The Contribution of a Collagen Patch Coated With Fibrin Glue in Sealing Upper Gastrointestinal Defects: An Experimental Study

GREGORY CHRISTODOULIDIS¹, ATHINA A. SAMARA¹, THEODOROS FLOROS¹,
KOSTAS-SOTIRIOS KARAKANTAS¹, PROKOPIS-ANDREAS ZOTOS¹,
ANTONIOS KOUTRAS² and KONSTANTINOS TEPETES¹

¹Laboratory of Experimental Surgery, University of Thessaly, Larissa, Greece;

²1st Department of Obstetrics and Gynecology, Alexandra Maternity Hospital,
National and Kapodistrian University of Athens, Athens, Greece

Abstract. *Background/Aim:* We aimed to evaluate the safety and efficacy of sealing primary upper gastrointestinal (GI) perforations with a collagen patch coated with fibrin glue (Tachosil®). *Materials and Methods:* Thirty New Zealand rabbits were used in this study. An iatrogenic gastric perforation was created, and primary repair was performed on the control group. Tachosil®, without suturing the deficit, was used in the intervention group. *Results:* Leakage was observed in 3 (20%) and 2 rabbits (13.3%) in the control and intervention group, respectively; however, the difference was not statistically significant ($p=0.62$). Moreover, adhesions formed in 10/15 and all rabbits in the intervention and control group, respectively ($p=0.014$); however, based on the Zuhlke adhesion's classification, there was no statistically significant difference between the two groups. *Conclusion:* A collagen patch coated with fibrin glue is not a replacement but can be considered a safe option for the reinforcement of suturing, preventing leakages in the upper GI tract.

Gastrointestinal (GI) leakage is a common and significant complication in routine clinical surgical practice, with increasing morbidity and mortality (1). Leakages of the GI tract can be the result of a perforation (spontaneous or iatrogenic) or a surgical anastomosis complication (2). Wide-ranging complications are associated with GI leakages, including local abscess formation, sepsis, and even multiple organ system

failure in cases of delayed diagnosis (3). Furthermore, prolonged hospitalization due to anastomotic leakages has been associated with long-term decreased quality of life (4).

Several clinical and experimental studies have been conducted to increase the safety of anastomoses and primary repaired injuries (gastric or duodenal perforation), thereby decreasing the incidence of these complications (5, 6). Through several trials, a variety of materials have been evaluated to decrease the incidence of leakage and any related consequences (7-9). Experimental studies have provided promising findings; however, results are not easily reproducible in human clinical studies.

Tachosil® (haemostatic surgical patch; Nycomed, Linz, Austria) is a fibrin sealant patch indicated for use as an adjunct in hepatic and cardiovascular surgery when control of bleeding using classic surgical techniques is ineffective or impossible (10). Several studies have examined the effectiveness of Tachosil® as an additional reinforcement to pancreatic stump closure, with unclear results (11). Conversely, Tachosil® has been associated with highly promising results when applied to the reinforcement of colorectal anastomoses in both experimental and clinical settings (12-14).

To the best of our current knowledge, Tachosil® has never been utilized as a reinforcement material in upper GI anastomoses and the primary closure of upper GI perforations. In the present experimental design using New Zealand rabbits, we aimed to determine and evaluate the safety and efficacy of sealing anastomoses and primary closure of upper GI perforations with a collagen patch coated with fibrin glue (Tachosil®).

Materials and Methods

Animal models. Thirty female New Zealand rabbits - an appropriate and validated model for experimental studies and research - were

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Correspondence to: Athina A. Samara, Laboratory of Experimental Surgery, University of Thessaly, Larissa 41110, Greece. Tel: +30 2413501701, e-mail: at.samara93@gmail.com

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Figure 1. Intraoperative images from the control group (A, B). Management of the perforated stomach involved the placement of sutures (Dexon 3/0) in the defect.

used as animal models in this study. The animals were kept in the Laboratory of Experimental Surgery of the University of Thessaly under controlled conditions (temperature $25\pm 1^{\circ}\text{C}$, humidity $55\pm 5\%$, controlled light 12 h per day), with the rabbits fasting the evening prior to surgery.

Research involving animals. The present study was conducted in accordance with the Declaration of Helsinki concerning animal and Human Rights, and was approved by the Medical School of the University of Thessaly Ethics Committee.

Experimental protocol and surgical procedures. Rabbits were anesthetized by injecting intravenously a mixture of ketamine hydrochloride (15 mg/kg) and midazolam (1 mg/kg); anesthesia was maintained with intravenous infusion of ketamine hydrochloride at a rate of 60 mg/kg/h. Initially, the rabbits' abdomen was shaved and excess hair was removed with a piece of tape. Basic surgical principles including safety and minimization of surgical time were strictly followed to reduce suffering of the rabbits and prevent secondary infections, which could have affected the experiment's outcome. The rabbits' skin was disinfected and midline incisions (3.5-4.0 cm) of both the skin and muscle layer were performed. The surgeon created an artificial hole in the posterior gastric wall after identifying and pulling the stomach forward. The abdominal wall was closed using an absorbable running suture (Vicryl 2.0) in the muscle layer and a non-absorbable interrupted suture (Nylon 3.0) with inverted knots in the skin.

Management of the perforated stomach involved the placement of sutures (Dexon 3/0) in the control group ($n=15$) (Figure 1A and B), whereas in the intervention group, management required only the application of Tachosil®. Additionally, an omentoplasty was performed on each rabbit in the control group by the same surgeon, where a flap of the animal's greater omentum was used to cover the defect. In the intervention group ($n=15$), the yellow surface of the Tachosil® was placed directly on the created defect without any further handling. In accordance with the study's protocol, the patch was required to cover at minimum 1-2 cm beyond the margins of the defect (Figure 2).

Figure 2. Intraoperative image from the intervention group. Placement of the yellow surface of the Tachosil® directly on the created defect without any further handling. The patch covers a minimum of 1-2 cm beyond the margins of the defect.

Post-operatively, rabbit cages were placed in a room with an estimated temperature of $25-28^{\circ}\text{C}$, where the animals were carefully evaluated daily by a member of the medical team. On the 15th post-operative day, the animals were euthanized with exsanguination, following intravenous administration of sodium pentobarbital (30 mg/kg), in accordance with the Institutional Animal Care and Use Committees' (IACUC) guidelines. All euthanized rabbits were submitted to a second laparotomy for the purpose of evaluating both the peritoneal cavity and surgical site.

Fibrin sealant. Tachosil® is widely used for intraoperative hemostasis. It consists of an active yellow side with human thrombin (2.0 IU/cm²) and human fibrinogen (5.5 mg/cm²), as well as a supportive white side made of collagen. After thawing and

warming to 37°C, the two solutions were mixed and approximately 0.4 ml were applied directly on the defect.

Pathology analysis. Standard pathology methods and procedures were followed. Diagnosis was based on formalin fixed, paraffin-embedded hematoxylin and eosin-stained sections that were 4 µm thick.

Statistical analysis. Data were analyzed using SPSS v.25 (IBM, Chicago, IL, USA), with quantitative variables presented as absolute and relative frequencies. Chi-square tests were used for comparisons between the two groups. A *p*-value <0.05 was considered statistically significant.

Results

New Zealand rabbits were divided into two groups of 15 animals (group A – control, group B- Tachosil®). A total of two rabbits, one from each group, deceased on the 3rd and 4th postoperative day, respectively, due to abdominal sepsis from macroscopic leakage and peritonitis (*p*=1). In group A (control), an additional severe inflammatory reaction due to leakage was observed in two more animals (3 in total-20%), whereas in group B an intra-abdominal abscess due to leakage was detected in one rabbit (2 in total-13.3%) (Table I). There was no statistically significant difference regarding the presence of postoperative leakage between the two groups (*p*=0.62). The healing of the repaired defect was excellent in the animal that survived until the 15th postoperative day (n=14 and n=14, respectively).

Adhesions. Adhesions were evaluated using Zuhlke classifications (15); they were most commonly identified on the right upper quadrant of the abdomen. In group A (control), 46.6% of the animals had adhesions categorized as I-II according to the Zuhlke classification, with 26.6% classified as III and 20% as IV. Within group B (Tachosil®), 42.8% of rabbits showed adhesions categorized as I-II, with 21.5% classified as III, 0% as IV, and no adhesions were identified in 5 animals (Table II). There was a statistically significant difference regarding the presence of adhesions between the two groups (*p*=0.014), however, there was no statistically significant difference related to Zuhlke's classification.

Histopathological evaluation. Inflammation, revascularization and fibroblasts were present in the samples of both groups. Mucosal and serosal continuity was normal in all surviving models. (Figures 3 and 4).

Discussion

A significant number of trials have been conducted regarding the sealing of GI anastomoses, particularly in colonic anastomoses and duodenal trauma related defects (16, 17). Most of these trials have been experimental and suggest

Table I. *Complication rates in each group.*

	Group A (control)	Group B (Tachosil)	<i>p</i> -Value*
Mortality	1/15 (6.7%)	1/15 (6.7%)	1
Leakage	3/15 (20%)	2/15 (13.3%)	0.62

*Chi-square test.

Table II. *Macroscopic adhesion classification according to Zuhlke.*

	Group A (control)	Group B (Tachosil)	<i>p</i> -Value*
No adhesions	0/15 (0%)	5/15 (35.7%)	0.014
I – II Zuhlke	7/15 (46.6%)	6/15 (40%)	0.71
III Zuhlke	4/15 (26.6%)	3/15 (20%)	0.66
IV Zuhlke	3/15 (20%)	0/15 (0%)	0.06

Bold value indicates statistical significance.

sealing with different materials such as omental flaps, meshes, amniotic membranes or small intestinal submucosa (18-20). A few human studies have also been performed with conflicting results (11-12).

A study by Karagoz-Avci *et al.* (21) compared classic primary surgical repair and suture-less repairing techniques utilizing fibrin glue and a polyethylene–glycol hydrogel material, used as an adjunct to standard methods of dural repair (Duraseal®). The authors evaluated both the pathological and histological findings, measuring the bursting pressure of the repaired area by insufflating air. The results were similar between the groups and no harmful effects were observed when suture-less techniques were used (21).

Saygun *et al.* (22) evaluated the reinforcing ability of Eptfe grafts with histoacryl glue in an experimental model of duodenal trauma. No leakages, abscesses or deep surgical site infections were found in the Eptfe-HAG group, and the integrity of the graft into the intestinal serosa remained still strong ten days after the repair (22). Another experimental study evaluated the use of human amniotic membrane (HAM) in repairing duodenal perforation in animal models (23); this study revealed better histological results compared with simple suturing, but not significantly improved surgical findings (23).

Furthermore, Hoepfner *et al.* (24) examined the use of small intestinal submucosa (SIS) in sealing staples of colonic anastomoses in a porcine model. No significant differences were found regarding complications or adverse effects. The same research team also applied SIS on a colonic anastomosis of a rodent model and detected significantly improved anastomotic healing, with no significant complications in the study group (25).

Figure 3. Histopathology image of the defect healing in the control group.

Figure 4. Histopathology image of the defect healing in the intervention group.

A few studies have been published related to the use of Tachosil® for the sealing of colorectal anastomoses (12-14). Poomergaard *et al.* conducted a study with technically insufficient mice colon anastomoses and found a significantly lower leakage rate in the group where Tachosil® was applied (26). However, most of the studies were experimental and conducted on animal models, with only one clinical study using Tachosil® in 24 patients (12). Additional clinical studies, especially randomized clinical studies in humans should be performed to investigate and evaluate the safety and efficacy of this material.

On the other hand, there has been only one study that evaluated the use of Tachosil® on upper GI anastomoses or defects (27). Application of the fibrin coated patch on esophageal anastomoses was found to be feasible on both esophagogastrostomy and esophagojejunostomy.

Animal models are valuable for evaluating pathophysiological mechanisms and may be used to test measures, which aim to prevent the risk of leakage, such as external anastomotic coating. This study presents our initial experience with intraoperative topical application of Tachosil® on perforated gastric ulcers, where our primary goal was to prove that its application is technically feasible. This goal was proven in all experimental animals, and highly promising results regarding leakages and no severe adverse events such as adhesion formation or stenosis were obtained.

An important result of the present study is the fact that the Tachosil® group showed fewer adhesions than the control group. Similar results were reported by two experimental studies where Tachosil® reduced postoperative adhesions following laparoscopic surgery on a rat model and did not provoke adhesion formation in a pig model (28, 29).

The results of the present study demonstrate that while Tachosil® cannot replace conventional surgical techniques, it can play a supportive role in reinforcing primary suturing of upper GI deficits in patients at risk for postoperative leakage. Specifically, when risk factors including old age, obesity, malnutrition, severe comorbidities (particularly diabetes), prolonged surgery time, perioperative blood transfusions and chemoradiotherapy are present, surgeons must consider the use of Tachosil® as a reinforcement preventing leakages.

Before the appraisal of our results, several limitations must be considered. This is an experimental study with a small number of animals; thus, the generalization of results is limited. To our current knowledge, this is the first study demonstrating the application of Tachosil® for upper GI perforations. Additionally, all procedures were performed by the same team which reduced heterogeneity of the study.

In conclusion, a collagen patch coated with fibrin glue is not a replacement but can be considered a safe option for the reinforcement of suturing, preventing gastric leakage in cases of stomach perforations and leaks in anastomoses located in the upper GI tract.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

All Authors have contributed equally to the study design, conduction of the experiments, and drafting and revising the manuscript.

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