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Management of Postoperative Gastrointestinal Leakage With Autologous Stromal Vascular Fraction

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To assess the efficacy of using autologous stromal vascular fraction (SVF) to promote healing of controlled fistula tracts in the management of postoperative upper gastrointestinal leakage. This is an experimental study conducted on 10 experimental rabbits. Animal models were divided into the SVF group which received an autologous SVF and the control group which did not receive the implantation. Surgery was performed on both groups to induce a gastric leak and create a controlled fistula tract between the leakage site in the stomach and the skin. After 2 weeks, surgery was performed on the SVF group to harvest, process and then implant the autologous SVF in the fistula tract. Animal models were followed up and their fistula tracts were evaluated for healing by gross and microscopic examination of the fistula tracts before the SVF implantation and at 24 hours, 1 week, 2 weeks and 3 weeks after implantation. The control group revealed no closure of fistula tracts by the 3rd week after implantation and there were no signs of inflammation or drainage. On the other hand, the SVF group showed signs of healing process with progressive closure of the fistula tract to about 95% by the 3rd week after implantation. The use of autologous SVF implantation to promote

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the healing of controlled fistula tracts seems to be a novel, safe and effective method in the management of postoperative upper gastrointestinal leakage. It could prevent reoperation and reduce hospital stay, morbidity and mortality. These results are promising and provide support for further clinical studies.

Key words: Stromal vascular fraction – SVF – Adipose stem cells – Gastrointestinal leakage – Postoperative leakage

Gastrointestinal leakage is one of the most serious postoperative complications that leads to morbidity and mortality.[1] Several studies have reported leakage incidence after surgeries including esophageal surgeries (7%),[2] colorectal surgeries (8.7%),[3] pancreatic surgeries (0–25%),[4] laparoscopic sleeve gastrectomy (1.4–2.5%),[5] and Roux-en-Y gastric bypass surgeries (0.7–20%).[6] It poses a challenge for surgeons worldwide as it is difficult to manage, is usually associated with long hospital stay, and has high mortality rates that range from 4.8 to 75%.[7]

Management of postoperative upper gastrointestinal leakage is usually achieved by reoperation and primary closure of the leakage site, or conservatively by using drains or stents. Converting the leakage site to a controlled fistula in the management of upper gastrointestinal leakage is effective, but usually requires long hospital stay in order to achieve the closure of the leakage site.

Stem cells are undifferentiated cells that have the capacity to self-renew and the capability to differentiate into many types of cells.[8] There are two types of stem cells that are either embryonic or adult stem cells. The embryonic stem cells have ethical, immune, and source limitations. In contrast, adult stem cells have no such limitations as they are abundant in many body tissues such as bone marrow, adipose tissue, and blood. There is also no immune-rejection as they can be obtained from the same patient, they do not proliferate to tumors and are easy to harvest with local anesthesia.[9] Stem cells usually remain dormant until damage or injury occurs. In this case, they travel to the injured site, replicate, differentiate and start the process of healing.[10] Researchers have studied the adipose stem cells in form of expanded isolated cultured cells or as stromal vascular fraction and both forms are found to be effective.[11,12]

Recent studies showed high efficacy of autologous adipose stem cells and stromal vascular fraction in many different clinical applications and showed good outcomes in regenerative medicine and surgery. These applications include plastic reconstructive surgeries following resection (*e.g.*, mastectomy) or trauma and filling aging wrinkles.[13] They also have been used in treatment of lipodystrophy, repair of fractures, clavicular defects, traumatized joints, osteoarthritis, muscular dystrophies, myocardial infarctions, ischemic limbs, urinary incontinence, renal impairment, and healing of diabetic foot ulcers.[14] Furthermore, more recent clinical trials showed promising results in the healing of Crohn's fistulae.[15]

In our study, we evaluated the efficacy of using autologous stromal vascular fraction to promote the healing of controlled fistula tracts, which will helps in early closure of the fistula without the need of reoperation or using stents. This is a promising novel method that can be used in the management of postoperative upper gastrointestinal leakage.

Materials and Methods

This is an experimental laboratory study on 10 New Zealand rabbits that weighed 4 to 5 kg and their ages ranged from 6 to 10 months. The study was conducted at King Fahad Medical Research Center (KFMRC) in King Abdulaziz University, Jeddah, Saudi Arabia. All experimental protocols were approved by the local ethical committee of KFMRC.

The animal models were divided into 2 groups: the stromal vascular fraction (SVF) group (n = 5) that received the autologous adipose stem cell implantation and the control group (n = 5) that did not receive the implantation.

Creation of the fistulae

In the first stage of the study, both groups underwent the same operation to induce gastric leakage and to create a fistula-like tract from the leakage site in the stomach to the skin. This was performed under a completely sterile environment and general anesthesia (Ketamine 10%, IM, 35 mg/kg and xylozine 2%, IM, 5 mg/kg). The surgical site

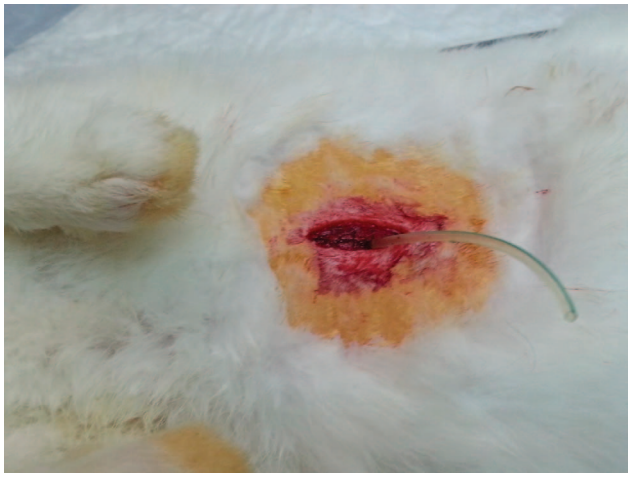

Fig. 1 The tube was inserted into the leakage site in the stomach and fixated.

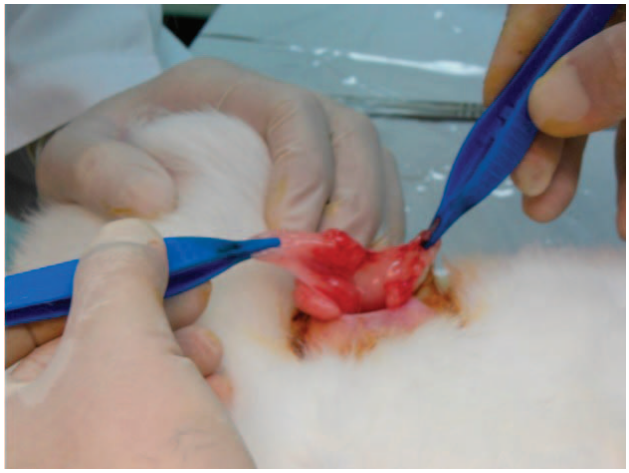

Fig. 2 Harvesting inguinal adipose tissue.

was shaved and sterilized with povidone iodine 10% wt/vol. An upper midline incision (2 cm), just inferior to the xyphoid process, was made in the skin and underlying muscles. The stomach was identified and a small gastric incision was created and left for 2 hours to form gastric leakage. A tube (8 Fr.) was then inserted through the gastric incision as a drain and was fixated (Fig. 1). The distal end was kept outside the body. The skin and muscles were then sutured around the tube with Vicryl sutures (3/0) and the site was dressed. A postoperative prophylactic dose of antibiotic was administered (enrofloxacin 5%, SC, 0.5 mL). The animal models in both groups were kept under observation for 2 weeks to allow formation of the fistula tract between the leakage site in the stomach and the skin.

Harvesting of adipose tissue and stromal vascular fraction implantation

After 2 weeks, we started the second stage in which the gastric tubes were removed from both groups and the stromal vascular fraction was implanted in the fistula tracts of the SVF group. The group with SVF underwent second surgery to harvest the adipose tissue from the inguinal pad, and process and implant it in the fistula tract. Again, the operation was performed under a completely sterilized environment and general anesthesia. Inguinal skin (right or left) was shaved and sterilized. A 2-cm incision was made, underlying adipose tissue was exposed, and about 2 g of the adipose tissue was harvested (Fig. 2). The surgical wound was then sutured and dressed. The adipose tissue was minced into small cubes and washed extensively with phosphate buffered saline. The mixture was centrifuged at 500 × g for 5 minutes. After that, the centrifuged sample was digested with 0.075% collagenase IA solution (Sigma-Aldrich, St. Louis, Missouri) at 37° on a shaker for 30 minutes then filtered with 100-μm Nylon mesh filter. It was centrifuged again and the stromal vascular fraction was obtained (Fig. 3). The gastric tubes were removed from both groups and biopsies were obtained from fistula tracts in animal models (1 from each group) to be examined by histopathology. In the control group, the fistula tract was left open; while in the SVF group, the stromal vascular fraction were implanted into the fistula tract (Fig. 4).

Fig. 3 Processing adipose tissue.

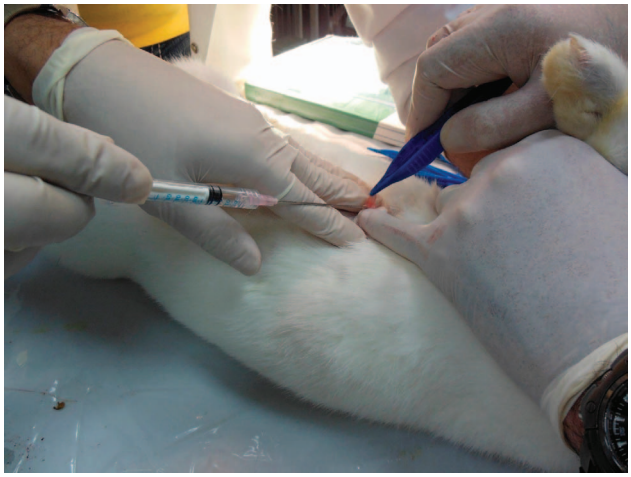

Fig. 4 Implanting the adipose stem cells in the fistula tract.

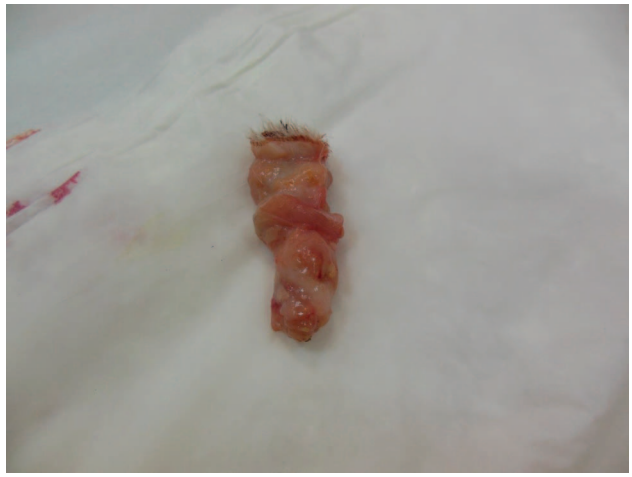

Fig. 5 Excised fistula tract for histopathology examination.

Evaluation of healing (gross and microscopic examination of the fistulae)

Animal models were followed up and their fistula tracts were evaluated for healing by gross examination of the external opening for closure; drainage; and signs of inflammation (redness, hotness, and swelling), and by microscopic examination of biopsies that were obtained from fistula tracts in both groups for closure and types of cells that were found at 24 hours and 1, 2, and 3 weeks after implantation (Fig. 5). The closure percentage was calculated by comparing the surface area of sections in the SVF group with sections in the control group (sections have the same sizes as the inserted tubes had the same size in all animals) at same time point. The surface area of sections was also compared in SVF group at the different time points.

Specimens were fixed at 10% neutral-buffered formalin for at least 24 hours. Tissues were dehydrated in graded alcohols, cleared with xylene, and infiltrated and embedded in paraffin. Tissues embedded in paraffin were cut at 4 micrometers and mounted on glass slides. Sections were stained with hematoxylin and eosin and examined under a light microscope.

Statistical Analysis

The data were analyzed using statistical software (SPSS, version 20.00, SPSS, Inc, Chicago, Illinois). We calculated the percentages of animal models that showed closure, drainage, and signs of inflammation (gross and microscopic) and the average percentages of microscopic closure at 24 hours, and 1, 2, and 3 weeks after implantation.

Results

In the creation of fistulae stage, the procedure was successfully performed for all 10 animal models and was well tolerated. Mean duration of the procedure was 38 ± 6.4 minutes (range, 25–45 minutes) and there were no intra- or postoperative complications.

In the second stage, all gastric tubes were removed easily and the fistula tracts were palpable in the control group and the SVF group. The adipose tissues were harvested, processed, and implanted successfully into the fistula tracts of the SVF group. The procedure duration was about 90 minutes for the whole process.

One of the control group animals experienced wound infection in the inguinal surgical wound on the 4th day after procedure. This was managed with an antibiotic (enrofloxacin) course for 5 days. Otherwise, inguinal surgical wounds were completely healed at the 7th day after surgery.

Healing

The results of gross examination of the fistula tracts' external opening are displayed in Fig. 6. In the control group, there was no closure of the fistula tracts by the 3rd week and there were some signs of inflammation and drainage. On the other hand, the SVF group fistula tracts achieved almost complete healing of the external opening by the 3rd week with no signs of inflammation or drainage and no adverse effects.

The results of microscopic examination of the fistula tracts are displayed in Fig. 7. The control group showed no closure by the 3rd week (Fig. 8).

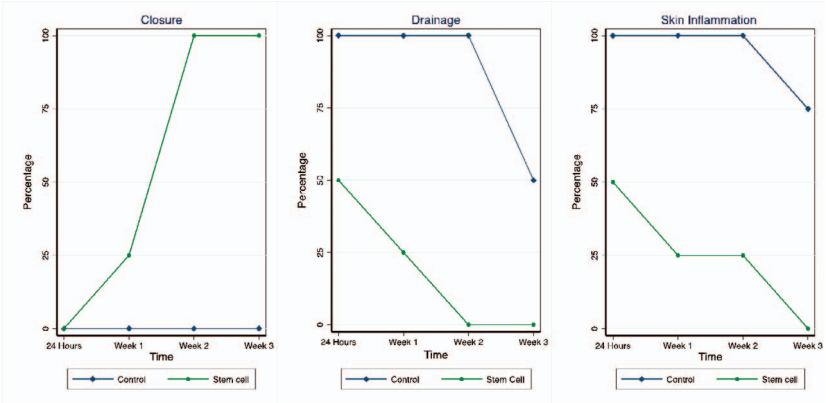

Fig. 6 Gross examination of tract external openings at 24 hours and 1, 2, and 3 weeks after implantation. Comparison of the percentage of animal models with tract closure, drainage, and inflammation of the skin around the opening between the stem cell group and control group.

The group with SVF showed partial closure of the fistula tract lumen at the 1st week specimen (mean: 50%). Healing and progressive closure of the fistula tracts were observed at the 2nd week (mean: 70%). After the 3rd week, the lumen was filled with numerous neutrophils, fat cells and undifferentiated stem cells with almost complete closure (mean 95%; Fig. 9).

Discussion

Postoperative gastrointestinal leakage can be managed using different methods depending on patient status and time of detection. When gastrointestinal leakage is detected early, within the first 72 hours, patients have to undergo a second surgery in order to clean and close the leakage site; but this second surgery is still associated with high mortality rates and repeated laparotomies (24%) as stated by Klein.[16] When gastrointestinal leakage is detected late, it is usually managed conservatively using drains, keeping the patient null per os on parenteral nutrition and broad-spectrum antibiotics. There is also need for regular evaluation of the leakage site. This may take up to several months in the intensive care unit (ICU).[17–19] Leenders[20] investigated the use of self-expanded stents and found it to be effective in certain cases only. Moreover, it is associated with many complications such as migration as well as strictures. Difficult leaks also can be managed by controlled fistula but usually takes long time to heal and needs stenting or reoperation to close the fistula.

In this experimental study, a novel method using autologous stromal vascular fraction was applied and evaluated in the management of postoperative upper gastrointestinal leakage. Autologous stromal vascular fraction were found to be a safe and effective tool in stimulating the healing process and closure of the leakage site. It can be used in difficult early and late postoperative gastrointestinal leakage. This is the first study to investigate the use of autologous stromal vascular fraction in the management of postoperative upper gastrointestinal leakage, which is a promising method that requires the support of further clinical studies.

Recent studies reported the use of autologous stromal vascular fraction and adipose stem cells in

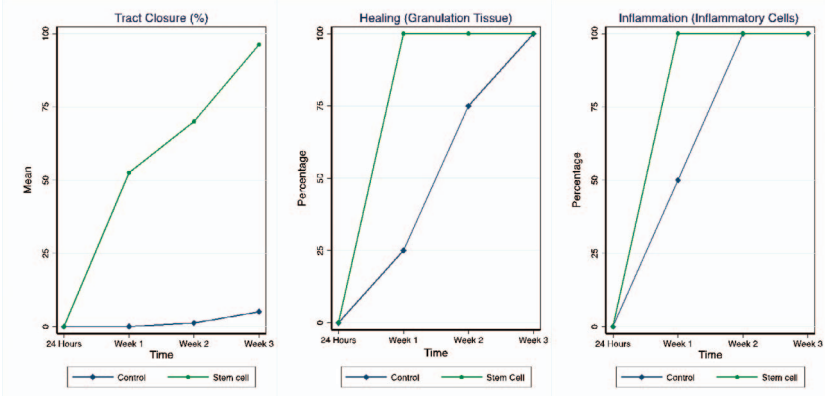

Fig. 7 Microscopic examination of tract external openings at 24 hours and 1, 2, and 3 weeks after implantation. Comparison of the average percentage of tract closure and the percentage of animal models with healing (granulation tissue) and inflammation (inflammatory cells) between the stem cell group and control group.

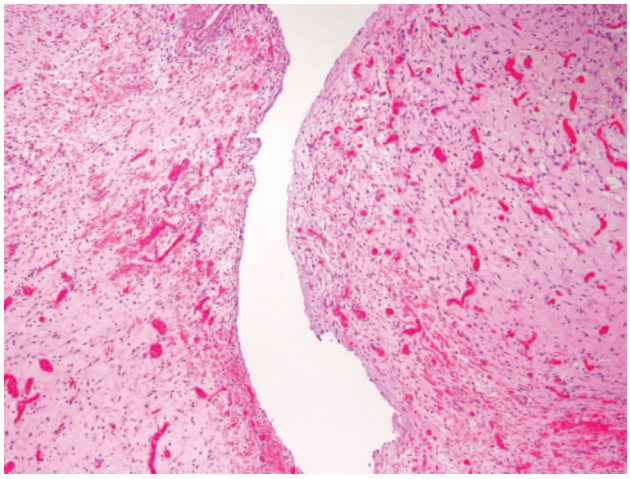

Fig. 8 Control fistula tract (3rd week): the lumen is patent and the wall is formed of granulation tissue (hematoxylin and eosin stain, ×4).

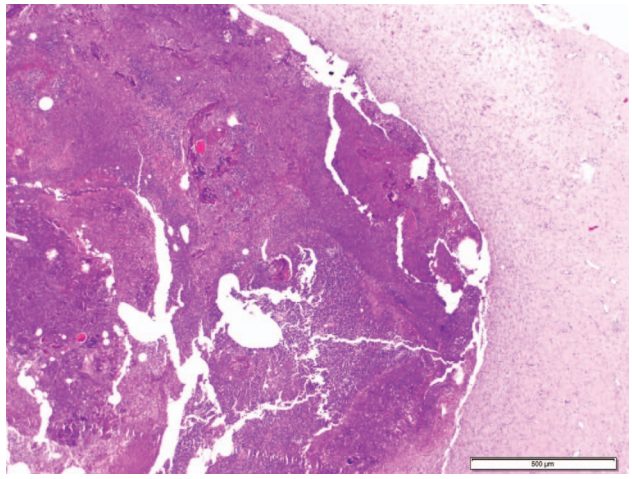

Fig. 9 Fistula tract implanted with adipose stem cells (3rd week): overview showing distended lumen filled with different types of cells.

treatment of Crohn fistula tracts, but no similar studies were conducted in the management of postoperative leakage. Clinical trials by Cho,[21] Garcia,[11] and Lee[12] showed that the closure rate of Crohn fistulae were 50, 75, and 82%, respectively by week 8.

In our study, almost complete closure of the controlled fistula tracts were achieved in the SVF group by the 3rd week in comparison with the control group that showed no closure. This shorter period of healing, in comparison to the Crohn fistulae, might be attributed to the different types of fistula tracts. The Crohn fistula is considered a diseased fistula while the surgically created fistula in the management of postoperative upper gastrointestinal leakage is healthier.

Conclusion

The use of autologous stromal vascular fraction implantation to promote the healing of controlled fistula tracts seems to be a novel, safe, and effective method in the management of difficult, upper postoperative gastrointestinal leakage. It could prevent reoperation and reduce hospital stay, morbidity, and mortality. These results are promising and provide support for further clinical studies.

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References

1. Choi H, Lee B, Kim JJ, Kim JH, Song J, Ji JS, et al. The temporary placement of covered self-expanded metal stents to seal various gastrointestinal leaks after surgery. *Gut Liver* 2013;**7**(1):112–115
2. Xu Q, Yin G, Chen S, Jiang F, Bai X, Wu J. Fluoroscopically guided nose tube drainage of mediastinal abscesses in postoperative gastro-esophageal anastomotic leakage. *Br J Radiol* 2012;**85**:1477–1481
3. Daams F, Luyer M, Lange J. Colorectal anastomotic leakage: Aspects of prevention, detection and treatment. *World J Gastroenterol* 2013;**19**(15):2293–2297
4. Machado N. Pancreatic fistula after pancreatectomy: definitions, risk factors, preventive measures and management-review. *Int J Surg Oncol* 2012:**2012**:602478
5. Dakwar A, Assalia A, Khamaysi L, Kulger Y, Mahajna A. Late complication of laparoscopic sleeve gastrectomy. *Case Rep Gastrointest Med* 2013:**2013**:136153
6. Gill R, Whitlock K, Mohamed R, Sarkhosh K, Brich D, Karmali S. The role of upper gastrointestinal endoscopy in treating postoperative complications in bariatric surgery. *J Interv Gastroentrol* 2012;**2**(1):37–40
7. Seo K, Yoon K, Lee S, Shin Y, Choi K, Hwang H. Amylase, lipase and volume of drainage fluid in gastrectomy for the early detection of complications caused by pancreatic leakage. *J Korean Surg Soc* 2011;**81**:402–407

8. Zuk P. Adipose-derived stem cells in tissue regeneration. *ISRN Stem Cells* 2013:1–21.
9. Mizuno H, Tobita M, Uysal C. Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine. *Stem Cells* 2012;**30**:804–810
10. Gimble J, Bunnell B, Ghiu E, Guilak F. Concise review: adipose-derived stromal vascular fraction cells and stem cells: let's not get lost in translation. *Stem Cells* 2011;**29**:749–754
11. Garcia D, Herreros D, Pascual M, Pascual I, De-la P, Trepol J, *et al*. Treatment of enterocutenous fistula in Crohn's disease with adipose derived stem cells: a comparison of protocols with and without cell expansion. Int J Colorectal Dis 2009;**24**(1):27–30
12. Lee W, Park K, Cho Y, Yoon S, Song K, Kim D, et al. Autologous adipose tissue-derived stem cells treatment demonstrated favorable and sustainable therapeutic effect for Crohn's fistula. *Stem Cells* 2013;**31**:2575–2581
13. Locke M, Windsort J, Dunbart R. Human adipose-derived stem cells: isolation, characterization and applications in surgery. *ANZ J Surg* 2009;**79**(4):235–244
14. Gir P, Oni G, Brown S, Mojallal A, Rohrich R. Human adipose stem cells: current clinical applications. Plast Reconstr Surg 2012;**129**(6):1277–1290
15. Gimble J, Katz A, Bunell B. Adipose-derived stem cells for regenerative medicine. *J Am Heart Assoc* 2007;**100**:1249–1260
16. Klein M. postoperative use of non-steroidal anti-inflammatory drugs in patients with anastomotic leakage requiring reoperation after colorectal resection. *Br Med J* 2012;**354**:1–13
17. Lipska M, Pissett I, Parry B, Merrie A. Anastomotic leakage after lower gastrointestinal anastomosis. *ANZ J Surg* 2006; **76**(7):579–585
18. Daams F, Sleiker J, Tedja A, Karsten T, Lange J. Treatment of colorectal anastomotic leakage. *Diagnostic Surg* 2012;**29**(6):516–521
19. Genser L, Manceau G, Karoui M, Breton S, Brevart C, Rousseau J, et al. Post-operative and long term outcomes after redo surgery for failed colorectal or coloanal anastomosis. *Dis Colo Rectum* 2013;**56**(6):747–755
20. Leenders B, Stronkhorst A, Smulders F, Nieuwenhujizen J, Gillisen L. Removable and repositionable covered metal self-expandable stents for leaks after upper gastrointestinal surgery. *Surg Endosc* 2013;**27**(8):2751–2759
21. Cho YB, Lee WY, Park KJ, Kim M, Yoo HW, Yu CS. Autologous adipose-derived stem cells for the treatment of Crohn's fistula: a phase I clinical study. *Cell Transplant* 2013;**22**(2):279–285