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Crystalloid fluid suspension results in decreased adhesion burden when compared to bioresorbable membranes in a rat model☆

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ABSTRACT

Introduction: Adhesion formation represents a major cause of long-term morbidity. Suspension of intra-abdominal contents in fluid medium may effectively prevent adhesion formation. We compare saline hydro-flotation (NS) to hyaluronate bioresorbable membranes (HBM) for adhesion prevention following surgery.

Methods: Animals were randomized to four groups: sham (no injury, n = 5), control (injury without intervention, n = 5), HBM (n = 20) or 10 cc NS (n = 21). Interventions were administered after standardized surgical trauma to the cecum and abdominal wall. Necropsies at two weeks were completed to compare adhesion burden using a customary scoring algorithm.

Results: Significant adhesion burden was noted in all rats. HBM sustained a more significant adhesion burden with higher total adhesion scores (HBM = 10 vs NS = 8.1/15, p = 0.02). Gross adhesion scores were lower with NS (5.6/9) compared to HBM (7.1/9, p = 0.01). Neo-vascularity was more common in HBM at 2.6/3 versus 1.9/3 with NS (p = 0.01). Percent of the cecum encased with adhesion was higher with HBM (42%) compared to NS (31%, p = 0.05).

Discussion: Fluid based anti-adhesion methods should be considered for abdominal adhesion formation prevention.

Introduction

Intra-abdominal insult resulting damage to the peritoneum can trigger a natural inflammatory cascade that results in inter-organ fibrin formation causing adhesion formation. Complications from adhesions account for ~6% of all hospital readmissions costing US healthcare over one billion dollars annually.[1] Despite the dramatic scope of this problem, there has been minimal advancement in terms of preventative strategies, with no one method that is universally accepted or effective.

Arguably the most commonly used barrier in the general surgery suites to date is a hyaluronic barrier membranes (HBM) best known as Seprafilm® (Genzyme Biosurgery, Framingham, MA). Literature supporting Seprafilm's efficacy in reduction of adhesion related morbidity remains equivocal although there are certainly anecdotal benefits. The overall absence of an anti-adhesion product variety leaves much room for innovation and study in this field.[2]

Fluid suspension using crystalloid or hypertonic products allows for intraabdominal contents to remain oriented in such a way that they do not contact each other during the critical formation of fibrin-thus preventing inter-loop and inter-organ adhesion formation. Its use was first described in the 1970s with normal saline and lactated ringers. Gynecological surgery is currently the only field where hydro-flotation methods are being studied. Despite some promising results, its use has not been pursued in the greater surgical community.

Preclinical evaluation of adhesion products is reliably observable after peritoneal disruption in rat models and is clinically comparable to adhesion formation in humans.[3,4] We hypothesize

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that hydro-flotation of abdominal contents by instillation of normal saline after peritoneal violation would provide anti-adhesive effects in a rat model when compared to controls and standard adhesion barrier methods.

Materials and methods

Institutional Animal Care and Use Committee (IACUC) protocol approval was obtained and in vivo experiments were completed in an American Association for Laboratory Animal Science accredited large animal research facility. Animals utilized included all adult male Sprague-Dawley rats weighing between 350 and 450 g. Prior to experiments, animals were housed at the facility for a minimum 5 days, under the supervision of a licensed veterinary staff. General care did not vary among animals, no animals required unexpected medical attention prior to participation and their diet was ad libetum.

A prospective pilot study was carried out using fifty-one animals, computer randomized to four groups including sham ($N = 5$), control ($N = 5$), 10 cc normal saline (NS, $N = 21$), and HBM (Seprafilm, $N = 20$). Number of animals in each group were based on the estimated power analysis that was extracted from injury rate determined during the development of the surgical injury. A single surgeon completed all experiments and was blinded to treatment group until after injury creation was completed.

Animals underwent a 14-day survival period followed by necropsy. Fourteen days was chosen as it was observed as the peak of adhesion formation in our development phase and because it correlates to peak adhesion formation seen clinically. Primary outcomes evaluated gross and histological adhesion severity and tenacity. Secondary outcomes focused on abdominal wall dehiscence, perforation and small bowel obstruction.

Surgical procedures were conducted using aseptic technique.[5] Injury was created through a 3 cm celiotomy. Celiotomy was followed by immediate closure and survival completed in sham animals. In experimental groups, the cecum was isolated and the peritoneum was lifted from the cecal wall and removed using surgical gauze followed by violation of abdominal wall peritoneum. There was no full thickness injury made to the bowel or abdominal musculature. After this standard injury had been completed, an assistant would reveal the randomized treatment group. Control rats were simply closed after injury and experimental animals received instillation of either 10 cc of normal saline or a 3×2 cm piece of HBM. Closure was completed using a running 3-0 prolene stitch, skin was approximated using surgical staples, and animals were survived for 14 days.

Necropsy was performed after 14-day survival. Adhesion scoring was performed at time of necropsy by a blinded surgeon who was uninvolved with the index operation. After completion of gross scoring, en-bloc resection of adhered tissue was harvested and sent to a blinded pathologist for histological scoring. Scores were based on a semi-quantitative scoring system that included gross percent of the cecum involved (0–4), neovascularization (0–3), tenacity (0–3) and histologically for inflammation (0–3), and fibrosis (0–3).[6–9]

Adhered tissue was prepared for histological analysis with fixation in 10% buffered formaldehyde (formalin) for at least 24 h. Specimens were then sectioned and paraffin embedded using hematoxylin and eosin staining for pathological scoring. Examples of gross and histopathological tissues examined can be seen in Fig. 1.

SPSS v22 (IBM, Chicago, IL) was used for data collection and analysis. Groups were analyzed with ANOVA and Chi-Square analyses for parametric and nonparametric data respectively. Tukey post-hoc analysis was completed for all ANOVA comparisons between groups. Kruskal Wallis testing was utilized for nonparametric data which used median values and was followed by pairwise analysis for significant findings between groups. All p-values <0.05 were considered to be statistically significant.

Results

Fourteen-day survival was accomplished in all 51 animals. Our injury model was validated by a complete lack of adhesions in the sham group. There was no abdominal wall dehiscence or bowel perforation in any animal across all groups. Overall gross and total scores showed significant differences within and between groups on Kruskal Wallis comparison with $p = 0.001$ and $p = 0.004$ respectively. Comparisons of each individual score revealed significant differences for the percent of cecum involved ($p < 0.001$) and neo-vascularity of adhesions ($p = 0.01$). Values for comparison on post-hoc analysis for gross, histological and total scoring can be seen in Fig. 2. Fig. 3 shows individual score comparisons of the three groups with Fig. 4 displaying comparison of percent of adhered cecum.

Saline was found to have lower gross and total scores when compared to both HBM and control groups (Fig. 2). Further, percent of cecum involved as well as neo-vascularity was significantly decreased in saline groups compared to both control and HBM groups (Figs. 3 and 4). Gross number of adhesions trended toward significance on post hoc for saline at a median of two compared to four for controls ($p = 0.06$).

Discussion

Though the pathophysiology of intra-abdominal adhesion formation has never been strictly described, the collective assumption is that they are acquired via the processes associated with any wound healing after surgical insult. In short, the inflammatory cascade results in fibrin deposition and ultimately inappropriately adhering intra-abdominal contents to each other.[10] These adhesions can result in pain, infertility in women and bowel obstruction which can lead to prolonged hospital admissions, reoperation, perforation, and in some cases death. Adhesions form in over 90% of patients who have had abdominal surgery and over one third of those patients suffer a complication due to their adhesive disease.[11,12]

An answer to the question of ‘how can we prevent adhesion formation’ has been sought for over half a century with effectively minimal success. As of now, the best recommendation is for meticulous atraumatic surgical technique and the use of minimally invasive methods whenever possible.[13] Unfortunately, despite the wide adherence to these principles, there has yet to be any benefit shown in terms of adhesion-related morbidity.[14]

Membrane barriers containing hyaluronic acid-based compounds, such as our comparison group, are perhaps the best studied adjuncts in terms of preclinical research with supporting prospective randomized trials.[15,16] Like many of the products proposed for adhesion prevention, the adhesion number is decreased but the overall morbidity remains unchanged showing similar rates of small bowel obstructions and infertility.[16–20]

In our study, we observed an overall gross and total adhesion score benefit in the NS group. The authors are aware that crystalloid installation has been found to be ineffective and not feasible in human trials in the past.[2,14,21] Crystalloids failure has been accredited to its rapid reabsorption and the propensity for fluids to leak out of midline incisions. However, the concept of ‘hydro-flotation’ still holds value.[22] Intra-operative instillation of 4% dextran solutions that have the ability to remain within the peritoneal cavity for >48 h have shown equivalent outcomes to HBMs. However, the use of dextran solutions is far less frequently

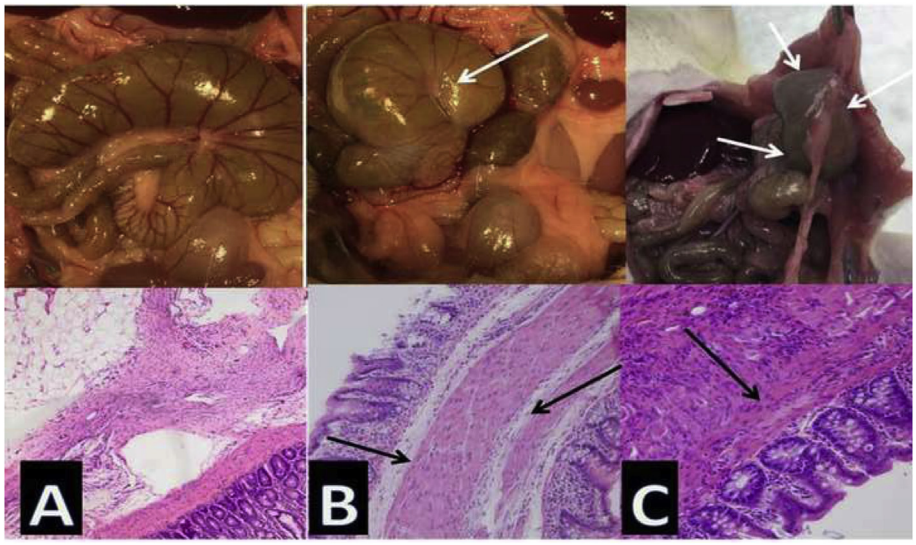

Fig. 1. Three columns showing adhesion formation with increasing severity and corresponding histology. (A) No gross or histological adhesions. (B) Simple cecal to cecal adhesion with minimal fibrosis. (C) Dense adhesions of the cecum to the abdominal wall with concomitant infiltration of inflammatory cells and layered fibrosis.

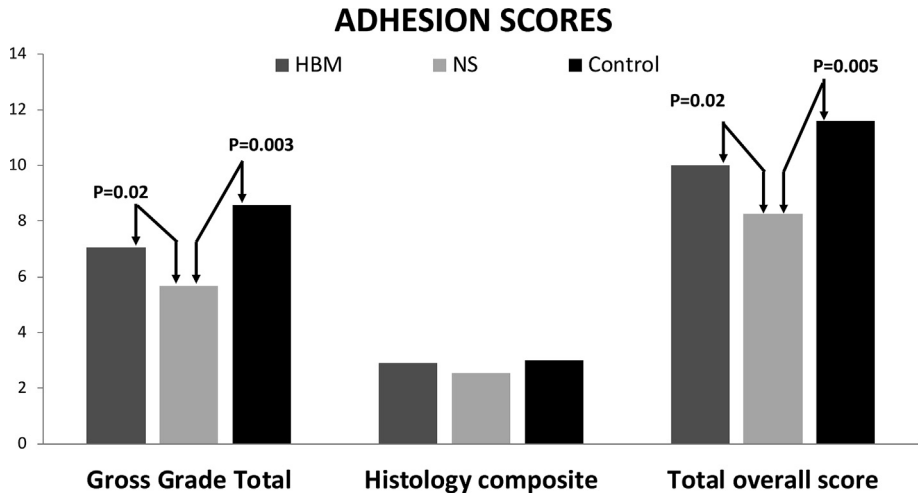

Fig. 2. Total adhesion scores showed significantly reduced overall and gross scores for the saline group when compare to HBM and control groups.

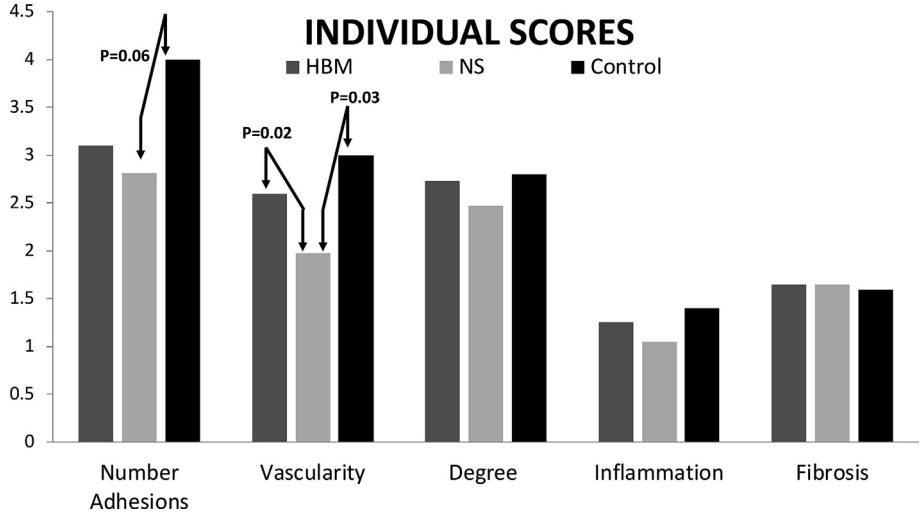

Fig. 3. Component median scores were significantly higher for neo-vascularity in the control and HBM groups compared to saline.

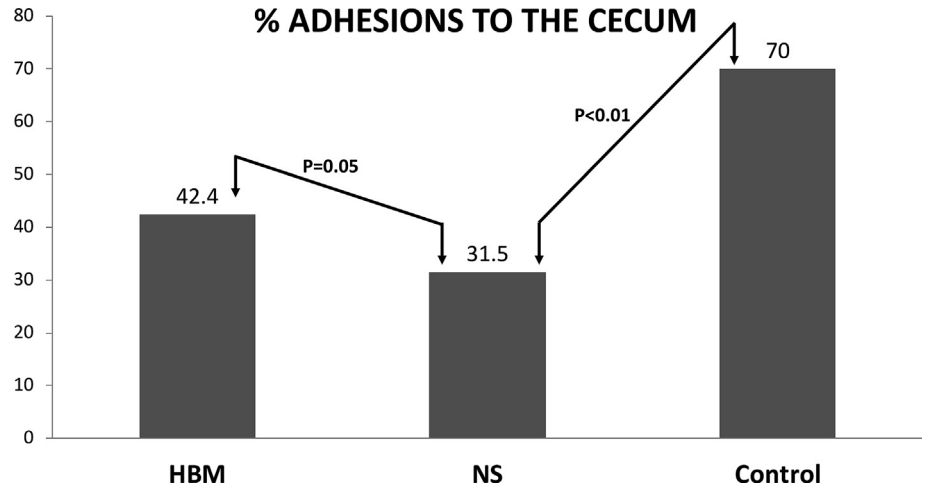

Fig. 4. The percent of involved cecum was significantly lower in groups treated with saline as opposed to both HBM and control groups.

employed.[23,24] A recent Cochrane review evaluating fluid based anti-adhesion methods concluded that on the whole, they are effective at preventing adhesion formation and further studies utilizing fluid-based agents should be pursued.[2] Numerous reviews of studies evaluating intra-abdominal adhesion formation increasingly call for a combination of various adjuncts in hopes that there is a cooperative effect of these products.[12,13,25]

Our findings found that the most commonly used adhesion barrier used in abdominal surgery today performs significantly worse than simple normal saline instillation. We do not surmise or suggest from this data that normal saline or other simple fluids be solely utilized or that this information would be directly transferable to a clinical setting. Our findings do however bolster the inclination to more aggressively and thoroughly pursue the benefits of fluid based anti-adhesion products. Therefore, we propose that in the battle against adhesion formation, which the surgical community is currently losing, we consider the benefits of a fluid-based product either alone or in synergy with membrane-based methods. This may be accomplished by the development and clinical evaluation of a potential anti-adhesion products suspended in a non-absorbable fluid media.

There are several important limitations to be discussed. First, this is a pre-clinical study completed in a rat model and the translation of these findings to a clinical setting is uncertain. For example, the amount of crystalloid fluid required for human application would be approximately 2 L and reabsorption would be expected within 24–48 h making this impractical. As such, we do not submit direct clinical application, but rather the serious consideration of developing and evaluating more applicable fluid medias for adhesion prevention in the future. This study represents an adhesion environment limited to the 14-day survival period. It is possible that adhesion burden and comparisons may be different at alternate survival periods.

Conclusion

Adhesion formation represents one of the most challenging issues still afflicting modern abdominal surgery. Crystalloid fluid suspension was found to be superior to a bioresorbable membrane in preventing abdominal adhesions with an overall decrease in adhesion burden. These findings suggest that a fluid-based product may be more useful than previously thought as we continue to find the solution to the significant clinical challenge of intra-abdominal adhesion formation.

Disclosures

The results and opinions expressed in this article are those of the authors, and do not reflect the opinions or official policy of the United States Army or the Department of Defense. None of the authors have any relevant financial interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amjsurg.2018.12.014.

References

1. Stanciu D, Menzies D. The magnitude of adhesion-related problems. *Colorectal Dis: Off J Assoc Coloproctol Great Britain Ireland*. 2007;9(Suppl 2):35–38.
2. Ahmad G, Mackie FL, Iles DA, et al. Fluid and pharmacological agents for adhesion prevention after gynaecological surgery. *Cochrane Database Syst Rev*. 2014;(7), Cd001298.
3. Poehnert D, Grethe L, Maegel L, et al. Evaluation of the effectiveness of peritoneal adhesion prevention devices in a rat model. *Int J Med Sci*. 2016;13(7):524–532.
4. Urkan M, Ozerhan IH, Unlu A, et al. Prevention of intraabdominal adhesions: an experimental study using mitomycin-C and 4% icodextrin. *Balkan Med J*. 2017;34(1):35–40.
5. Yuan F, Lin LX, Zhang HH, Huang D, Sun YL. Effect of carbodiimide-derivatized hyaluronic acid gelatin on preventing postsurgical intra-abdominal adhesion formation and promoting healing in a rat model. *J Biomed Mater Res*. 2016;104(5):1175–1181.
6. Vlahos A, Yu P, Lucas CE, Ledgerwood AM. Effect of a composite membrane of chitosan and poloxamer gel on postoperative adhesive interactions. *Am Surg*. 2001;67(1):15–21.
7. Wurster SH, Bonet V, Mayberry A, Hoddinott M, Williams T, Chaudry IH. Intraperitoneal sodium carboxymethylcellulose administration prevents reformation of peritoneal adhesions following surgical lysis. *J Surg Res*. 1995;59(1):97–102.
8. Du X, Hong G, Sun P, Liu G. Zn2+-SCMC versus HA for preventing intraperitoneal adhesions: a rat model study. *Int J Med Sci*. 2012;9(6):467–471.
9. Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. *Arch Surg (Chicago, Ill: 1960)*. 1974;108(6):849–853.
10. Maciver AH, McCall M, James Shapiro AM. Intra-abdominal adhesions: cellular

mechanisms and strategies for prevention. *Int J Surg*. 2011;9(8):589–594.
11. Cheong YC, Laird SM, Li TC, Shelton JB, Ledger WL, Cooke ID. Peritoneal healing and adhesion formation/reformation. *Hum Reprod Update*. 2001;7(6):556–566.
12. Liakakos T, Thomakos N, Fine PM, Dervenis C, Young RL. Peritoneal adhesions: etiology, pathophysiology, and clinical significance. Recent advances in prevention and management. *Dig Surg*. 2001;18(4):260–273.
13. Ouaissi M, Gaujoux S, Veyrie N, et al. Post-operative adhesions after digestive surgery: their incidence and prevention: review of the literature. *J Visc Surg*. 2012;149(2):e104–e114.
14. diZerega GS. Contemporary adhesion prevention. *Fertil Steril*. 1994;61(2):219–235.
15. Becker JM, Dayton MT, Fazio VW, et al. Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective, randomized, double-blind multicenter study. *J Am Coll Surg*. 1996;183(4):297–306.
16. Zeng Q, Yu Z, You J, Zhang Q. Efficacy and safety of Seprafilm for preventing postoperative abdominal adhesion: systematic review and meta-analysis. *World J Surg*. 2007;31(11):2125–2131. discussion 2132.
17. Diamond MP, Burns EL, Accomando B, Mian S, Holmdahl L. Seprafilm((R)) adhesion barrier: (2) a review of the clinical literature on intraabdominal use. *Gynecol Surg*. 2012;9(3):247–257.
18. Hayashi S, Takayama T, Masuda H, et al. Bioresorbable membrane to reduce postoperative small bowel obstruction in patients with gastric cancer: a randomized clinical trial. *Ann Surg*. 2008;247(5):766–770.
19. Tan A, Argenta P, Ramirez R, Bliss R, Geller M. The use of sodium hyaluronate-carboxymethylcellulose (HA-CMC) barrier in gynecologic malignancies: a retrospective review of outcomes. *Ann Surg Oncol*. 2009;16(2):499–505.
20. Kuckelman JP, Kononchik J, Smith J, et al. Human-derived amniotic membrane is associated with decreased postoperative intraperitoneal adhesions in a rat model. *Dis Colon Rectum*. 2018;61(4):484–490.
21. Wiseman DM, Trout JR, Diamond MP. The rates of adhesion development and the effects of crystalloid solutions on adhesion development in pelvic surgery. *Fertil Steril*. 1998;70(4):702–711.
22. Colvin HS, Rajab TK. Adhesion prevention by hydroflotation. *Colorectal Dis: Off J Assoc Coloproctol Great Britain Ireland*. 2010;12(6):606.
23. Catena F, Ansaloni L, Di Saverio S, Pinna AD, P.O.P.A. study: prevention of postoperative abdominal adhesions by icodextrin 4% solution after laparotomy for adhesive small bowel obstruction. A prospective randomized controlled trial. *J Gastrointest Surg: Off J Soc Surg Aliment Tract*. 2012;16(2):382–388.
24. Sakari T, Sjodahl R, Pahlman L, Karlbom U. Role of icodextrin in the prevention of small bowel obstruction. Safety randomized patients control of the first 300 in the ADEPT trial. *Colorectal Dis: Off J Assoc Coloproctol Great Britain Ireland*. 2016;18(3):295–300.
25. Risberg B. Adhesions: preventive strategies (577). In: *The European journal of surgery Supplement: = Acta chirurgica Supplement*. 1997:32–39.