Int J Clin Exp Med 2014;7(5):1255-1261
www.ijcem.com /ISSN:1940-5901/IJCEM0000401

Original Article

A novel sutureless colonic anastomosis with self-gripping mesh: an experimental model

Gokhan Cipe[1], Fatma Umit Malya[1], Mustafa Hasbahceci[1], Pinar Atukeren[2], Nur Buyukpinarbasili[3], Oğuzhan Karatepe[1], Mahmut Muslumanoglu[1]

[1]Department of General Surgery, Faculty of Medicine, Bezmialem Vakif University, Vatan Street, Fatih, 34093 Istanbul, Turkey; [2]Department of Biochemistry, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey; [3]Department of General Pathology, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

Received April 1, 2014; Accepted May 20, 2014; Epub May 15, 2014; Published May 30, 2014

Abstract: Introduction: Anastomotic leakage is one of the most feared complications of colorectal surgery. High morbidity and mortality rates are related to this complication and several studies had been performed to test new techniques which are suggested to reduce leakage rates. The aim of our study was to evaluate the safety and effectiveness of a novel technique sutureless anastomosis with self-gripping mesh in an animal model by examining wound healing process in anastomosis. Methods: In this study sixteen Norwegian Wistar Albino female rats were used. The rats' weights ranged from 250 to 300 g. The rats were divided into control and study groups. The control group underwent a colocolic anastomosis using the conventional method of hand-sewing with single-layer interrupted nonabsorbable sutures. The study group underwent a colocolic anastomosis using self-gripping mesh without sutures. These rats were sacrificed on the 10th postoperative day. The sample pieces obtained from the groups were subjected to anastomotic bursting pressure tests, to a test for hydroxyproline levels in the tissue and to histopathological examinations. The tissue was evaluated in terms of quantity of inflammatory cells, fibroblasts, neovascularization level and collagen content and classified according to the Ehrlich-Hunt model. Statistical analysis was done by using Mann-Whitney U test. Results: The burst pressure mean ± range of control and study groups were 162 ± 78 and 123 ± 35, respectively (P = 0.049). The mean peritoneal adhesion grades were 3.2 ± 0.7 in the study group and 2.3 ± 0.7 in the control group (P = 0.036). The operative time was significantly shorter in the study group. The difference between the groups by mean of hydroxyproline levels was found to be significant (P = 0.001). According to histopathological examinations by means of the Ehrlich-Hunt model, the fibroblast activation and collagen fiber ratio were higher in the study group and the difference between these measurements was statistically significant (P = 0.006; P = 0.028). Conclusion: This study showed that use of self-gripping meshes for colocolic anastomosis in rats is a safe and feasible method. It is suggested that the most important advantage of this technique is the shorter operative time.

Keywords: Colonic anastomosis, mesh, experimental model

Introduction

Anastomotic leakage following colorectal surgery is a significant cause of morbidity and mortality. The incidence of reported anastomotic leakage varies between 6.4 and 11.6% [1, 2].

Sepsis associated with anastomotic leakage constitutes more than 50% of postoperative deaths in colorectal surgery [3]. For this reason, many types of anastomotic techniques have been described.

Ideal anastomosis should be associated with less complication, less cost and short procedure time. Currently, the use of staplers for anastomoses has been growing and is becoming commonly accepted in most countries because of the short procedure time [4]. But, hand-sutured anastomoses are still commonly used due to its cost-effectivity.

Recently, self-gripping meshes have been introduced, and used the groin hernia repair. The current studies conclude that, the use of the self-gripping mesh for hernioplasties offers a potential benefit in short and medium-term period [5].

The aim of the present study was to evaluate the safety and effectiveness of a novel technique sutureless anastomosis with self-gripping

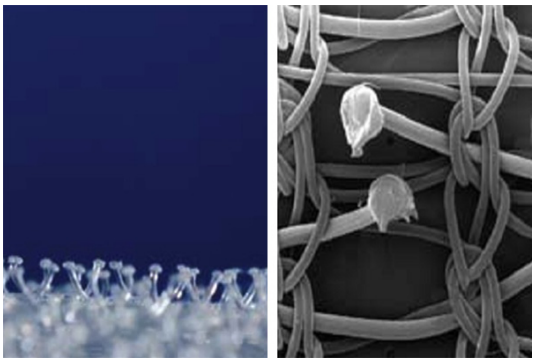

Figure 1. Appearance of self-gripping mesh.

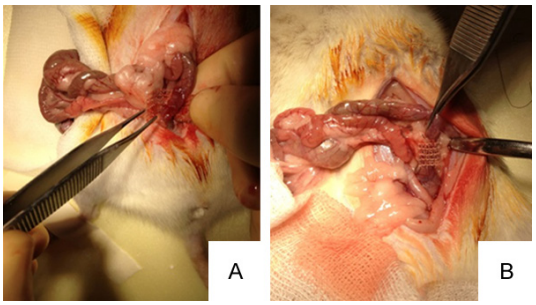

Figure 2. A and B: Sutureless anastomosis with self-adherent mesh.

mesh versus hand-sutured anastomosis in an animal model by comparison of wound healing between these two styles of anastomoses.

Materials and methods

This study was conducted at the Experimental Research and Animal Laboratory at the Research Center of Bezmialem Vakif University. This study had been approved by the Bezmialem Vakif University Animal Research Ethics Committee (Number: BAV.27.11.2011/60).

Self-adherent mesh

Parietex ProGrip™ Mesh (monofilament polyester mesh, Covidien Commercial Ltd., UK) is the self-gripping, semi-resorbable, lightweight mesh available for use of inguinal hernia repair [6, 7]. Parietex ProGrip™ Mesh is the bicomponent mesh comprised of monofilament polyester and a resorbable polylactic acid gripping system (**Figure 1**). It has been showed that hydrophilic polyester monofilament results in fast and intimate tissue in-growth. Polyester's large pore size and monofilament fiber composition works with the body's natural systems to improve tissue response and reduce foreign material reaction [8].

Design of the study

Sixteen Norwegian Wistar Albino female rats were used in this study. The rats' average age was 3 months and their weights ranged from 250 to 300 g. The bowel preparation was not used. The rats were divided into two equal groups - a control group and an experimental group. The control group consisted of 8 of the 16 rats that underwent a colocolic anastomosis using the conventional method of hand-sewing with single-layer interrupted nonabsorbable sutures. The rats of control group were sacrificed on the postoperative 10th day. Tissue bursting pressure test was conducted on sample pieces of the anastomotic lines of all rats. Subsequently, these materials were sent to the biochemistry laboratory for measurement of hydroxy-proline levels and to the pathology laboratory for measurement of fibroblastic activity, inflammation, neovascularization and collagen levels. The similar procedure was performed for the other 8 rats in the experimental group which underwent a colocolic anastomosis using self-gripping mesh without sutures. These rats were sacrificed on the 10th postoperative day. The values achieved were statistically compared with values gained for the control group for each parameter to determine any significant differences between the control group and the experimental group.

Experiment procedure

The animals were administered a sugared water diet and left without water for the last hour. General anaesthesia was administered using 100 mg/kg ketamine (Ketalar®, Parke-Davis & Co., Detroit, Michigan, USA) and 10 mg/kg xylazine (Rompun®, Bayer Ag, Leverkusen, Germany). After shaving the operation site, antisepsis was administered using povidone iodine. A midline incision was performed and 1 cm descending colon was excised and colocolonic single-layer anastomosis was performed with 3/0 polypropylene suture (Prolene®, Kurtsan A.S.). Anastomoses performed on the 8 rats in the experimental group consisted of the application of self-gripping mesh starting from the mesenteric border of the two colon ends on which anastomosis was to be performed so as to completely cover the anas-

Table 1. Comparison of two groups in terms of bursting pressure, adhesion score, operation time and hydroxyproline levels

	Control group	Study group	*P* value
Bursting pressure (mmHg)	162 ± 78	123 ± 35	0.172
Adhesion score	2.3 ± 0.7	3.2 ± 0.7	0.036
Operation time (min)	16 ± 4	10 ± 2	0.003
Hydroxyproline levels	0.76 ± 0.1	1.3 ± 0.2	0.001

Figure 3. Appearance of anastomotic leakage in the control group.

tomotic line (**Figure 2A** and **2B**). Then, the abdomen was closed by suturing the fascia and skin separately with 3/0 silk sutures.

Animal was housed 21°C with a 12 h Day-Night cycle. They had free access water and standard laboratory chow.

The subjects in study and control groups were re-operated on postoperative 10th day under a general anesthesia and the anastomotic colonic segments were removed by resection so that 2 cm of healthy colon. Afterwards, the rats were killed by overdose anesthesia.

The sample pieces obtained from the groups were initially subjected to anastomotic bursting pressure tests followed by preparation of 1 g specimens containing the anastomotic segment stored in a -70°C in a freezer for biochemical measurement of hydroxyproline levels. 1-2 cm specimens containing the anastomotic line were prepared and stored in a 40% formaldehyde solution at a temperature of +4°C for histopathological examinations.

Anastomotic bursting pressure

The distal ends of all resected anastomotic colon pieces were securely tied using 2/0 silk suture. A polyethylene catheter was placed into the lumen at the proximal end with the other end of the catheter attached to a transducer and an air pump. A setup required to view the intraluminal pressure in millimeters of mercury (mmHg) was thus achieved. The anastomotic colonic segment was put into a bowl filled with water. Air was blown into the colonic lumen at an insufflation rate of 2 ml/min. The first air leakage from the anastomotic line was documented as the anastomotic bursting pressure.

Hydroxyproline determination

The colonic segment including the anastomosis was excised and the specimen were kept at -70°C in a freezer until assay. The samples were weighed and homogenized in serum physiologic solution (1/10, w/v) using a tissue grinder fitted with a Teflon pestle (Heidolph- RZR 2021, Germany). Homogenates were centrifuged at 1500 rpm for 15 min and obtained supernatants were hydrolyzed by adding hydrochloric acid (37%) and incubating at ~100°C for 16-18 h. The amino acid is oxidized forming a pyrrole derivative, which is colored with Ehrlich's reagent and quantitatively determined spectrophotometrically at 560 nm using the hydroxyproline kit (Hypronosticon, Organon, Holland) and the results were interpreted as μg/mg wet tissue.

Histopathological examinations

Specimens were fixed in formaldehyde and embedded in paraffin wax. Sections were cut into 5 μm in thickness and stained with hematoxylin and eosin. Examples were histopathologically classified according to Ehrlich-Hunt model. The tissue was evaluated in terms of quantity of inflammatory cells, fibroblasts, neovascularization level and collagen content.

Statistics

All data were recorded on SPSS II for windows 11.0.1J program (IBM Japan, Tokyo, Japan) and were presented as means ± standard deviation. Statistical analyses were done by using

Table 2. Histopathologic evaluations of control and study groups

	Grades	Control group		Study group		
		n	%	n	%	
Inflammation	1	0	0	0	0	
	2	5	62.5	3	37.5	
	3	3	37.5	2	25	
	4	0	0	3	37.5	$P = 0.152$
Fibroblast activation	1	4	50	0	0	
	2	4	50	4	50	
	3	0	0	3	37.5	
	4	0	0	1	12.5	$P = 0.006$
Neovascularization	1	1	12.5	2	25	
	2	7	87.5	4	50	
	3	0	0	2	25	
	4	0	0	0	0	$P = 0.700$
Collagen deposition	1	5	62.5	1	12.5	
	2	3	37.5	5	62.5	
	3	0	0	2	25	
	4	0	0	0	0	$P = 0.028$

Mann-Whitney U test. A P value < 0.05 was considered to be statistically significant.

Results

The burst pressure mean ± range of control and study groups were 162 ± 78 and 123 ± 35, respectively. The results show a statistically significant difference between the groups (P = 0.049) (**Table 1**). The average measurements of burst pressure were higher in the control group. There was an anastomotic leakage in the control group (**Figure 3**) whereas no anastomotic leakage was observed in the study group. The mean peritoneal adhesion grades were 3.2 ± 0.7 in the study group and 2.3 ± 0.7 in the control group (P = 0.036) (**Table 1**). The operative time was significantly shorter in the study group. (16 ± 4 min; 10 ± 2 min; P = 0.003).

Values of hydroxyproline levels measured in tissue samples of the subjects obtained in study groups were higher than those obtained in control groups. The difference between the groups by mean of hydroxyproline levels was found to be significant (P = 0.001) (**Table 1**).

The histopathological findings were based on the Ehrlich-Hunt model, which measures the score of the regeneration of the anastomotic line via the inflammation, fibroblastic activity, neovascularization and collagen formation. These findings are shown in **Table 2**. Although, marked inflammation and fibroblastic proliferation were observed in study group (**Figure 4**), mild inflammation and fibrotic proliferation were observed in the control group (**Figure 5**). The difference between the groups in term of inflammation and neovascularization did not reach the statistically significant level (P = 0.152, P = 0.7). The fibroblast activation and collagen fiber ratio were higher in the study group and the difference between these measurements was statistically significant (P = 0.006; P = 0.028).

Discussion

Anastomotic leakage after colonic anastomosis is thought to be the most important complication causing morbidity and mortality in human beings [9]. For that reason, there have been many different methods or reinforcements used previously. Peritoneal and omental graft, dura mater and different types of synthetic meshes are important examples for this purpose [10, 11].

Besides the general use of meshes for reinforcement of abdominal wall during hernia surgery, they have been used for reinforcement of the gastrointestinal anastomoses in animal studies. Aysan et al showed that polypropylene mesh caused high anastomotic burst pressure in rabbit colonic anastomosis model [9, 11]. Although nonabsorbable meshes are thought to cause higher burst pressures due to permanent fixation to the serosal surfaces, they may also increase the risk of peritoneal adhesion and anastomotic stenosis. There were some studies using absorbable meshes for the reinforcement of the colonic anastomosis [9]. In these studies, it has been shown that anastomotic burst pressures were found to be lower than the control group. Therefore, nonabsorbable meshes were used in the present study.

Fixation of the meshes during reinforcement of colonic anastomosis is another controversial issue. In literature, there were many studies focusing on this. Some researchers used meshes to cover merely all anastomotic surfaces by

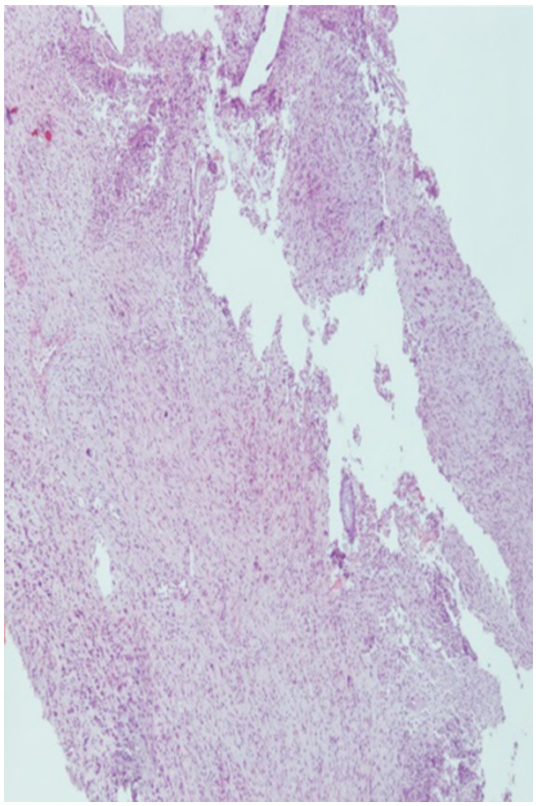

Figure 4. Marked inflammation and fibroblastic proliferation were observed in study group.

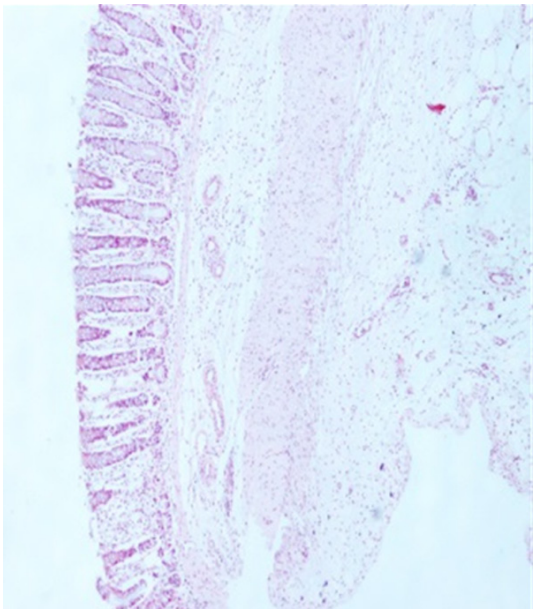

Figure 5. Mild inflammation and fibrotic proliferation were observed in the control group.

fixing them to the intestinal wall [12, 13]. However, others used the method in which fixation of the mesh over the anastomosis only by extended suture ends [9]. Such modifications were thought to be important to perform a dynamic anastomotic model for prevention of mesh migration caused by strong peristaltic movements.

Intestinal anastomoses can be divided into two broad groups as suture/stapling and sutureless techniques. An alternative to conventional hand-sewing colocolic anastomosis: anastomosis with absorbable surgical barrier film without sutures [14]. There have been a variety of many different suture/stapling techniques in which none of them were shown to be the best. In addition, there were also some different methods for sutureless anastomosis including some specific instruments and materials like biofragmentable anastomotic ring, polypropylene rings, magnetic rings and fibrin adhesive materials [15-20].

The present technique as a modification of sutureless anastomosis was shown to be an effective way to perform a safe and durable anastomosis. Lack of anastomotic stenosis which is the most commonly encountered problem with sutureless methods was another advantage of our technique [21].

Self-gripping, semi-resorbable, lightweight meshes have been used for repair of inguinal hernia [22]. As an example of such meshes, Parietex ProGrip has been used for the last several years. The main advantage of this mesh is to eliminate or reduce the need for additional fixation by sutures by the resorbable microgrips endowing the mesh with self-gripping properties and penetrating the underlying tissues. In the present study, these meshes were used to reach a dynamic anastomotic model.

There are some mechanical, biochemical and histological parameters used for the evaluation of intestinal healing. For assessment of the anastomotic strength, the bursting pressure and breaking strength are used. However, it is still controversial that which one is superior to other [23].

Bursting pressure reflecting the intestinal anastomotic resistance to an increase in intraluminal pressure was evaluated in the present study.

Correlation of the anastomotic strength and leakage is another important problem which could not be shown by many studies [9]. Besides the higher bursting pressures, more anastomotic leakages have been defined by some studies. Therefore, it may be believed that surgical technique with regard to perform an anastomosis is the most important step for both strength and safety [23, 24].

Although the histopathological scores including inflammation and neovascularization were the same for both groups; higher hydroxyproline levels, higher ratios of fibroblast activation and collagen fiber were appraised as the histopathological evidences for a secure anastomosis. It is normally expected to encounter higher inflammatory scores with use of foreign bodies like meshes [9]. However, our findings in the study group showed that there was no difference in the inflammatory scores. Some characteristic features of the meshes like being light weight and semi-resorbable may be important to get such results.

In conclusion, this study showed that use of self-gripping meshes for colocolic anastomosis in rats is a safe and feasible. Self-gripping meshes did not increase the bursting pressure. However, the most important advantage was shorter operating time. Lack of anastomotic leakage might be regarded as an evidence for the adequate strength of the anastomosis although the values were less than that of the control group.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Fatma Umit Malya, Department of General Surgery, Faculty of Medicine, Bezmialem Vakif University, Vatan Street, Fatih, 34093 Istanbul, Turkey. Tel: 00905327982333; E-mail: fumitm@gmail.com

References

[1] Krarup PM, Jorgensen LN, Andreasen AH, Harling H; Danish Colorectal Cancer Group. A nationwide study on anastomotic leakage after colonic cancer surgery. Colorectal Dis 2012; 10: e661-7.

[2] Peeters KC, Tollenaar RA, Marijnen CA, Klein Kranenbarg E, Steup WH, Wiggers T, Rutten HJ, van de Velde CJ. Risk factors for anastomotic failure after total mesorectal excision of rectal cancer. Br J Surg 2005; 92: 211-216.

[3] Beard JD, Nicholson ML, Sayers RD, Lloyd D, Everson NW. Intraoperative air testing of colorectal anastomoses: a prospective, randomized trial. Br J Surg 1990; 77: 1095-1097.

[4] Suturing or stapling in gastrointestinal surgery: a prospective randomized study. West of Scotland and Highland Anastomosis Study Group. Br J Surg 1991; 78: 337-341.

[5] Pedano N, Pastor C, Arredondo J, Poveda I, Ruiz J, Montón S, Molina M, Hernández-Lizoain JL. Open tension-free hernioplasty using a novel lightweight self-gripping mesh: medium-term experience from two institutions. Langenbecks Arch Surg 2012; 397: 291-5.

[6] Kapischke M, Schulze H, Caliebe A. Self-fixating mesh for the Lichtenstein procedure--a prestudy. Langenbecks Arch Surg 2010; 395: 317-22.

[7] Chastan P. Tension-free open hernia repair using an innovative self-gripping semi-resorbable mesh. Hernia 2009; 13: 137-42.

[8] Cobb WS, Kercher KW, Heniford BT. The argument for lightweight polypropylene mesh in hernia repair. Surg Innov 2005; 12: 63-9.

[9] Aysan E, Bektas H, Ersoz F, Sari S, Kaygusuz A. A novel colonic anastomosis technique involving fixed polyglycolic acid mesh. Int J Clin Exp Med 2010; 3: 341-346.

[10] Gulati SM, Thusoo TK, Kakar A, Iyenger B, Pandey KK. Comparative study of free omental, peritoneal, Dacron velour, and Marlex mesh reinforcement of large-bowel anastomosis: an experimental study. Dis Colon Rectum 1982; 25: 517-521.

[11] Aysan E, Dincel O, Bektas H, Alkan M. Polypropylene mesh covered colonic anastomosis. Results of a new anastomosis technique. Int J Surg 2008; 6: 224-229.

[12] Dilek ON, Bakir B, Dilek FH, Demirel H, Yiğit MF. Protection of intestinal anastomoses in septic environment with peritoneal graft and polyglycolic acid mesh: an experimental study. Acta Chir Belg 1996; 96: 261-265.

[13] Henne-Bruns D, Kreischer HP, Schmiegelow P. Reinforcement of colon anastomoses with polyglycolic acid mesh: an experimental study. Eur Surg Res 1990; 22: 224-230.

[14] Nejdet B, Ayhan C, Doğan F, Hüseyin E, Gülay D, Mustafa G, Nagehan B. An alternative to conventional hand-sewing colocolic anastomosis: anastomosis with absorbable surgical barrier film without sutures. Colorectal Dis 2010; 12: 1260-7.

[15] Hardy TG Jr, Pace WG, Maney JW, Katz AR, Kaganov AL. A biofragmentable ring for sutureless bowel anastomosis. An experimental study. Dis Colon Rectum 1985; 28: 484-90.

[16] Rosati R, Rebuffat C, Pezzuoli G. A new mechanical device circular compression anastomosis. Preliminary results of animal and clinical experimentation. Ann Surg 1988; 207: 245-52.
[17] Jansen A, Brummelkamp WH, Davies GA, Klopper PJ, Keeman JN. Clinical applications of magnetic rings in colonic anastomosis. Surg Gynecol Obstet 1981; 153: 537-45.
[18] Hjortrup A, Nordkild P, Kiaergaard J, Sjøntoft E, Olesen HP. Fibrin adhesive versus sutured anastomosis: a comparative intraindividual study in the small intestine of pigs. Br J Surg 1986; 73: 760-1.
[19] Wu Z, Vakalopoulos KA, Kroese LF, Boersema GS, Kleinrensink GJ, Jeekel J, Lange JF. Reducing Anastomotic Leakage by Reinforcement of Colorectal Anastomosis with Cyanoacrylate Glue. Eur Surg Res 2013; 50: 255-261.
[20] Vuocolo T, Haddad R, Edwards GA, Lyons RE, Liyou NE, Werkmeister JA, Ramshaw JA, Elvin CM. A highly elastic and adhesive gelatin tissue sealant for gastrointestinal surgery and colon anastomosis. J Gastrointest Surg 2012; 16: 744-52.
[21] Di Castro A, Fausto B, Brocato R, Adami EA, Truosolo B, Massi G. Intestinal anastomosis with the biofragmentable anastomosis ring. Am J Surg 1998; 176: 472-4.
[22] Birk D, Hess S, Garcia-Pardo C. Low recurrence rate and low chronic pain associated with inguinal hernia repair by laparoscopic placement of Parietex ProGrip™ mesh: clinical outcomes of 220 hernias with mean follow-up at 23 months. Hernia 2013; 17: 313-20.
[23] Durães Lde C, Durães EF, Lobato LF, Oliveira PG, Sousa JB. Correlation between bursting pressure and breaking strength in colonic anastomosis. Acta Cir Bras 2013; 28: 447-52.
[24] Tajima M, Kono Y, Ninomiya S, Amin NT, Inomata M, Shiraishi N, Kitano S. Safety and effectiveness of mechanical versus hand suturing of intestinal anastomoses in an animal model of peritonitis. Exp Ther Med 2012; 4: 211-215.