Background and study aims: Secure transluminal closure is the most fundamental prerequisite for the safe introduction of natural orifice transluminal endoscopic surgery. The aim was to compare acute strength of various gastrostomy closure techniques in an in vitro porcine stomach model by assessing leak pressures.

Methods: Standardized gastrotomies were closed manually, without the use of an endoscope, by one of seven NOTES closure devices: (i) T tags, (ii) purse string modified T tags, (iii) Eagle Claw VIII, (iv) Resolution clips, (v) flexible stapler, (vi) purse string suturing device, and (vii) flexible Endostitch. After closure, each specimen was fixed on the experimental apparatus and the pressure could be determined in detail. We began by collecting gold standard reference values, by testing 15 gastrotomies by a transvaginal approach. We then compared the results of the transvaginal group with the results of the same gastrotomies fixed on the experimental apparatus and tested by an endoscopic approach.

Results: The Resolution clips (P < 0.001), Eagle Claw VIII (P = 0.0325), flexible stapler (P < 0.001), and flexible Endostitch (P = 0.002) produced a noninferior closure in comparison with the predetermined gold standard. T tags (P > 0.6775), purse string modified T tags (P > 0.999), and the purse string suturing device (P = 0.9975) resulted in inferior closures.

Conclusions: The Eagle Claw VIII, Resolution clips, flexible stapler and flexible Endostitch produced noninferior closures in comparison with surgical closure in this model. These techniques seem to be the prime candidates for further testing in animal experiments before human trials can be initiated.

Methods

Whole stomachs were harvested from freshly slaughtered adult pigs weighing

In vitro comparison and evaluation of seven gastric closure modalities for natural orifice transluminal endoscopic surgery (NOTES)

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50 - 70 kg. All experiments were performed on the day of harvesting without freezing of the specimen. The porcine stomachs were cut along the greater and lesser curvature, providing anterior and posterior specimens. A small central incision (2 mm) was created to allow access. The gastric opening was then dilated in a standardized fashion using a 15 - 18 mm dilating balloon (CRE balloon, Boston Scientific, Natick, Massachusetts, USA). The balloon was inflated to a pressure of 7 atmospheres, matching an outer diameter of 18 mm, for exactly 60 seconds and was then deflated.

Closure

Once the gastrotomy had been created and dilated, it was closed using one of the modalities listed below. Closures were performed by or under the supervision of an experienced therapeutic endoscopist (P.F.) or senior surgeon (M.vB.H.). Closure was performed manually on a bench, without the use of an endoscope (except for the Eagle Claw VIII device). This was done to create an optimal closure with each of the tested devices. Closure was achieved by placing sutures, clips, or staples until the gastrotomy appeared to be adequately closed at macroscopic inspection. The eight closure modalities comprised:

1. Hand-sewn interrupted suture with 3.0 polidoxane (PDS) II (Ethicon Endo-Surgery, Cincinnati, Ohio, USA). The surgical suture was considered to be the gold standard.
2. Resolution clips (Boston Scientific) (Fig. 1 a)). Nowadays, endoscopic clips are routinely used within the lumen of the gastrointestinal tract, mainly for the treatment of nonvariceal gastrointestinal bleeding. In many NOTES-related reports, endoclip has been used for endoscopic closure [5][6][11].
3. T tags (Ethicon Endo-Surgery) (Fig. 1 b)). This closure technique has frequently been applied in porcine NOTES experiments [10][19][20][21]. With this technique, a metal T bar and thread are loaded into a 19-gauge hollow needle, which is passed through the tissue a few millimetres to the side of the defect. After the anchor has been ejected beyond the wall, another T tag is placed on the opposite side of the incision. Subsequently the threads are tied together with a locking cinch.
4. Pursestring modified T tags (Cook Endoscopy, Winston-Salem, North Carolina, USA) (Fig. 1 c)). The modification of these 'purse string' T tags consists of an additional metal ring at the midpoint of the T bar. This loop allows four such fasteners to be deployed sequentially on the same suture, arrayed in a square around a gastronomic site [22].
5. Eagle Claw VIII (Olympus Corporation, Tokyo, Japan) (Fig. 1 d)). The Eagle Claw VIII device is attached to the tip of the endoscope. The device has two opposing jaws that move simultaneously, one fixing the target tissue and the other holding a curved needle to deliver the suture through the tissue. The penetrating needle tip is attached to the end of the curved needle and can detach and lock into the suture unit cartridge once the jaws are closed and the curved needle has penetrated the target tissue [24].
6. Flexible stapler (Power Medical Interventions, Langhorne, Pennsylvania, USA) (Fig. 1 e)). The flexible stapler is a computer-mediated, electromechanically powered, cutting and stapling device delivered on a flexible working shaft. Staples were 1.7 mm in height and 75 mm in length, and were partially deployed [25].
7. Purse string suturing device (LSI solutions, Victor, NY, USA) (Fig. 1 f)). This prototype device deploys a purse string suture. It utilizes vacuum suction to draw the gastric wall into a small chamber in which a 3-mm cutting blade can also be used to create an incision. After the sutures have been deployed, another device is used to tighten and secure the purse string with a titanium knot [26].
8. Flexible Endostitch (Covidien, North Haven, Connecticut, USA) (Fig. 1 g)). The jaws of the flexible Endostitch hold a double-ended, sharp, taperpointed needle that has a suture swaged at its midsection. Opening and closing the jaws toggles the needle back and forth with the needle passing through tissue. The running sutures were secured without tying knots, using a prototype 3 - 0 barbed monofilament suture. The key element of the suture is that the barbs engage with the tissue, negating the difficulty of endoscopic knot.

Leak testing

After closure, the gastric explants were fixed between two equal-sized aluminium rings and clamped onto the experimental apparatus (Fig. 2) with the mucosa facing down, creating an air-tight space within the jar. The pressure in this air-tight space was then raised in a controlled standardized manner, using a constant flow of water into the glass jar below the specimen. As the volume of the water increased, the pressure of the air trapped within this base was gradually raised. A standardized layer of water on top of the gastric explants showed air bubbles caused by leakage after pressurizing.

A pressure gauge (AE Sensors BV, type ATM 1 - 6 bar) was connected to the experimental apparatus and to a personal computer. The experiments were monitored using a system consisting of two camera views, one from above and one from beneath the fixed specimen, which recorded in synchrony with the analogue pressure data (VisionDAQ Video System). The three sources of information were displayed in one interface (Fig. 3). Video 1). Leakage was defined as the first appearance of air bubbles. After clear leakage the experiment was stopped and the data were saved. The combination of the pressure gauge with the two cameras meant that the
leak pressure and location of the leakage were determined with great accuracy when the recorded data were reviewed.

**Fig. 2** Experimental apparatus, with glass upper section (A), clamp (B), glass jar (D), three junctions (C, E, and F) and aluminum rings (F).

**Fig. 3** Digital interface showing two camera views and pressure curve. The fixed specimen with the closed gastrotomy is seen from below (left upper image) and from above (right upper image). Air bubbles can be seen at the site of the closed gastrotomy. The leak pressure can be read from the graph.

**Fig. 4** Closed incisions using: a) Resolution clips; b) T-tags; c) purse string modified T tag; d) Eagle Claw VIII; e) flexible endoscopic stapler; f) purse string suturing device; g) flexible Endostitch.

Outcome parameters and statistics

The primary outcome parameter was the leak pressure of closed gastrotomies. The secondary outcome parameter was leak location. All calculations were carried out using the Statistical Package for Social Sciences for Windows 12.0.1 software (SPSS Inc, Chicago, Illinois, USA). Data were normally distributed and expressed as mean with standard deviation (SD). Statistical significance was assessed using the Student's t test.

In the initial phase of this study we collected gold standard values by testing 15 gastrotomies, closed with interrupted surgical sutures with 3-0 PDS II. These showed a mean leak pressure of 206 mmHg (SD 59). Using a noninferiority design, with a margin of equivalence of -56, a power of 80 %, and alpha value of 0.05, a sample size of 11 specimens for each closure technique was determined for the second phase of our study. The data were drawn from populations with standard deviations of 59 and 45. The margin of equivalence was based on data showing peak gastric pressures between 125 and 180 mmHg during coughing and vomiting [26][27].

**Fig. 5** Mean leak pressures for each closure modality with 90 % confidence intervals (CIs).

Results

All 77 incisions were successfully closed macroscopically with one of the seven different endoscopic techniques ([Fig. 4 a - g]). The results showed no significant difference in leak pressures between the anterior and posterior stomach walls (P = 0.152).

The mean pressures for air leakage from the gastrotomies closed using the different devices were as follows: T tags, 138 mmHg (SD 68); purse string T tags, 73 mmHg (SD 14); Eagle Claw VIII, 187 mmHg (SD 56); Resolution clips, 202 mmHg (SD 68); flexible stapler, 244 mmHg (SD 55); purse string suturing device, 102 mmHg (SD 32); and flexible Endostitch, 231 mmHg (SD 71). The mean gastrotomy leak pressures with 90 % confidence intervals (CIs) are shown in [Fig. 5]. The one-tailed Student's t test, comparing each individual group with the gold standard minus margin of equivalence, showed noninferiority closure using the Eagle Claw VIII (P = 0.0325), Resolution clips (P = 0.0285), flexible Endostitch (P = 0.002), and flexible stapler (P < 0.001), and inferiority of gastrotomy closure using T tags (P = 0.6775), purse string T tags (P > 0.999), and the purse string suturing device (P = 0.9875).

After leak testing, each gastric closure and corresponding video was examined to assess the breach site. In more than half of the experiments (54 %) leakages occurred on the incision itself. In the majority of the experiments where
pressures above 300 mmHg were reached, rupture of the gastric wall occurred elsewhere before the gastrotomy closure became disrupted.

It should not be neglected that some of the leaks (16 %) occurred through needle holes and not between the sutures. Leakage through the needle holes occurred in 4/11 (36 %) of experiments using the T tags, in 3/11 (27 %) cases with the Eagle Claw VIII, in 3/11 (27 %) with the Endostitch, and in 2/11 (18 %) using the purse string T tags. All the gastrotomies closed with the purse string suturing device or Resolution clips leaked at the incision itself. In the case of the flexible stapler, all leakages occurred at the incision itself or through a rupture of gastric wall elsewhere.

Discussion

Since the first report on NOTES in 2004, the closure of the gastrostomy has remained one of the main challenges in the development of this novel technique. Several methods of gastrotomy closure have been described in small porcine series, with varying success [21,23,24,25,26,27]. However, no particular technique has demonstrated proven efficacy or has been shown to be more reliable than other closure modalities. This lack of data could ultimately hamper the progression of NOTES.

This report is the first to describe a comparison of a wide variety of NOTES gastric closure modalities. An in vitro model was chosen, because it is the most efficient and ethical method for the primary evaluation and comparison of novel techniques. It also helps the selection of only the most promising for in vivo evaluation. Moreover, detailed in vitro evaluation of the closure technique itself seems most sensible in this emergent stage of development of NOTES. By applying the closure manually in an in vitro model, optimal conditions were created to evaluate the technique itself and other variables, such as in vivo delivery problems of the technique, were avoided as much as possible.

Surprisingly, apart from the study of Ivu et al. [23], an in vitro phase of endoscopic gastric closure techniques for NOTES purposes has not been previously reported. The study of Ivu et al. included only two endoscopic techniques and was underpowered for detection of a significant difference. In the present study most of the currently used endoscopic closure modalities were tested and repeated in order to ensure adequate power.

The results of this study indicate that closing the gastric incision up to predetermined gold standard leak pressures can be achieved with endoscopic techniques. The closure techniques included can be classified into three different categories (Fig. 6). First, the gold standard surgical sutures provide a parallel closure of the gastric wall layers, including the serosa ([Fig. 6 a]). The endoscopic devices that mimic a surgical suture (Eagle Claw VIII and Endostitch) also produced parallel closures. Closure of the gastrotomy using the flexible stapler and Resolution clips was intended to result in inverted closure and possibly yield stronger closures. If these sutures could be tightened from the peritoneal side, this maneuver in vivo during a NOTES procedure in the small, confined space of the stomach. Additionally, in an in vivo model a small-caliber endoscope will be needed to provide vision. Similar drawbacks can be stated regarding the Eagle Claw device.

The superiority of inverted, serosa to serosa apposition, in comparison with everted, mucosa to mucosa closure of the gastrostomy would also be expected as regards long-term reliability. Namely, everted, mucosa to mucosa suturing does not induce permanent fusion between the joined gastric folds [28]. However, serosal rather than mucosal juxtaposition induces local ischemia, foreign body reaction, and permanent contact between serosal surfaces by fibrous adherence [29,30,31]. Resolution clips rarely provided serosa to serosa apposition but demonstrated a surprisingly strong closure under optimal in vitro conditions. However, in an in vivo model with widely spaced incision edges and tissue edema, closing the incision with standard Resolution clips could prove to be challenging [8]. Even if the Resolution clips could be placed successfully, permanent fusion might be jeopardized due to lack of serosal juxtaposition and due to the risk of dislocation of endoclips in the early phase of healing.

T tags, purse string modified T tags, and the purse string suturing device effected everted closures, with mucosa to mucosa apposition ([Fig. 6 c]). The Resolution clips, Eagle Claw VIII, flexible Endostitch, and flexible stapler devices produced closures which were noninferior compared with the predetermined gold standard. Apart from the Resolution clips, the results of this study indicated the superiority of full thickness, serosa to serosa closure with either parallel or inverted wall layers. The flexible stapler and Endostitch devices yielded the strongest closures. Furthermore, both endoscopic devices were relatively easy to use in vitro and the stapler, especially, produced a fast and adequate closure. The use of the barbed suture in combination with the Endostitch appears to be promising, because the combination of these devices avoids the challenge of endoscopic knot tying. However, both the Endostitch and stapler are still relatively large in diameter and may prove difficult to maneuver in vivo during a NOTES procedure in the small, confined space of the stomach.

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T tags, purse string modified T tags, and the purse string suturing device produced closures that were significantly inferior to the predetermined gold standard. All three techniques were designed to produce full thickness sutures. However, with the sutures being tightened from the mucosal side without a suture crossing the incision at the serosal side, all three techniques resulted in mucosa to mucosa juxtaposition ([Fig. 6 c]). Besides the lack of serosal juxtaposition, the relatively low leak pressures could also be caused by the resulting everted closure acting as a valve. The cone shape of the everted suture converges the pressure to a central point which allows relatively early leakage. If these sutures could be tightened from the serosal side, this would result in inverted closure and possibly yield stronger closures.
apposition force between each anchor pair, thereby encouraging a "cheese-cutting" or tissue pull-through effect [31]. Another important drawback of the in vivo use of T tags, which was obviously not evaluated in this in vitro model, is the blind puncture through the gut wall. This is potentially a dangerous technique, because it may lead to injury of adjacent organs [20][24]. Closure with the purse string modified T tags may also be complicated by the "cheese-cutting" effect if higher pressures are reached. The failure may be explained by the difficulty in pulling both ends of the suture tight enough to ensure secure closure. The porcine tissue appeared to be too rigid to allow proper lac ing. In experiments using the automated purse string suturing device, the tissue tore at the end of the incision in 8 out of the 11 closed gastrotomies. The relatively long unidirectional purse string closure held in a rigid model might have led to a stress concentration in the direction of the shortened stomach wall. Decreasing the length of the purse string might lead to higher leak pressures. However, the lack of sersal juxtaposition will then still be a potential limitation. In this study the gold standard was the leak pressure of surgically closed gastrotomies minus a margin of equivalence that was based on peak gastric pressures during coughing and vomiting [28][29]. However, it may be the case that any method that produces a closure strength in vivo above a certain critical leak pressure threshold will be sufficient for maintaining gastric wall integrity. Nevertheless, in this nascent stage of NOTES, the goal of maximizing the strength of the gastrotomy closure seems most appropriate. It should also be noted that none of the four "noninferior" techniques was leakproof in all 11 samples; although the model was testing the extreme, ideally there should be no leak after any closure technique before it is applied in humans. As already mentioned, adequate closure using the tested techniques may be challenging and potentially result in a different outcome when applied in vivo rather than in the optimal conditions of this in vitro model. Secondly, acute strength may not be an absolute predictor for long-term reliable closure; for example the influence of blood supply and ischemia on midterm leakage was obviously not assessed. Survival experiments are necessary to determine accurately the reliability of the most promising techniques. A final issue is to what extent fresh, but dead tissue can be compared with living tissue and to what extent the porcine and human stomach are comparable. For example, the wall of the porcine stomach is thicker than that of the human stomach. In conclusion, closure of gastric incisions up to predetermined gold standard leak pressures proved to be attainable with resolution clips, the Eagle Claw VIII, the flexible Endostitch, and the flexible stapler. The flexible Endostitch and flexible stapler yielded the strongest closures. In vivo acute and chronic animal experiments with adequate statistical power are needed to further evaluate the most secure gastric closure modalities. Only the best methods emerging from these studies should be considered for human indications. 

**Competing interests:** None

**References**


