scar tissue can be seen in representative rats from all three surgical groups in Figure 3. Note the small size of the hernias in the BH group compared to the SH group. A summary of the study data is

presented in Tables 1 and 2. EO and RA sections from the BH group contracted with significantly lower mean maximum forces than sections from the SHAM and SH groups (Table 2). Hernias, both rostral and caudal to the fibrous bridge that formed at the point where the suture had been placed, formed in all SH group rats and in 3 out of 6 BH group rats (Table 1). Rostral hernias in the BH group were shorter (craniocaudally) and narrower (transversely) than rostral hernias in the SH group (Fig. 4, 5). No significant differences in the formation of caudal hernias in the BH and SH groups were noted (Fig. 4, 5). When the rostral and caudal hernia lengths were added together, the total hernia length in the BH group was significantly shorter than the total hernia length in the SH group (Fig. 4). Similarly, the total hernia width in the BH group was significantly smaller than the total hernia width in the SH group (Fig. 5). Superficial and intra-abdominal adhesions were significantly thicker and more dense in the SH and BH groups compared to the SHAM group (Table 1).

Representative photographs of muscle tissue from EO and RA muscles from all three surgical groups were taken at 160x magnification (Fig. 6). All photographs are representative of longitudinal sections taken at a deep plane. Microscopic inspection of all muscle sections found no sections with indications of infection or fiber regeneration. Muscle fiber atrophy was observed in the SH and BH group EO and RA muscles. There were no obvious signs of increased extra cellular matrix for any of the muscles in any of the groups.

Discussion

Based on the data, we conclude that treatment with Botox® at the time of laparotomy repair decreases abdominal muscle contractile force, thereby reducing the incidence and size of ventral midline incisional hernias. Botox® injections decreased *in vitro* muscle forces compared to SHAM and saline-injected muscles while causing no appreciable muscle cell damage or necrosis upon microscopic examination. Adhesion formation was not affected by muscle paralysis.

Larger size of the defect is correlated with higher risk of recurrence.^{18, 46} Paralysis suppressed both the baseline tone and active forces in the abdominal musculature, allowing the incision edges to rest more closely approximated to each other during the recovery period. This positioning likely helps ameliorate shear and distractive forces at the fascial repair site and enables the incision to heal with less physical interruption. This is in accordance with a recent study by Ibarra-Hurtado *et al.* (2009), demonstrating pre-treatment with Botox® in patients with abdominal wall herniation significantly reduces the transverse hernia defect diameter and facilitating fascial closure with no recurrences at a mean follow-up of 9 months.⁴⁷ In another study, Botox® was similarly injected in patients with open abdomen after a damage control laparotomy. Primary fascial closure was achieved in 89% of patients who received Botox® within 24 hours after the initial open abdomen procedure.⁴⁸ Botox® in patients with incisional hernia repair has also shown to significantly improve pain scores and reduce need for opioid analgesia.^{49, 50}

No significant difference between EO and RA contractile forces in the SHAM and SH groups was observed. Given the otherwise identical treatment of the SH and BH groups, the

significant loss of force in BH group muscle sections is attributed to the pharmacologic effect of Botox®. These sections retained some contractile function, however, suggesting that the paralysis was incomplete. This finding is in accordance with the expected result of Botox® treatment, which has been shown to leave skeletal muscle with as little as 12% of its original contractile force generation ability.^{38, 39, 51} In this study, EO and RA sections treated with Botox® were still able to produce 25% and 50% of their baseline contractile force, respectively, suggesting that doses higher than 10 units could be used to produce an even greater magnitude of paralysis. It remains unknown whether differing levels of paralysis would have variable effects on incisional hernia formation and size.

The placement of a single suture in the animal model allowed for the formation of two unique hernia sites with variable loads on local musculature. Rostral to the suture, considerable strain was placed on the two halves of the abdominal wall by the rigid costal margin, the underlying abdominal organs, and the movement of the diaphragm. Upper abdomen midline incisions are reported to have a higher incidence of incisional herniation than lower abdominal incisions.^{10, 52} Comparatively, the left and right slips of the rectus abdominis muscle arise from the symphysis pubis and cross the midline in the pubic area, the two muscles overlap and thus anatomy helps to avoid herniation in the caudal area. The abdominal wall caudal to the suture would be under less mechanical strain. This premise is supported by our results: In the BH group, rostral hernias formed in 100% of the rats while caudal hernias formed in only 50% of the rats. Furthermore, the average caudal hernia was 11 mm shorter and 6 mm narrower than the average rostral hernia in the SH group. In the BH group, the average caudal hernia was 11 mm shorter and 4 mm narrower than the average rostral hernia. However, we did not test to see if abdominal wall strain was different on these two locations, which is a limitation.

There was no statistically significant difference between the SH and BH groups with regard to hernia size caudal to the suture. Even though the average caudal hernia was smaller in the BH group compared to the SH group, the fact that caudal hernias were present in only 3 of 6 BH rats expanded the standard deviation of the mean hernia size. This made statistically significant differences unlikely. The small sample size is a limitation of our study, a larger sample size may permit the observation of a statistically significant difference in caudal hernia size and the number of hernias between the SH and the Botox groups.

Adhesions are a complication of abdominal surgery, with an incidence of 93% after general abdominal surgeries.⁵³ Comparable presentation of adhesions, both superficial and intraperitoneal, was observed in the SH and BH groups. These findings suggest that decreasing movement in the abdominal wall does little to affect adhesion development.

Given the difficulty of recreating a human incisional hernia in an animal model, some assumptions were made in this study. First, it was assumed that the incomplete closure of the abdominal cavity in our model closely approximates the human incisional hernia environment where the laparotomy incision is closed with many stitches. It was also assumed that the Botox® solution was able to diffuse throughout the entire abdominal wall and partially paralyze the internal oblique and transversus abdominis muscles as well as the EO and RA muscles, thereby alleviating strain from the linea alba.

The short follow-up of this study can be considered as a limitation, since hernias can develop months or years after surgery or may arise from small hernias. Although we believe incisional hernias typically develop in the early postoperative period, a longer follow up would be needed to see if Botox® leads to a reduction in incidence or a delay in hernia formation. The clinical effect of Botox® is manifest after 2 days, reaches its maximum at 14 days and has a duration of effect for 6–9 months.⁵⁴ These effects may therefore further aid in wound healing. We did not notice any adverse reactions; however, systemic spread of Botox® is a rare but serious complication with symptoms with symptoms consistent with botulin actions including generalized muscle weakness. Future work is needed to confirm our findings, establish a proper dose-response relationship, and determine the time course of hernia formation.

The purpose of this study was not to suggest a possible therapeutic approach to abdominal incisional hernia repair, but rather to help define the pathogenesis of these defects. Based on our results, incisional hernias are likely related to abdominal muscle contractions. It may be useful to consider our conclusions when pre-operatively explaining the risk of a laparotomy incision to patients or discussing the pathogenesis of incisional hernias with a patient that has already developed one. Given our results, future interventions for incisional hernia prophylaxis may be targeted at limiting abdominal muscle activity.

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References

1. DuBay DA, Wang X, Adamson B, Kuzon WM Jr, Dennis RG, Franz MG. Progressive fascial wound failure impairs subsequent abdominal wall repairs: a new animal model of incisional hernia formation. *Surgery*. 2005; 137:463–71. [PubMed: 15800496]
2. Millikan KW. Incisional hernia repair. *Surgical Clinics of North America*. 2003; 83:1223–34. [PubMed: 14533912]
3. Mudge M, Hughes LE. Incisional hernia: a 10 year prospective study of incidence and attitudes. *Br J Surg*. 1985; 72:70–1. [PubMed: 3155634]
4. National Hospital Discharge Survey, 1995. Ann Arbor, Mich: Inter-University Consortium for Political and Social Research [distributor]; 1998.
5. Anthony T, Bergen PC, Kim LT, Henderson M, Fahey T, Rege RV, et al. Factors affecting recurrence following incisional herniorrhaphy. *World J Surg*. 2000; 24:95–100. discussion 1. [PubMed: 10594211]
6. Luijendijk RW, Hop WC, van den Tol MP, de Lange DC, Braaksma MM, JNII, et al. A comparison of suture repair with mesh repair for incisional hernia. *N Engl J Med*. 2000; 343:392–8. [PubMed: 10933738]
7. Korenkov M, Sauerland S, Arndt M, Bograd L, Neugebauer EA, Troidl H. Randomized clinical trial of suture repair, polypropylene mesh or autoderma hernioplasty for incisional hernia. *Br J Surg*. 2002; 89:50–6. [PubMed: 11851663]
8. van der Linden FT, van Vroonhoven TJ. Long-term results after surgical correction of incisional hernia. *Neth J Surg*. 1988; 40:127–9. [PubMed: 3148112]
9. Liakakos T, Karanikas I, Panagiotidis H, Dendrinis S. Use of Marlex mesh in the repair of recurrent incisional hernia. *The British journal of surgery*. 1994; 81:248–9. [PubMed: 7710471]

10. Langer I, Herzog U, Schuppisser JP, Ackermann C, Tondelli P. Preperitoneal prosthesis implantation in surgical management of recurrent inguinal hernia. Retrospective evaluation of our results 1989–1994. *Der Chirurg; Zeitschrift für alle Gebiete der operativen Medizen*. 1996; 67:394–402.
11. McLanahan D, King LT, Weems C, Novotney M, Gibson K. Retrorectus prosthetic mesh repair of midline abdominal hernia. *Am J Surg*. 1997; 173:445–9. [PubMed: 9168086]
12. Park A, Birch DW, Lovrics P. Laparoscopic and open incisional hernia repair: a comparison study. *Surgery*. 1998; 124:816–21. discussion 21–2. [PubMed: 9781006]
13. Schumpelick V, Klosterhalfen B, Müller M, Klinge U. Minimized polypropylene mesh for preperitoneal net plasty (PNP) of incisional hernias. *Der Chirurg; Zeitschrift für alle Gebiete der operativen Medizen*. 1999; 70:422–30.
14. Toniato A, Pagetta C, Bernante P, Piotta A, Pelizzo MR. Incisional hernia treatment with progressive pneumoperitoneum and retromuscular prosthetic hernioplasty. *Langenbeck's archives of surgery/Deutsche Gesellschaft für Chirurgie*. 2002; 387:246–8.
15. Read RC, Yoder G. Recent trends in the management of incisional herniation. *Arch Surg*. 1989; 124:485–8. [PubMed: 2649047]
16. Manninen MJ, Lavonius M, Perhoniemi VJ. Results of incisional hernia repair. A retrospective study of 172 unselected hernioplasties. *The European journal of surgery = Acta chirurgica*. 1991; 157:29–31. [PubMed: 1675878]
17. Paul A, Korenkov M, Peters S, Kohler L, Fischer S, Troidl H. Unacceptable results of the Mayo procedure for repair of abdominal incisional hernias. *European J Sur Acta chirurgica*. 1998; 164:361–7.
18. Luijendijk RW, Lemmen MH, Hop WC, Wereldsma JC. Incisional hernia recurrence following “vest-over-pants” or vertical Mayo repair of primary hernias of the midline. *World J Surg*. 1997; 21:62–5. discussion 6. [PubMed: 8943179]
19. Burger JW, Luijendijk RW, Hop WC, Halm JA, Verdaasdonk EG, Jeekel J. Long-term follow-up of a randomized controlled trial of suture versus mesh repair of incisional hernia. *Ann Surg*. 2004; 240:578–83. discussion 83–5. [PubMed: 15383785]
20. Xing L, Culbertson EJ, Wen Y, Franz MG. Early laparotomy wound failure as the mechanism for incisional hernia formation. *J Surg Res*. 2013; 182:e35–42. [PubMed: 23036516]
21. Franz MGK, MA, Nguyen K, Wang X, Ko F, Wright TE, Robson MC. Early biomechanical wound failure: a mechanism for ventral incisional hernia formation. *Wound Rep Reg*. 2001; 9:140–1.
22. Franz MG. The biology of hernia formation. *The Surgical Clinics of North America*. 2008; 88:1–15. vii. [PubMed: 18267158]
23. Pollock AV, Evans M. Early prediction of late incisional hernias. *Br J Surg*. 1989; 76:953–4. [PubMed: 2804595]
24. Burger JW, Lange JF, Halm JA, Kleinrensink GJ, Jeekel H. Incisional hernia: early complication of abdominal surgery. *World J Surg*. 2005; 29:1608–13. [PubMed: 16311846]
25. Bendavid, R. *Prostheses and Abdominal Wall Hernias*. Austin: RG Landes Co; 1994.
26. DuBay DA, Choi W, Urbanchek MG, Wang X, Adamson B, Dennis RG, et al. Incisional herniation induces decreased abdominal wall compliance via oblique muscle atrophy and fibrosis. *Ann Surg*. 2007; 245:140–6. [PubMed: 17197977]
27. Jost WH. Other indications of botulinum toxin therapy. *European journal of neurology: EFNS*. 2006; 13 (Suppl 1):65–9.
28. Truong D, Duane DD, Jankovic J, Singer C, Seeberger LC, Comella CL, et al. Efficacy and safety of botulinum type A toxin (Dysport) in cervical dystonia: results of the first US randomized, double-blind, placebo-controlled study. *Movement Disorders*. 2005; 20:783–91. [PubMed: 15736159]
29. Wissel J, Masuhr F, Schelosky L, Ebersbach G, Poewe W. Quantitative assessment of botulinum toxin treatment in 43 patients with head tremor. *Movement Disorders*. 1997; 12:722–6. [PubMed: 9380055]
30. Deuschl G, Lohle E, Heinen F, Lucking C. Ear click in palatal tremor: its origin and treatment with botulinum toxin. *Neurology*. 1991; 41:1677–9. [PubMed: 1922818]

31. Schnider P, Binder M, Auff E, Kittler H, Berger T, Wolff K. Double-blind trial of botulinum A toxin for the treatment of focal hyperhidrosis of the palms. *Br J Dermatol.* 1997; 136:548–52. [PubMed: 9155956]
32. Schurch B, Stohrer M, Kramer G, Schmid DM, Gaul G, Hauri D. Botulinum-A toxin for treating detrusor hyperreflexia in spinal cord injured patients: a new alternative to anticholinergic drugs? Preliminary results. *J Urol.* 2000; 164:692–7. [PubMed: 10953127]
33. Brisinda G, Bentivoglio AR, Maria G, Albanese A. Treatment with botulinum neurotoxin of gastrointestinal smooth muscles and sphincters spasms. *Movement Disorders.* 2004; 19 (Suppl 8):S146–56. [PubMed: 15027068]
34. Annese V, Bassotti G, Coccia G, Dinelli M, D’Onofrio V, Gatto G, et al. A multicentre randomised study of intrasphincteric botulinum toxin in patients with oesophageal achalasia. GISMAD Achalasia Study Group. *Gut.* 2000; 46:597–600. [PubMed: 10764700]
35. National Academy of Sciences. Guide for the Care and Use of Laboratory Animals. 8. Washington, D.C: The National Academies Press; 2011.
36. Kutschenko A, Reinert MC, Klinker F, Paulus W, Hesse S, Liebetanz D. Botulinum toxin-induced focal paresis in mice is unaffected by muscle activity. *Muscle Nerve.* 2011; 44:930–6. [PubMed: 22102464]
37. Bambrick LL, Gordon T. Comparison of the effects of botulinum toxin in adult and neonatal rats: neuromuscular blockade and toxicity. *Can J Physiol Pharmacol.* 1989; 67:879–82. [PubMed: 2598123]
38. Ma J, Elsaidi GA, Smith TL, Walker FO, Tan KH, Martin E, et al. Time course of recovery of juvenile skeletal muscle after botulinum toxin A injection: an animal model study. *Am J Phys Med Rehabil.* 2004; 83:774–80. Quiz 81–3. [PubMed: 15385786]
39. Dodd SL, Selsby J, Payne A, Judge A, Dott C. Botulinum neurotoxin type A causes shifts in myosin heavy chain composition in muscle. *Toxicon.* 2005; 46:196–203. [PubMed: 15975617]
40. Korenkov M, Paul A, Sauerland S, Neugebauer E, Arndt M, Chevrel JP, et al. Classification and surgical treatment of incisional hernia. Results of an experts’ meeting. *Langenbecks Arch Surg/Deutsche Gesellschaft fur Chirurgie.* 2001; 386:65–73.
41. Segal SS, Faulkner JA, White TP. Skeletal muscle fatigue in vitro is temperature dependent. *J Appl Physiol.* 1986; 61:660–5. [PubMed: 3745058]
42. Luna, LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. In: Luna, LG., editor. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3. New York: Mc-Graw-Hill Book Company; 1968. p. 1-47.
43. Butler CE, Navarro FA, Orgill DP. Reduction of abdominal adhesions using composite collagen-GAG implants for ventral hernia repair. *J Biomed Mater Res.* 2001; 58:75–80. [PubMed: 11153001]
44. Xu X, Pappo O, Garbuzenko E, Bischoff SC, Rivkind A, Levi-Schaffer F. Mast cell dynamics and involvement in the development of peritoneal adhesions in the rat. *Life Sci.* 2002; 70:951–67. [PubMed: 11853232]
45. Butler CE, Prieto VG. Reduction of adhesions with composite AlloDerm/polypropylene mesh implants for abdominal wall reconstruction. *Plast Reconstr Surg.* 2004; 114:464–73. [PubMed: 15277815]
46. Hesselink VJ, Luijendijk RW, de Wilt JH, Heide R, Jeekel J. An evaluation of risk factors in incisional hernia recurrence. *Surg Gynecol Obstet.* 1993; 176:228–34. [PubMed: 8438193]
47. Ibarra-Hurtado TR, Nuno-Guzman CM, Echeagaray-Herrera JE, Robles-Velez E, de Jesus Gonzalez-Jaime J. Use of botulinum toxin type a before abdominal wall hernia reconstruction. *World J Surg.* 2009; 33:2553–6. [PubMed: 19771472]
48. Zielinski MD, Goussous N, Schiller HJ, Jenkins D. Chemical components separation with botulinum toxin A: a novel technique to improve primary fascial closure rates of the open abdomen. *Hernia.* 2013; 17:101–7. [PubMed: 23001400]
49. Smoot D, Zielinski M, Jenkins D, Schiller H. Botox A injection for pain after laparoscopic ventral hernia: a case report. *Pain Medicine.* 2011; 12:1121–3. [PubMed: 21668748]

50. Zendejas B, Khasawneh MA, Srwantstyan B, Jenkins DH, Schiller HJ, Zielinski MD. Outcomes of chemical component paralysis using botulinum toxin for incisional hernia repairs. *W J Surg.* 2013; 37:2830–7.
51. Cichon JV Jr, McCaffrey TV, Litchy WJ, Knops JL. The effect of botulinum toxin type A injection on compound muscle action potential in an in vivo rat model. *Laryngoscope.* 1995; 105:144–8. [PubMed: 8544593]
52. Fassiadis N, Roidl M, Hennig M, South LM, Andrews SM. Randomized clinical trial of vertical or transverse laparotomy for abdominal aortic aneurysm repair. *Br J Surg.* 2005; 92:1208–11. [PubMed: 16175532]
53. Menzies D, Ellis H. Intestinal obstruction from adhesions--how big is the problem? *Ann R Coll Surg Engl.* 1990; 72:60–3. [PubMed: 2301905]
54. Truong, DHM. *Manual of Botulinum Toxin Therapy.* 2. Cambridge CB2 8bs, United Kingdom: Cambridge University Press; 2009. p. 12-6.

Appendix 1. Adhesion Grading System

Grade		Criteria:
Superficial (To Skin)	Deep (To peritoneal surface)	
0	0	No adhesion.
1	1	Thin (flimsy) adhesion, avascular, usually limited to one area, easily separable (not requiring sharp dissection).
2	2	Thin (flimsy) adhesion, vascular, limited to one area, moderately sharp dissection required.
3	3	Thin (flimsy) adhesion, more than one place, mostly vascular, difficult to separate; or a single firm adhesion involving bowel, omentum, liver, mesentery or pelvic fat.
4	4	Thick, firm adhesion, more than one place (superficial). Thick, firm adhesion, more than one place, involves bowel, omentum, liver, mesentery or pelvic fat, vascularized, requiring sharp dissection (deep).
5	5	A dense adhesion mass in which individual bowel loops could not be separated, and/or intestinal obstruction — some of intestinal loops are dilated; sometimes, bleeding in peritoneal fluid may occur (superficial and deep).

Appendix 1 References.

- Xu X, Pappo O, Garbuzenko E, Bischoff SC, Rivkind A, Levi-Schaffer F. Mast cell dynamics and involvement in the development of peritoneal adhesions in the rat. *Life Sci.* 70(8): 951–67, 2002.
- Butler CE, Prieto VG. Reduction of adhesions with composite AlloDerm/polypropylene mesh implants for abdominal wall reconstruction. *Plast. Reconstr. Surg.* 114(2): 464–73, 2004.
- Butler CE, Navarro FA, Orgill DP. Reduction of abdominal adhesions using composite collagen-GAG implants for ventral hernia repair. *J. Biomed. Mater. Res.* 58(1): 75–80, 2001.

Figure 1.

Rat incisional hernia model, based on work by Dubay *et al.*¹ A single fast-absorbing chromic gut suture closes the laparotomy incision at its midpoint.

Figure 2. Peroneal, left lateral deep view of the abdominal wall after 5 injections (at white arrows) of 0.03 mls at each site. A 1% aqueous solution of Evan's Blue was injected orthogonal to the linea alba. Photo visualizes the immediate spread of fluid through the muscle tissue.

Figure 3.

Gross view of a representative abdominal wall from the **a)** SHAM group, **b)** Saline Hernia group, and **c)** Botox Hernia group at post-operative day 14. **a)** Note absence of any hernias. White and black outlines represent where the left EO and RA muscle sections are taken, respectively. Identical sections are also taken from the right side. **b)** and **c)** Rectangles on either side of the rectus sheath (white arrows) indicate where external oblique sections were removed bilaterally for contractile force testing. Note the difference in hernia size between the Saline Hernia and Botox Hernia groups and the amount of abdominal contents allowed through the hernia defects in either group (transparent arrows).

Figure 4.

The effect of the surgical group on the length (mm) of the hernias formed on both sides of the midline suture through the abdominal wall. For total hernia length, the rostral and caudal lengths are added together. The SHAM group had no hernias and therefore is not plotted.

*Indicates significantly different than Saline Hernia group ($p < 0.05$).

Figure 5.

The effect of the surgical group on the width (mm) of the hernias formed on both sides of the midline suture through the abdominal wall. For total hernia length, the rostral and caudal lengths are added together. The SHAM group had no hernias and therefore is not plotted.

*Indicates significantly different than Saline Hernia group ($p < 0.05$).

Figure 6. Hematoxylin and Eosin-stained longitudinal sections through representative samples of external oblique (EO) and rectus abdominis (RA) sections from all three surgical groups. Although the Botox® treated sections of muscle show mild atrophy, there are no obvious signs of necrosis or cellular regeneration.

Table 1

Hernia occurrence and adhesion rating data by surgical group.

Dependent Variables	Surgical Group		
	SHAM (n = 6)	Saline Hernia (n = 5)	Botox Hernia (n = 6)
Number of Rats with Rostral Hernias	0	5 [*]	6 [*]
Number of Rats with Caudal Hernias	0	5 [*]	3
Total Caudal and Rostral Hernias	0	10 [*]	9
Superficial Adhesion Score	0.3 ± 0.5	3.5 ± 0.5 [†]	2.8 ± 1.0 [†]
Intraperitoneal Adhesion Score	0.0 ± 0.0	3.5 ± 0.5 [†]	2.7 ± 0.8 [†]

* Indicates significantly different from SHAM group when occurrence was tested using the χ^2 test with equal expected hernia occurrence, (p < 0.05).

[†] Indicates significantly different from SHAM group when the scores were tested using a GLM with Bonferroni multiple comparison, (p < 0.05), n = number of rats in each surgical group.

Table 2

Muscle strip volume and contractile force data for external oblique and rectus abdominis muscle sections by surgical group

Muscle	Surgical Group		
External Oblique	SHAM (n = 12)	Saline Hernia (n = 12)	Botox Hernia (n = 8)
Muscle Length (mm)	17.7 ± 1.5	19.6 ± 1.7 [*]	16.6 ± 1.3 [†]
Muscle Width (mm)	7.8 ± 0.7	6.7 ± 1.3 [*]	8.2 ± 0.1 [†]
Muscle Thickness (mm)	0.9 ± 0.1	1.0 ± 0.2	0.7 ± 0.1 ^{*†}
Muscle Volume (mm ³)	152.6 ± 20.2	148.5 ± 17.0	112.5 ± 20.3
Wet Muscle Mass (g)	129.9 ± 21.5	113.7 ± 26.4	76.4 ± 14.5 ^{*†}
<i>In Vitro</i> Force (mN)	809.8 ± 215.5	638.8 ± 326.5	168.2 ± 63.2 ^{*†}
<i>In Vitro</i> Specific Force (kN/m ²)	113.0 ± 26.7	104.5 ± 56.9	28.8 ± 11.1 ^{*†}
Rectus Abdominis	SHAM (n = 12)	Saline Hernia (n = 12)	Botox Hernia (n = 10)
Muscle Length (mm)	19.4 ± 1.1	19.1 ± 1.4	17.5 ± 0.9 ^{*†}
Muscle Width (mm)	10.0 ± 1.2	8.0 ± 1.2 [*]	7.7 ± 0.9 [*]
Muscle Thickness (mm)	2.0 ± 0.4	2.3 ± 0.4	2.0 ± 0.2
Muscle Volume (mm ³)	412.8 ± 78.3	415.2 ± 139.3	286.4 ± 46.4 ^{*†}
Wet Muscle Mass (g)	412.5 ± 45.6	379.7 ± 79.5	260.8 ± 45.0 ^{*†}
<i>In Vitro</i> Force (mN)	2230.1 ± 603.4	2323.1 ± 350.4	1261.1 ± 207.9 ^{*†}
<i>In Vitro</i> Specific Force (kN/m ²)	116.6 ± 42.9	136.2 ± 39.4	81.5 ± 12.9 [†]

^{*} Indicates significantly different from SHAM group; (p < 0.05).

[†] Indicates significantly different from Saline Hernia group; (p < 0.05). Data presented as Mean ± SD. n = number of samples.