

Tissue Reaction and the Formation of Adhesion after the Use of DS Clip in Laparoscopic Appendectomy

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ABSTRACT

Background and Objectives: Titanium DS clips are made to secure the appendiceal stump during laparoscopic appendectomy. The assumption is that they behave like stapler clips in the body, being made from titanium. However, the construction and weight of DS clips differs from staplers. Their biocompatibility may have implications for their clinical use.

Methods: One hundred and twenty rats were randomized into four experimental groups: in the first group the appendiceal stump was secured by Vicryl® ligature, in the second by linear staplers, in the third by Hem-o-lok® plastic clips, and in the fourth by DS titanium clips. Ten animals from each group were sacrificed 7, 28, and 60 days post-surgery. Histopathological data and adhesion formation were assessed.

Results: On postoperative day 7, a statistically significant difference was found in the occurrence of inflammation between the Hem-o-lok® and stapler groups. The adhesion score was significantly higher in the Vicryl® and Hem-o-lok® in comparison to the DS and stapler groups. On postoperative day 28, a statistically significant difference was found in the occurrence of inflammation between the Vicryl® and stapler groups. The adhesion

score was significantly higher in the Vicryl® than in the DS and stapler groups. On postoperative day 60, there were no statistical differences between any of the groups. The adhesion score was significantly higher in the Hem-o-lok® group in comparison to the DS and stapler groups.

Conclusion: Staplers and DS clips have advantages over other methods of securing the appendiceal stump, because of their high biocompatibility and lower adhesion score.

Key Words: Stapler, Endoloop, Clips, Laparoscopic appendectomy.

INTRODUCTION

Several techniques have been described for use in securing the appendiceal stump during laparoscopic appendectomy. Each of them has their own advantages and disadvantages. The standard technique for securing the appendiceal stump is an endoloop ligature made from Vicryl® (polyglactin 910).¹ It is simple to apply and affordable, and the surgery takes less time. The use of staplers with titanium clips is more expensive, but it has advantages, and is particularly well indicated in situations when the appendiceal base cannot be secured using the endoloop method.² Plastic nonresorbable clips are an alternative method which has shown some advantages.³ The biocompatibility of each of these methods has already been demonstrated.⁴

Titanium DS® clips are made specifically to secure the appendiceal stump.⁵ However, their biocompatibility and the formation of adhesion after application are still unknown. We may assume that they will behave like stapler clips in the body, since they are all made from titanium. However, the construction and weight of DS clips differs from staplers. Their biocompatibility may have implications for their clinical use.

In order to determine this, we conducted a study, using a rodent surgical model, to establish the intensity of the inflammatory reaction of the tissue and the formation of

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adhesion to DS clips, and compared this with endoloop, staplers and Hem-o-lok[®] plastic clips.

MATERIALS and METHODS

The experimental study was undertaken at the Department of Pathophysiology of the Veterinary Faculty. This study was reviewed and approved by the Institutional Review Board. The rats (Wistar albino) weighing 250 – 300 grams were kept in standard plastic cages, with sawdust-covered floors, at a constant temperature (temperature 20 to 24°C), 55 – 60 constant humidity, in a controlled environment, with 12-hour light-dark cycle. One hundred and twenty rats were randomized, 1:1:1:1, into four experimental treatment groups of 30 animals each.

The Surgical Procedure

After the rats had been anaesthetized (using ketamine 850 mg/kg), they were laid on a heated pad and fixed on the operating table in a supine position with adhesive tape, and their abdomen was shaved, disinfected with povidone-iodine solution. Laparotomy was performed using a medial incision. After locating the cecum, a large pouch in the lower third of the abdominal cavity which has a closed end and imitates the appendix in humans, was resected.

In group I (n = 30) the closed end of the cecum (approximately 0.7 cm in length) was closed using a Vicryl[®] 2-0 ligature (polyglactin 910) and the distal part of the bowel was resected.

In group II (n = 30) a 45 mm stapler was applied to the closed end of the cecum (Ethicon, Endosurgery, Cincinnati, OH) and resection was performed in that way.

In group III (n = 30) Hem-o-lok XL[®] clip (Hem-o-lok, Weck Closure Systems, Research Triangle Park, NC, USA) was applied to the closed end of the cecum, and the distal part of the bowel was resected.

In group IV DS titanium clips (Braun, Germany) were applied to the closed end of the cecum, and the distal part of the bowel was resected.

Laparotomy and closure of the skin were performed using a 3-0 continuous suture. No antibiotic therapy was used either during or after the experiment. Throughout the entire observation period all the animals were monitored and subjected to clinical testing to check for any local or systemic complications.

Parameters of Monitoring

Ten animals from each group of subjects were sacrificed on days 7, 28, and 60 postsurgery. Relaparotomy was performed using a left paramedial incision, to gain an overview of the entire abdomen.

Histopathological Analysis

Histopathological analysis was performed at the Pathology Clinic. Part of the tissue with the clips was subjected to histological testing. Sections of tissue were placed in 10% buffered formalin and fixed for 24 hours. After that the preparations were molded into paraffin blocks, and three thin sections 4 microns thick were cut from each block. The sections were stained using the hematoxylin eosin method for histopathological testing, to establish the presence and extent of any tissue reaction to the clips, whereby any inflammatory response was semiquantitatively graded as mild, moderate, or severe.

Formation of Adhesion

After opening the abdomen, the formation of adhesion was assessed according to the Surgical Membrane Study Group score (**Table 1**), after sacrificing on days 7, 28, and 60.

Statistical Analysis

The Fisher exact probability test was used for analysis of the differences between the distribution of histopathological changes. Due to multiple comparisons (a total of 6 between 4 groups) differences were considered significant at $P = .05$ corrected by Bonferroni factor $f = 1/6$. The Kruskal-Wallis test was used to compare SMSG scores in the Vicryl[®], stapler, Hem-o-lok[®], and DS clip groups. Post hoc analysis was performed using the Mann-Whitney test with a corrected P value again using the Bonferroni correction method.

RESULTS

All the operated rats survived (100% survival). No complications were noticed, such as wound infection, intestinal obstruction, or wound dehiscence.

Inflammatory Reaction

We analyzed the tissue reaction in the area around the Vicryl[®] ligature, stapler clip, plastic, or titanium DS clip (**Table 2**). Fisher's test was used to test the significance of differences in the occurrence of mild, moderate, or severe

Table 1.

The Surgical Membrane Study Group Score (SMSG)

Character of the Adhesion	Score
The size of area affected by adhesion	
None	0
< 25% of the surface	1
< 50% of the surface	2
< 75% of the surface	3
100% of the surface	4
Type of adhesion	
None	0
Thin, transparent, avascular	1
Opaque, translucent, avascular	2
Opaque, capillaries present	3
Opaque, large vessels present	4
Tenacity of adhesion	
None	0
Adhesion separates easily	1
Adhesion separates under traction	2
Adhesion requires sharp dissection	3

inflammation in the four groups. For the reasons already mentioned, correction was performed using Bonferroni's factor ($f = 1/6$), and differences were deemed significant when $P < .008$.

Table 2.

Histopathological Findings of the Cecum Stump

	Vycril	Stapler	Hem-o-Lok	DS
7 th postoperative day				
Mild inflammation		3		
Moderate inflammation	6	6	1	3
Severe inflammation	4	1	9	7
28 th postoperative day				
Mild inflammation		4	1	1
Moderate inflammation	2	6	4	7
Severe inflammation	8		5	2
60 th postoperative day				
Mild inflammation	5	5	4	3
Moderate inflammation	1	5	3	6
Severe inflammation	4		3	1

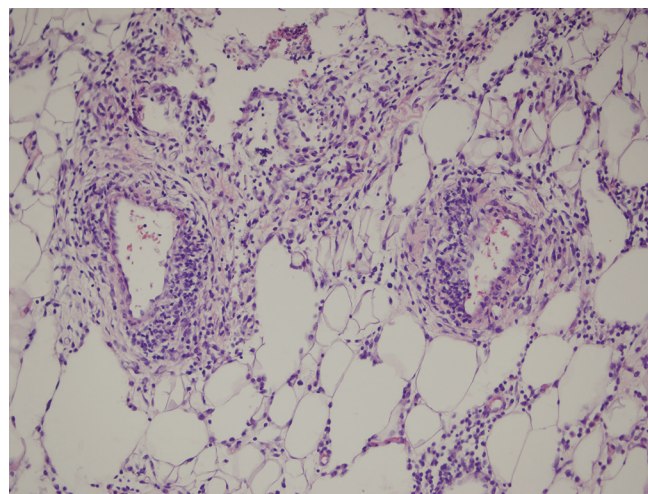


Figure 1. Moderate inflammation, Stapler, Day 7. (Hematoxylin-eosin stain, original magnification x 300).

Postoperative Day 7

The Fisher's test revealed a statistically significant difference in the occurrence of mild, moderate, and severe inflammation between the Hem-o-lok[®] and stapler groups ($P = .001$) (**Figure 1**).

No statistically significant differences were found in any other cases (Vycril[®]-Hem-o-lok[®], $P = .057$; Vycril[®]-DS, $P = .370$; Vycril[®]-stapler, $P = .015$; Hem-o-lok[®]-DS, $P = .303$; Hem-o-lok[®]-stapler, $P = .580$; DS-stapler, $P = .020$).

Postoperative Day 28

A statistically significant difference was found in the occurrence of mild or moderate (severe) inflammation between the Vycril[®] and the stapler groups ($P = .001$). No statistically significant difference was found in any other cases (Vycril[®]-Hem-o-lok[®], $P = .349$; Vycril[®]-DS, $P = .023$; Hem-o-lok[®]-DS, $P = .349$; Hem-o-lok[®]-stapler, $P = .033$; DS-stapler, $P = .474$) (**Figure 2**).

Postoperative Day 60

No statistically significant differences were found between any of the groups compared (Vycril[®]- Hem-o-lok[®], $P = .615$; Vycril[®]-DS, $P = .106$; Vycril[®]-stapler, $P = .015$; Hem-o-lok[®]-DS, $P = .576$; Hem-o-lok[®]-stapler, $P = .087$; DS-stapler, $P = .473$) (**Figure 3**).

The Formation of Adhesion

The Kruskal-Wallis test was used to test the differences in the adhesion scores for the Vycril[®], stapler, Hem-o-lok[®], DS

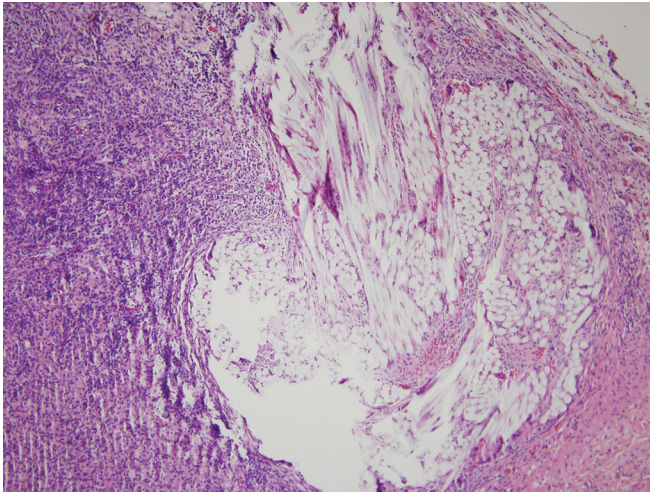


Figure 2. Severe inflammation, Vycril®, Day 28. (Hematoxylin-eosin stain, original magnification x 300).

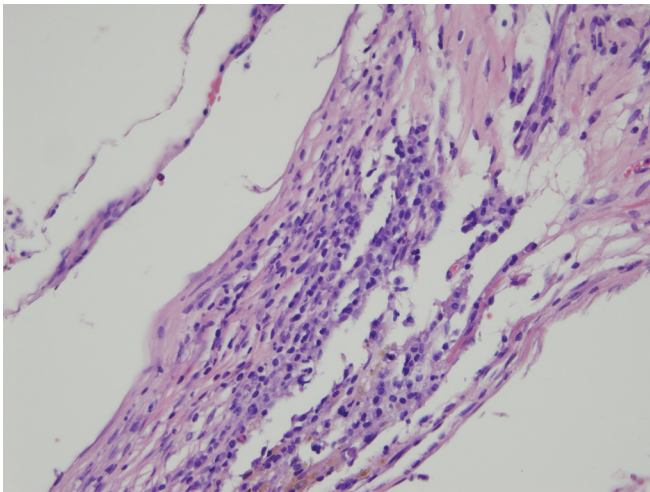


Figure 3. Moderate inflammation, DS clips with fibrosis, Day 60. (Hematoxylin-eosin stain, original magnification x 300).

groups after 7, 28, and 60 days. Statistically significant differences were found between the groups after 7 days (Kruskal-Wallis $H = 22.01$, $df = 3$, $P < .001$), 28 days (Kruskal-Wallis $H = 18.52$, $df = 3$, $P < .001$), and 60 days (Kruskal-Wallis $H = 15.51$, $df = 3$, $P = .001$).

In order to determine if any differences existed between individual groups, post hoc testing was performed using the Mann-Whitney test, where the level of significance, P , was multiplied by the appropriate Bonferroni factor $f = 1/6$ (a total of 6 comparisons between 4 groups, which means that after multiple testing, the level of significance fell to $P = .008$).



Figure 4. Vycril®, Day 7. Adhesion covered 100% of the stump, firmly, difficult to separate.

Since we were interested in whether the adhesion score in one group was higher or lower than the corresponding value in another group, where there was a statistically significant difference, the results of *unilateral* testing are given.

Postoperative Day 7

On postoperative day 7 no significant differences were found in the adhesion scores between the Vycril® and Hem-o-lok® groups ($U = 460.5$, $P = .796$), or between the DS and stapler groups ($U = 450.5$, $P = .754$). The adhesion score was significantly higher in the Vycril® group in comparison to the DS ($U = 50.0$, $P < .0001$) and stapler ($U = 40.5$, $P < .0001$) groups. Moreover, the adhesion score was significantly higher in the Hem-o-lok® group in comparison to the DS ($U = 10$, $P < .001$) and stapler ($U = 11$, $P < .001$) groups (**Figures 4 and 8**).

Postoperative Day 28

On postoperative day 28, a statistically significant differences were observed in the adhesion score between the Vycril® and Hem-o-lok® groups ($U = 120.5$, $P < .0001$). No significant differences were found between the DS and Stapler groups ($U = 490.0$, $P = .921$). The adhesion score was significantly higher in the Vycril® group in comparison to the DS ($U = 5$, $P < .0001$) and stapler ($U = 10$, $P < .0001$) groups. No significant differences were observed in the scores for the Hem-o-lok® and DS groups ($U = 22$, $P = .029$), or the Hem-o-lok® and stapler groups ($U = 35$, $P = .228$). (**Figures 5 and 8**).



Figure 5. Vycril®, Day 28. Adhesion covered 100% of the stump, firmly, difficult to separate.

Postoperative Day 60

On postoperative day 60, no statistically significant differences were observed in the adhesion scores between the Vycril® and Hem-o-lok® groups ($U = 340.5, P = .257$) or between the DS and stapler groups ($U = 330.5, P = .222$). No significant differences were found in the adhesion score between the Vycril® and DS groups ($U = 33, P = .193$), but the score was statistically significantly higher in the Vycril® group than in the stapler group ($U = 14, P = .002$). In addition, the adhesion score was statistically significantly higher in the Hem-o-lok® group than in the DS ($U = 19, P < .008$) and stapler ($U = 40.5, P < .0001$) groups. (Figure 6, 7 and 8).

DISCUSSION

In practice, there are various methods used for securing the appendiceal stump during laparoscopic appendectomy. However, different materials cause different degrees of inflammation. It would be ideal to use material(s) which cause the minimum tissue response(s).^{6,7} On postoperative day 7, the mildest degree of inflammation was noticed in the stapler group, which is in line with our earlier studies.⁴ However, if we consider the formation of adhesion, on all the days of the test, the lowest score was noted in the stapler and DS clips groups.

Stapler clips are made of titanium, which causes a minimal reaction in tissue, and they are extremely biocompatible.⁸ The construction of DS clips is different from the smaller titanium stapler clips. DS clips are made in one piece, and they weigh two and a half times more than staplers.⁹ A



Figure 6. Stapler, Day 60. There is no adhesion on the stump.



Figure 7. Vycril®, Day 60. Adhesion covered 100% of the stump, adhesion lysed with traction.

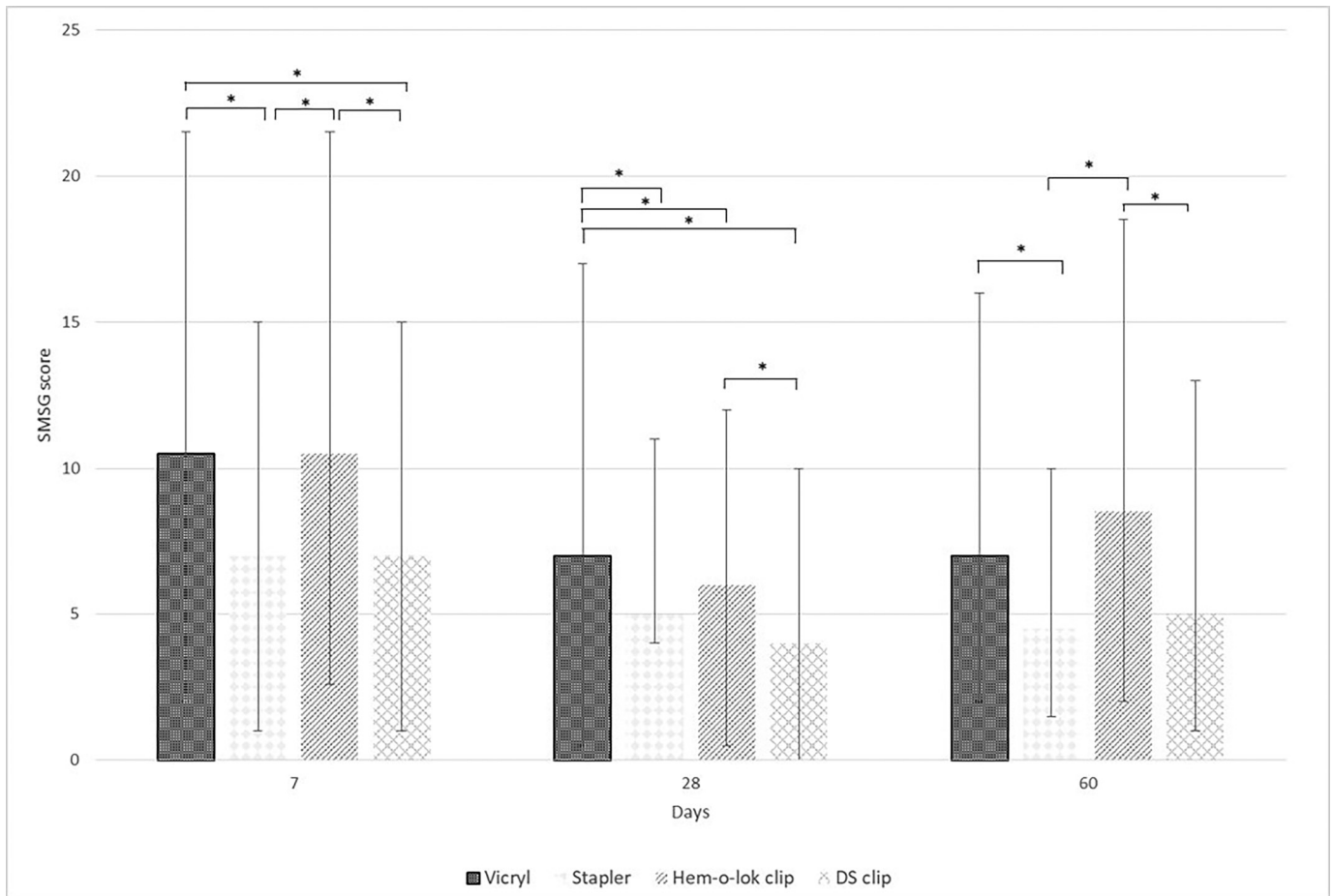


Figure 8. Surgical Membrane Study Group (SMSG) score on postoperative Days 7, 28, and 60. *Statistically significant difference.

smaller inflammatory reaction and less formation of adhesion correlate directly with the smaller size of the implanted material,¹⁰ which may explain the milder degree of inflammation with staplers in comparison with DS clips.

Hem-o-lok[®] clip and Vycryl[®] (endoloop) exhibit higher degree of inflammation and formation of adhesion than DS clip and stapler; however, have advantages in certain clinical situations (e.g. when the clip cannot encompass the inflamed appendix, endoloop, or stapler can be used).

In our study, there were no significant differences on postoperative day 60. However, inflammatory reactions and the formation of adhesion peaked on postoperative day 7¹¹ when we would normally expect postoperative complications in patients.

Suture materials may act as foreign bodies inside the abdomen, causing an inflammatory reaction, leading to the formation of adhesion.¹² This may result in the acute obstruction of the small intestine, which may be linked to the anchoring of

a clip onto neighboring tissue.¹⁰ Moreover, the surface of the clip may cause entanglement with adjacent tissue, creating a rough surface on the peritoneal surface, which may cause the formation of adhesion. Hem-o-lok[®] clips have this kind of surface, with tiny bulges, and they may cause the anchoring of neighboring tissue.¹³ The joining of visceral organs to the clips may potentially lead to erosion of the intestines and the formation of fistulas, so the use of this kind of clip should be avoided. On the other hand, DS clips have a completely straight and smooth external surface.

The lower amount of adhesion found with some methods may affect the patients' postoperative recovery. It is well recognized that up to 66% of laparotomies in high volume surgical centers result in repeat surgeries.¹⁴ Therefore, it is important to use methods that cause the lowest possible amount of adhesion. An ideal suture should enable high tension force and security, be resistant to inflammation and contamination, and react minimally in the tissue in which it is located.¹⁵

The limitations of this study are characteristic of animal studies, and one of the most important questions is how far the results of this study can be applied to humans. It is also impossible to determine how far the short incision in the anterior abdominal wall and its closure provoked the formation of adhesion, but the length of the incision was the same in all the animals. Another limitation of this study is the relatively small sample (with all the weaknesses of small samples). Of course, this is the result of our effort to adhere to the principles of the ethical use of animals in testing (including the use of methods which enable researchers to obtain comparable results using a small number of animals). Finally, the study did not have an additional sham control group, which would have involved just making an incision but leaving the cecum untouched/healthy. However, considering that the rats were allowed to recover for up to 60 days, the exterior surgery should not have had a major effect on the cecum itself. What our study does allow us to do however is conclude that titanium clips, either in the form of staplers or the cheaper DS® clips, were superior to other tested methods used to close the appendiceal stump from the point of view of biocompatibility.

Titanium DS clips are made specifically to close the appendiceal stump. The internal length of their prongs encompasses all average sized phlegmonous, gangrenous, and perforated appendices.¹⁶

In light of the results of this study, from the point of view of biocompatibility, we may conclude that staplers and DS clips have certain advantages over other tested methods used to close the appendiceal stump, because they exhibited a high level of biocompatibility and lower adhesion score. Their clinical use depends on other aspects however, including affordability, time need for their application, technical characteristics, and reliability. However, the results of this study imply the direction in which the development of various methods of closing the appendiceal base should go.

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