Adipose derived mesenchymal stem cells in gastrointestinal system anastomosis: a narrative review

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Background and Objective: Gastrointestinal (GI) system anastomosis is an artificial connection procedure after a resection of all or part of the digestive organs. GI system anastomosis may lead to many complications, including anastomotic leakage (AL), anastomotic dehiscence, or stenosis. AL from anastomosis is one of the most important and fatal complication of any GI resection. Prevention of AL has been a hot topic of ongoing research for decades.

Methods: To elucidate recent advances on therapeutic efficacy of adipose-derived mesenchymal stem cells (ADMSCs) in anastomosis, we performed a review of the published literature in English from September 2008 to February 2022 by independent searches using publicly available databases, including NIH National Library of Medicine PubMed, Web of Science, MEDLINE and conferences on this topic.

Key Content and Findings: Physical reinforcement of the anastomosis with supporting materials is considered as an effective method to prevent leakage. Liquid-based sealants have also been explored as one of the preventive methods. Finally, manipulating the interaction between the gut microbiome microenvironment and anastomotic healing has also been studied as a means to reduce leakage rates. However, although various surgical techniques have been developed to reduce AL, it remains to be one of the most serious and fatal postoperative complications. Recently, ADMSCs have been popularly used for accelerating anastomotic wound healing through their angiogenesis, immunomodulatory effects and tissue repair ability.

Conclusions: An understanding of above developing advances will be important for all surgeons who operate on the GI systems. Here, our review discusses recent advances in the application of various updated techniques, especially ADMSCs transplantation in GI system anastomosis that may stimulate future human studies exploring these new and exciting avenues.

Keywords: Gastrointestinal system (GI system); anastomosis; mesenchymal stem cells (MSCs)
Introduction

Gastrointestinal (GI) disease is the most common contributor of death from cancer, the leading cause of hospitalization, and the third most common cause of death (from: British Society of Gastroenterology, clinical services 2007). In the conditions of tumor, intestinal obstructions, diverticulitis, and Crohn’s disease, a procedure involving a resection of all, or part of the digestive organs will be considered. After that, a connection must be made through an artificial procedure called anastomosis. One of the most important and fatal complication of GI surgery is anastomatic leakage (AL). There are many factors contribute to impaired wound healing, including technical failures, ischemia, and uncontrolled inflammation (1,2). A large percentage of incidences of the gastrointestinal tract anastomosis were performed in the area of colon. In historic studies, AL rates of up to 30% in colonic anastomosis were reported, while more recent studies have reported rates under 3% (3-5). The incidence of AL has been reported to be approximately 10% in rectal surgery (6). AL causes peritonitis, increasing the chance of re-operation, long-term fasting, and long-term hospitalization. Furthermore, AL has a negative impact on the survival of patients (7). Therefore, various surgical techniques have been developed in an attempt to reduce leaks after anastomosis. For example, indocyanine green (ICG) fluorescence (8) is used to assess intestinal blood flow, improvement of stapling devices (9) and transanal tube placement for decompression of anastomosis (10). However, AL in GI surgery remains to be one of the most serious postoperative complications (11).

Recently, adipose-derived mesenchymal stem cells (ADMSCs) are increasingly being studied because they can be isolated from adult with a relatively low burden on donors compared to bone marrow derived stem cells (12). In addition to potential of differentiation into several cell types (13), ADMSCs also secrete significant levels of growth factors, including vascular endothelial growth factor (VEGF), which improve vascularity in anastomotic site (14,15). Therefore, the use of ADMSCs for acceleration of angiogenesis and wound repair has stimulated interest as a potential prevention strategy for AL.

The purpose of this review is to describe the progress in the application of ADMSCs in gastrointestinal system anastomosis. We have separated this review into the following four chapters: anastomosis in gastrointestinal surgery and potential complications; anastomatic leak risks assessment through intraoperative confirmation; traditional management techniques for preventing anastomatic leak; adipose derived mesenchymal stem cells in gastrointestinal system anastomosis. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dmr.amergroups.com/article/view/10.21037/dmr-22-21/rc).

Methods

To elucidate recent advances on therapeutic efficacy of ADMSCs in anastomosis, we performed a thorough review of the literature published from January 2000 to February 2022 by independent searches using publicly available databases, including NIH National Library of Medicine PubMed, Web of Science and MEDLINE, to search indexed and published articles. In order to include new research that was not published in standard journals but presented in international conferences and meetings into this review, we searched abstracts and proceedings at the three major digestive disease conferences on this topic: the Asia Pacific Digestive Week (APDW), the United European Gastroenterology Week (UEGW), and the Digestive Disease Week (DDW). Expert opinions, editorials, and technical reports were excluded from our literature searching. Details are presented in Table 1.

Briefly, fifty-nine articles were identified after searching in the PubMed, Web of Science, MEDLINE and digestive disease conferences. Out of these, 57 articles were selected based on our predefined filters (articles published after 2,000 and published in English) and articles were reviewed independently by two readers, based on the content of the abstract. Only studies employing adipose-derived stem cells on digestive anastomosis in hollow viscera or GI perforation sutures were finally included. Of these, only 14 primary studies referred to the use of ADMSCs on digestive anastomosis were included (Figure 1), and 4 review articles were eligible for a further analysis.

Anastomosis in gastrointestinal surgery and potential complications

Gastrointestinal surgery includes the treatment for diseases of the body organs related to digestion, such as the esophagus, stomach, small intestines, colon, liver, pancreas, gallbladder, and so on (16). After a procedure involving a resection of all or part of the digestive organs, a connection must be developed through an artificial procedure called anastomosis, which may lead to AL,
Table 1 The search strategy summary

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<th>Items</th>
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<td>Date of search</td>
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<tr>
<td>Timeframe</td>
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<tr>
<td>Inclusion and exclusion criteria</td>
<td>Inclusion criteria: clinical trials, animal study, review, conferences, and meetings. The search was restricted to English literature. Exclusion criteria: expert opinions, editorials, and technical reports</td>
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<td>Selection process</td>
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<td>Any additional considerations, if applicable</td>
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![Flowchart](https://example.com/flowchart.png)

Figure 1 Flowchart.

Anastomotic dehiscence, or stenosis. AL, which is mostly seen during the first 7 postoperative day, is one of the most fatal complications of anastomosis and is the main contributor of postoperative mortality and morbidity with mortality rates of up to 22% (7). It is reported that colon was involved in a large percentage of events of AL occurs in the gastrointestinal tract (17). If the contents of the GI lumen leak into the abdomen, the patient may develop fever, sepsis, abscess, metabolic disorder, or multiple organ failure, which increase the need for re-operation, increase the risk of local recurrence, increase the chance of morbidity and mortality, and generally reduce the quality of life (18,19). It is reported that about one-third of deaths related to colorectal anastomotic dehiscence are related to the extravasation of inner contents after surgery (20). Previous studies have reported a range of 1% to 19% leak rate in colorectal anastomosis. The anastomosis site is the most significant risk factor for AL and further distal site is related to higher risk of leak. The leakage rate of ileocolic anastomosis is from 1% to 4%, while the leakage rate of colorectal anastomosis is 0.5% to 19% (3-5).

Anastomotic healing plays a key role in a successful anastomosis and AL is likely to occur when wound healing is unsatisfactory. Take the colon as an example, the colon wall consists of four layers: mucosa, submucosa, muscularis propria, and serosa. Among them, the most important histology layer in wound healing is the submucosa, which consists mainly of collagen and elastin fibers and is a layer with the highest tensile strength (21). Definition of large bowel anastomotic leak is “leak of luminal contents from a surgical connection between two hollow segments” (22). AL has been an unsolved problem in GI surgery for more than a century. Although various prevention techniques and methods have been developed in recent 30 years to tackle this problem; leakage continues to be a complication in an unforeseeable future and the complication can be challenging to manage for the surgeon (23). Another potential complication of anastomosis is stenosis, which is also caused by hypoperfusion of anastomoses but occurs in the late postoperative phase (1–6 months) (24). It reduces the life quality of patients and may require resection treatment or dilatation (25).

Anastomotic leak risks assessment through intraoperative confirmation

Studies have identified that inadequate blood supply is one
of the most known risk factors related to postoperative AL (26-28). Therefore, a technique identifying anastomosis as with sufficient or insufficient blood supply may help reduce the risk of developing AL after gastroenterectomy. Methods for perfusion assessment intraoperatively have already been introduced and fluorescence angiography with indocyanine green (ICG-FA) seems to be the most promising one (29-31). Harmony et al. retrospectively reviewed 196 patients who had colorectal surgery and demonstrated that the use of ICG-FA for evaluation of anastomosis perfusion significantly reduced the AL incidence from 6% to 0% (32). Another retrospective study found a significantly higher incidence of postoperative AL in the ICG (−) group compared with the ICG (+) group [HR =10.0384, 95% confidence interval (CI): 1.7969–188.2576, P<0.01], which gives the information that it is feasible to use ICG fluorescein imaging to evaluate the blood flow of anastomoses sites and taking further actions to ICG (−) regions intraoperatively to reduce AL rate (33).

Air-leak testing (ALT) is considered to be the most commonly applied intraoperative technique to assess anastomotic continuity in Europe (34), a positive ALT demonstrates a lack of anastomotic integrity (35). A retrospective analysis found that the intraoperative ALT (+) rate was about 5% in laparoscopic left-sided colon resections, but after taking further methods actions the postoperative AL rate was significantly lower compared with the group without intraoperative ALT (2.5% vs. 5.8%, P=0.025) (36).

Another evaluating method is intraoperative endoscopy (IOE), which was firstly reported in 1973 for identifying the lesions that were not palpable by laparotomy (37). And now, IOE is used for the assessment of colorectal anastomosis. Comparing with ALT, IOE enables direct visualization to assess anastomotic failure, bleeding, and mucosal conditions around the anastomosis surface (38). Yang et al. retrospectively reviewed 1,266 patients with low anterior resection of rectal cancer and demonstrated that the AL rate was significantly reduced in the IOE group with 4.3% compared with 11.7% in the non-IOE group (39). However, some researchers question the effectiveness of these intraoperative testing methods which might possibly weaken the anastomosis (40).

### Traditional management techniques for preventing anastomatic leak

Various strategies have been developed to prevent anastomatic leak in last few decades, including the administration of perioperative drugs and interoperative local reinforcement strategies (41).

#### Prevent anastomatic leak through administering perioperative drugs

Many meta-analyses and clinical studies suggested that the application of non-steroidal anti-inflammatory drugs (NSAIDs) was significantly related to increased AL rate (42,43). However, the effect of NSAIDs on AL rate is still conflicting. The Enhanced Recovery After Surgery (ERAS®) Society found no sufficient evidence to discontinue NSAIDs as a component of postoperative multimodal analgesia (44) and it raises questions on whether stopping using NSAIDs could reduce the gastrointestinal leak rate.

Oral antibiotics has been administrated routinely for gastrointestinal operations, especially for the colon surgery. An RCT study performed on more than 300 patients concluded that patients who had ingested kanamycin sulfate and metronidazole orally before underwent elective colon cancer surgery had a significant lower leak rate (45). Besides, a meta-analysis including 40 studies of which 28 were randomized controlled trials with 69,517 patients found that oral antibiotic decontamination preparation for colorectal anastomosis significantly reduced the AL rate (46). In conclusion, current evidence suggests a potentially significant role of oral antibiotics preparation in the prevention of postoperative AL. However, Antibiotics might be related to the risk of stimulating antimicrobial resistance and increasing the risk of Clostridioides difficile infection (47).

#### Prevent anastomatic leak through anastomosis reinforcement

Fibrin-based sealant is one of the anastomosis reinforcement methods. Fibrin-based sealants have been investigated for more than 30 years to reduce AL rate. Fibrin can be obtained from plasma and it is resistant to a certain amount of tension (48). Studies on large animals have demonstrated an obvious reduction of AL rate with extraluminal circular application of experimental fibrin sealant after the low anterior anastomosis of the rectum (49,50). In addition, other sealants such as cross-linking gelatin, platelet-rich plasma and small intestine submucosa have also shown its functions on the reinforcement of anastomosis (51-53). However, until now there is a lack of robust clinical trials to prove its effectiveness. Few
human trials of fibrin glue for prevention of colorectal AL have been reported while no association between fibrin application and AL was found (54,55).

An alternative means to reinforce the anastomosis is putting the buttressing material between opposing tissues and stapling them together. This method makes the stapled wall more apposite, which has greater tensile strength to resist intraluminal pressures (48). Animal experiments found a higher bursting pressure in colorectal anastomosis after putting the buttressing material which means the anastomosis has greater tensile strength (56,57). As more and more anastomoses have been made by stapler in clinical use, bioabsorbable buttressing materials have been explored in some clinical trials. A multicenter study conducted in the United States, found that a circular bioabsorbable buttressing material called SEAMGUARD loaded on the colorectal anastomoses circular stapler leaded to an AL rate at 3.4%, which was markedly lower than the approximately 6–12% or higher rates typically associated with low proctectomy (58). However, other studies showed that there was no significant difference in overall leak rates between the groups with or without a bioabsorbable buttressing material (59,60), which makes the benefits of this alternative means uncertain. Similar to buttressing materials, a biodegradable intraluminal sheath attached to the stapler after the formation of anastomosis, has been developed for many years while its effectiveness of preventing AL has not been confirmed (61,62).

Apart from adding foreign substances to the anastomosis, e.g., sealants and biomaterials, there is another easier and low-risk technique to reinforce anastomoses—Omental Flaps. The omentum is considered to deliver VEGF which accelerates anastomotic neovascularization (63,64). In this technique, the omentum was loosely wrapped around the anastomotic sutures and secured to the bowel wall with sutures if necessary (65). A retrospective study including 147 patients who had undergone pancreaticoduodenectomy found that omental flaps around various anastomoses can reduce the incidences of pancreatic leak rate (4.0% vs. 17%) and biliary leak rate (1.0% vs. 13%) compared with control groups (66). A randomized trial also demonstrated a strikingly lower leaks rate in patients with omentoplasty after rectal resection (67). However, a meta-analysis including three randomized controlled trials with totaling 943 patients found no statistically significant difference between the non-omentoplasty group and the omentoplasty group in radiological AL after colorectal anastomosis, but this result need to be confirmed by multicenter, well-designed trials (68).

### Adipose derived mesenchymal stem cells in gastrointestinal system anastomosis

#### Therapeutic potential of ADMSCs

Ischemia, which causes hypoxia in the anastomotic site and delays wound healing, is one of the most important risk factors for AL (69,70). As a new treatment for ischemic disease states, stem cell–based therapies are increasingly being studied. Among potential stem cells, mesenchymal stem cells (MSCs) are increasingly being studied because they can be isolated from adult donors and maintained in cell culture. These progenitor cells are identified by positive surface molecular markers CD44, CD73, CD90, and CD105, while are negative for the hematopoietic lineage markers such as CD3, CD34, CD45 and CD11b (71). ADMSCs are better candidates than bone marrow–derived stem cells for stem cell therapy because of their relative ease of harvest from donors or patients. ADMSCs are pluripotent cells extracted from adult adipose tissue. ADMSCs have the ability of multilineage differentiation and strong paracrine activity of releasing cytokines, chemokines, and other factors (13), hence widely applicable for tissue regeneration and damage repair (72-74). ADMSCs were popularly used for treating inflammatory diseases because of their immunomodulatory effects and tissue repair ability (75,76). Previous studies have also shown that MSCs promote angiogenesis via increasing the expression of growth factors such as VEGF (15). It is therefore of great interest to promote anastomotic healing via ADMSCs administration (77).

#### Colon and colorectal anastomosis

The investigation of the use of ADMSCs to reduce the risk of AL in gastrointestinal surgery has been a hot topic and most studies focused on the prevention of colonic AL. Pascual et al. (78) published the first study in 2008 and introduced the first use of stem cell coated biosutures. Dosage of 1.5×10^6 MSCs were coated on the 30 cm braided polyglactin sutures during culturing. A total of 40 syngeneic BDIX rats were divided into four groups according to sacrifice date: sacrificed on 4, 7, 14 and 21 d post-surgery (n=10 each). In each group, 5 rats received anastomosis with biosutures and 5 with normal sutures. The right colon was sectioned and then received end-to-end anastomosis with
6 interrupted stitches. Histopathological features, adhesion formation and anastomotic strength were analyzed. Animals in ADMSCs-coated biosutures group found a lower adhesion index on postoperative (PO) d 4 (P=0.025) and 7 (P=0.006), while there were no significant differences in dilatation, obstruction, inflammation and ABP at any time point compared with conventional sutures.

Pascual et al. (79) conducted another related biosutures research in 2010. The difference from previous study is that the colonic anastomosis was kept adhesion free by intraperitoneal instilling 4% icodextrin. They modelled a higher leakage risk colonic anastomosis by a single strand suture. Three groups were designed: G1, anastomosis without 4% icodextrin; G2, anastomosis with 4% icodextrin; G3, anastomosis with 4% icodextrin and biosutures. AL, adhesion formation, and anastomotic strength were analyzed on PO d 4 after operation. No significant differences appeared in AL and adhesion index. A decrease in the adhesion index (P=0.01) and a lower ABP (P=0.15) were observed in G2 compared with G1. When G2 were compared, those in G3 had a higher ABP (P=0.008) with a similar adhesion index (P=0.48). To conclude, biosutures might improve the strength of adhesion-free anastomosis.

In 2012, Yoo et al. (15) modelled ischemic colonic anastomosis by ligating remote marginal vessels from the anastomotic site. Blood flow was monitored by Doppler flowmetry until the blood flow near the anastomosis reduced to <50% of normal. A total of 60 male Sprague-Dawley (SD) rats were divided into 2 groups (n=30 each): G1, without treatment; G2, 1×10⁶ MSCs coated with fibrinogen and thrombin were locally injected around the anastomosis. Body weight, infection, AL, mortality, adhesion, ileus, anastomotic fibrosis, ABP, histology features, and microvascular regeneration were assessed on PO d 7. No significant differences in lesion infection, AL, death rate, adhesions, or ulcer size were found between two groups. G2 showed more favorable anastomotic healing, less colitis manifestations, and enhanced ABP (P<0.01). Regarding histology, G2 augmented collagen deposition (P<0.05) and angiogenesis (P<0.05) compared with G1.

The following publication was from Sukho et al. (80) in 2017. The author suggested that injecting ADMSCs in the form of suspension or biomaterials (such as fibrin, collagen, or gelatin) is either related to insufficient cell retention or insufficient distribution of transplanted cells. Thereby, a human-derived MSC sheet was introduced for cell transplantation to improve cell retention for preventing leakage of sutured colorectal anastomosis in rats. Sixty rats were randomly distributed into 4 groups (n=15 each): G1, MSC sheet group analyzed on d 3; G2, MSC sheet group analyzed on d 7; G3, control group without MSC sheet analyzed d 3; G4, control group analyzed on d 7. One cell sheet was attached around the anastomosis in each treatment group. AL, abscess formation, adhesion formation, ABP, and histology were evaluated. Regarding macroscopy, MSC sheet application in G1 significantly reduced disruption (P=0.002), intraabdominal adhesions (P=0.043) compared to G3. Significantly more rats in the G4 had anastomosis abscesses (P=0.04) compared to G2. There were no significant differences regarding ABP both on PO d 3 and 7. For histology, there were no differences in angiogenesis and fibrosis between groups both on d 3 and 7. A significantly higher number of T-regs (P=0.001) and M2 macrophages (no P value provided) in the G2 compared to G4. In conclusion, MSC sheets treatment resulted in more anti-inflammatory cells at the anastomotic site, without increased adhesion formation.

In 2017, Van de Putte et al. (14) reported an evaluation of allogeneic adipose MSCs on high-risk colonic anastomosis after local irradiation in SD rats. Rats received high dose of irradiation of the colon. Four weeks later, the damaged colon was sectioned, and end-to-end anastomosis was conducted with interrupted suture. The study included 3 groups: G1, control group (n=4), anastomosis after sham irradiation; G2 (n=10), anastomosis after irradiation. Same dosage of phosphate buffered saline (PBS) was injected at the same time points as G3; G3 (n=10), injected (IV) 5×10⁶ MSCs in 500 mL PBS 3 wk after radiation. MSCs (5×10⁶ in 300 mL PBS) were injected locally around anastomosis. Two more IVs of 5×10⁶ MSCs were injected on PO d 10 and d 20. Ulcer size, mucosal vascular density, hemorrhage, and inflammation process were analyzed. With colonoscopy, G3 showed less amounts of necrotic tissue, less bleeding compared to G2 (no P value provided). For histology, the ulcer region in G3 was significantly smaller compared to G2 (P<0.05). At week 8, G3 had the highest number of M2 macrophages compared to G2/G1 (no P value) and the vessel density in G3 was statistically enhanced (P=0.007) compared with G2.

In 2019, Alvarenga et al. (81) introduced another high-risk colonic anastomosis model by using 2,4,6-trinitrobenzene (TNBS) sulfonic acid. Wistar rats were designed as follows: G1, TNBS induced colitis (n=11); G2, laparotomy only (n=11); G3, anastomosis only (n=14); G4, TNBS-colitis followed by anastomosis and applied of 1×10⁶ MSCs around anastomosis (n=15); G5, TNBS-
colitis followed by anastomosis and applied of acellular culture solution around anastomosis (n=15). AL, mortality, histology, and RNA were analyzed after 1 week. Less PO deaths occurred in G4 compared to G5 and G3 (0%, 27%, 7%; P=0.028). No MSC related adverse effects (death, fistula, abscess, peritonitis) appeared in G4. Regarding histology, a decrease in histological score was observed in G4 compared with G5 (P=0.011). For mRNA results, there was a significant difference between G4 and G5 (higher) in IFN-γ levels (P=0.02). In conclusion, ADMSCs decreased mortality and alleviated inflammation associated with high-risk anastomosis via its immunomodulatory activity.

Morgan et al. (82) in 2020 found that xenogeneic ADMSCs significantly enhanced the healing of ischemic colorectal anastomosis and reduced the risk of AL. Ischemia was developed by ligating the vessels of the mesocolon 2 cm to the anastomosis proximally and distally. Three groups were divided: G1, control (anastomosis only); G2, absorbable gelatin sponge (gelfoam); G3, MSC (gelfoam coated with 1x10⁶ MSCs). Each group was sacrificed on PO d 3 or d 7 (n=8 per subgroup). Macroscopy, histology, IHC were performed after sacrifice. No death or relevant adverse events occurred in this study. In macroscopy, MSCs significantly reduced AL compared to G1 on PO d 3 and 7 (P=0.02 for both) and with G2 (P<0.01 for both time points). The mean ABP in G3 was higher compared with G1 on d 3 (P=0.08) and on d 7 (P=0.30). Regarding histology, G3 significantly enhanced angiogenesis and collagen formation compared with G1/G2 (P<0.01 for both time points). In addition, IHC suggested the significantly increased endothelial marker CD31 for both time points in G3 (no P value provided).

**Gastric perforations**

The suture perforations in the stomach are also prone to AL. Currently, there are 2 studies using ADMSCs for preventing AL in stomach. In 2013, Komiyama et al. (83) developed a gastrotomy closure model in the gastric greater curvature of 40 male Wistar rats. Animals were divided into four groups with of 10 rats each: MSCs 7 d, PBS 7 d, MSCs 28 d, PBS 28 d. Sutured gastric perforations in MSC group were treated with locally transplanted 1.0x10⁷ autologous CM-DII-labeled ADMSCs and rats were sacrificed on PO 7 d or 28 d. Controls were treated with PBS injection and sacrificed on PO d 7 or d 28. Histopathologic features included assessment of angiogenesis and collagen deposition were evaluated in the four groups. Labelled ADMSCs were successfully detected on PO d 7 and 28 in the sutured area, but no differentiation was found in all groups. The mean ABP of the MSC treatment group was higher than that in controls on d 7 (121±30 vs. 291±14.8 mmHg, P<0.01). For histology, at d 7 after surgery, angiogenesis (P<0.01) and connective tissue (P<0.01) were denser in MSC groups. By contrast, at d 28 after surgery, density of connective tissue was significantly reduced in MSC group (P<0.01). The author proposed that these phenomena may be explained by paracrine mechanisms of ADMSCs. Secretion of VEGF, HGF, and TGF-β enhance tissue regeneration by enhancing angiogenesis and fibrosis (early period). ADMSCs prevent fibrosis formation by assisting with the regeneration of natural tissue structure (late period).

In 2015, Liu et al. (84) developed a sutured gastric perforation model at the gastric body in 204 female SD rats. Animals were divided into four experimental groups of 48 rats each and 12 sham operation rats: G1, control group; G2, fibrin group; G3, sprayed MSC group; G4, injected MSC group; G5, sham group. Sutured gastric perforation in G4 received a submucosal injection of 5x10⁷ MSC cells. The same dosage of MSC was applied in G3 combined with fibrin glue. G1 received submucosal injection of same volume PBS and G2 received topical fibrin glue. ABP, wound adhesions, AL and histology changes were assessed. Regarding histology, MSC treatment reduced the number of neutrophils and promoted granulation and re-epithelialization at d 5 and 7, being better in G4. In addition, results showed that local injection was a better approach compare with delivering MSCs with fibrin glue on enhancing bursting pressure, promoting granulation, re-epithelialization, reducing adhesions, AL and inflammation.

**Small bowel anastomosis**

Transplantation of ADMSCs can also improve anastomatic healing of small intestinal. In 2017, Maruya et al. (85) induced an ischemia small intestine anastomosis in 11 miniature female pigs by ligating vessels and delayed healing by injecting mitomycin C. Pigs were randomized into two groups: G1, delayed wound healing (n=4); G2, MSC sheets under delayed wound (n=7). For G2, eight anastomotic sites in each pig were randomly divided into MSC sheet subgroup (dressing with three MSC sheets but dosage not clearly described) and untreated control subgroup. Autologous MSC cell sheets were transplanted onto the serous membrane after suturing. ABP, hydroxyproline expression, and mRNA expression of collagen-1 α1 and
collagen-3 α1 were evaluated. Results showed that MSC sheets enhanced intestine bursting pressure (P<0.05) and up-regulated the mRNA expression of collagen-1 and collagen-3. Hydroxyproline expression was significantly higher in MSC sheet subgroup on PO d 7 (P<0.01) but not PO d 5.

In 2020, Pan et al. (86) introduced tissue fusion technology with allogenic ADMSCs in sixteen healthy pigs. Pigs were divided in two groups: MSCs group (7 or 14 d) and a control group (7 or 14 d). Five anastomotic sites were established using Liga-Sure ForceTriad (Covidien, MA, USA) in each animal. Vehicle solution with or without 5×10⁶ MSCs was administered subserosally at each anastomotic site. Postoperative complications, ABP and histological changes were evaluated. There were no significant differences in postoperative complications, AL and ABP between groups. For histology, MSC group demonstrated total re-epithelialization, more connective tissue, and higher proliferating cell nuclear antigen (PCNA) (P=0.021). But no differences in angiogenesis, inflammation and collagen fiber structure were found.

In addition, Navarro-Zorraquino et al. (87) reported that local implantation with ADMSCs was able to reduce risk of rejection after small bowel transplantation by inducing an immunomodulatory response. In 2020, small bowel transplantation was performed in 40 Wistar Han rats. Animals were allotted to two groups: control group receiving saline solution and MSC group receiving subserosa injected 1×10⁶ MSC cells. Rejection risk was evaluated by histological study. Numbers of immune cell and cytokine expression were also assessed. MSCs reduced the recipient death by 92.8% due to rejection in PO d 7 (P<0.002). MSC group also increased the percentages of Treg cells (P<0.05) and TGF-β1 levels in peripheral blood (P<0.05).

Biliary anastomosis leakage/stenosis

There were two studies focus on autologous ADMSCs treatment on biliary anastomosis healing. The first one is from Zhang et al. in 2020 (88). A total of 9 domestic white pigs were divided into three groups: G1, plastic stents wrapped in unseeded Vicryl mesh; G2, stents coated with 4×10⁶ MSCs seeded mesh; G3, non-wrapped stents with topically applied 4×10⁶ MSCs suspension. On d 0, procedures involved common bile duct (CBD) transection, suturing, stent insertion with or without MSCs application. Complications, fibrosis, and angiogenesis were assessed. There were no other complications except one pig in G2 died from acute cholangitis on PO d 3. No symptoms or cholangiography suggesting clinical biliary strictures in the surviving pigs. G3 showed significantly higher number of MSC cells engraftment compared with G1/G2 (P<0.05 for both). Histopathological analysis showed a smaller number of inflammatory cells, and less collagen fiber based on trichrome stain were identified in the bile duct wall in G3. In conclusion, ADMSC-wrapped mesh stents were locally engrafted within the porcine bile duct and showed beneficial effects on alleviating fibrosis and promoting angiogenesis on the anastomotic site. Extraluminal immersion with MSCs seems more efficient than MSC-coated stents.

In another study published in 2020, Hara et al. (89) also introduced a cell sheet technology for the promotion of in anastomotic healing at the duct-to-duct biliary site. ADMSCs cell sheets were formed by seeding on temperature-responsive culture dishes and transplanted on the porcine biliary anastomotic site. A total of 11 pigs (20–25 kg) were divided into two groups: ADMSC sheet (n=6) and control group (n=5). On d 0, The CBD was completely transection and biliary anastomosis was performed subsequently with a suture. MSCs sheet was transplanted on the anastomotic site in the MSC group (no mention of dosage). One pig in MSC group received an PKH26-labeled cell sheet with to track the survival of transplanted cells. Macroscopic changes, infiltration of inflammatory cells, and collagen content were evaluated on PO d 14. Labeled cells were confirmed to remain in CBD wall (n=1). Regarding macroscopic evaluation, no leakages or abscesses were found in each group. MSC group alleviated adhesions around the liver hilum compared with controls (P=0.07). Decreased diameter and cross-sectional area of the bile duct wall were shown in MSC group (P<0.02). For histology, MSC group demonstrated less inflammatory cells, less collagen fibers formation in the bile duct wall, and enhanced angiogenesis (P value not provided). However, long-term follow-up is necessary to assess the effect of preventing stricture.

Security concerns of ADMSCs

However, despite exhibiting promising results in preclinical studies for improving anastomotic healing, MSCs transplantation is still totally restricted to the experimental stage due to its critical disadvantages including safety issues, few cell survival, immunological rejection, inconsistency among preclinical studies and low cost-effectiveness (90). Given that transplanted ADMSCs are viable cells with both...
hallmark self-renewal properties and multipotent capacities, their conventional transplantation may lead to undesired differentiation of MSCs like ossification, calcification and tumor formation (91,92). The most concerning potential safety hazards is tumorigenesis involved in the transportation of stem cells (93).

There is growing evidence supports that the paracrine activities and secretory components of stem cells make the most prominent contribution to the process of organ repair, while stem cell differentiation in-vivo and definite engraftment play a secondary role (94,95). ADMSC secretome is mainly composed of small molecules and extracellular vesicles (EVs), which are responsible for mediating dynamic communication between MSCs and other cells. Xia et al. (95) evaluated the paracrine role of ADMSCs in the gastric mucosal injury. Conditioned medium from hypoxia-conditioned ADMSCs was injected to the submucosa around and results suggested the promotion of gastric mucosal healing through the angiogenesis and re-epithelization. ADMSC-derived EVs, which exactly represent the main mediators of paracrine communication of ADMSC, are considered to be much more effective and safer than stem cells themselves. EVs are extracellular particles separated by bilayer lipid membranes, and their main function is to serve as carriers for cellular interaction, transporting a variety of biologically active molecules, including proteins/peptides, lipids, mRNA and miRNA (96). Therefore, introducing approaches based on ADMSC-derived EVs may offer an alternative therapy in overcoming the limitations and critical disadvantages observed in stem cell-based methods and thus the ADMSC-EVs may also provide a far more beneficial therapeutic strategy for accelerating anastomotic healing. However, there are currently no studies investigating the role of stem cell-derived EVs in promoting anastomotic healing.

Discussion

There are many complications of gastrointestinal system anastomosis including AL, anastomotic dehiscence, stenosis, or even death. Considering these potential hazards, efforts have been made to develop several approaches for reducing anastomotic complications, either by interoperative local reinforcement strategies to control the local factors or by the administration of perioperative drugs in order to improve the general conditions. One of the new directions for reducing anastomotic leak incidence are prone to the use of ADMSCs in achieving better anastomosis healing either by direct injection or coating with biomaterials. Despite decades of technological advancement and diligent research, the failure of anastomotic healing far exceeds expectations. Our review provides a concise summary of the recent advances on the prevention of gastrointestinal systems anastomotic leak, especially the application of ADMSCs.

As shown in Table 2, there are currently only 14 studies on ADMSCs to promote the healing of gastrointestinal system anastomosis, including 7 studies on the colon, 3 studies on the small intestine, 2 studies on the bile duct, and 2 study on the stomach. One of the weaknesses of these studies is that all these studies were restricted on animal models, rather than on patients. Thereby, much more animal research is needed in this field and the promotion of anastomotic healing by ADMSCs is far from clinical application. In addition, 10 (71.4%) of these studies were performed on a small animal model, which was less convincing than big animal models. Anastomosis in the colon is the most concerned aspect of the gastrointestinal system, but all the studies on colorectal anastomosis were limited to rat models. Large animal models, such as porcine and canine models, are needed in the future to be studied in this filed.

Another unresolved issue is the best ADMSCs administration method, which influences stem cells colonization, survival, and function in wound area. The most employed method is direct transplantation. As shown in Table 2, seven studies mentioned locally transplantation method (injection or instillation), 3 studies introduced stem cell sheets and 6 studies coated stem cells with biomaterials (absorbable gelatin sponge, biosutures, fibrin glue, or plastic stents). Each method has its own advantages and disadvantages. Injection of ADMSCs as suspension is the easiest and most convenient method while has the risk of causing perforation. Combined with biomaterials such as fibrin, gelatin or biosuture is related to insufficient transplanted cells (80). Liu et al. (84) compared the method of local injection with fibrin glue coating method. ADMSCs colonization and differentiation was detected in the submucosa and granulation tissue of the gastric wound by both groups, but injection approach showed a better effect on enhancing anastomotic healing compared to coating with fibrin glue. Zhang et al. (88) compared the method of coating with cell seeded mesh with applying topical cell suspension. Topical MSCs showed significantly higher number of MSC cells colonization compared with stent coating method (P<0.05). Histopathological analysis showed a smaller number of inflammatory cells, and less
<table>
<thead>
<tr>
<th>Author</th>
<th>Publish Year</th>
<th>Animal model</th>
<th>Disease model</th>
<th>Organ</th>
<th>Transplant method</th>
<th>Endpoint</th>
<th>Result</th>
<th>Security concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrew Morgan</td>
<td>2020</td>
<td>Rat</td>
<td>Ischemic anastomosis</td>
<td>Colon</td>
<td>Coated with gelatin sponge</td>
<td>AL; VEGF</td>
<td>Decreased AL, increase VEGF</td>
<td>No</td>
</tr>
<tr>
<td>Jong Han Yoo</td>
<td>2012</td>
<td>Rat</td>
<td>Ischemic anastomosis</td>
<td>Colon</td>
<td>Coated with fibrinogen and thrombin + local injection</td>
<td>Body weight, infection, AL, mortality, adhesion formation, ileus, anastomotic stricture, anastomotic bursting pressure, histopathological features, and microvascular density</td>
<td>No differences in wound infection, AL, or mortality. Less body weight lost, ileus, and strictures. Augmented bursting pressure and collagen deposition. Favorable histopathological features, and higher microvascular density</td>
<td>No</td>
</tr>
<tr>
<td>Valter Alvarenga</td>
<td>2019</td>
<td>Rat</td>
<td>High-risk colonic anastomosis model by 2,4,6-trinitrobenzene</td>
<td>Colon</td>
<td>Instillation over anastomosis</td>
<td>AL, mortality, myeloperoxidase activity, fibrosis, epithelial integrity, NF-κB activation, expression of inflammatory cytokines, and extracellular matrix-related genes</td>
<td>Decrease AL and mortality, decreased deposition of collagen fibers, decreased myeloperoxidase activity, decreased accumulation of CD4+ T-cells and macrophages, decreased activation of NF-κB, IL-17, TNF-a, IFN-g, and metalloproteinases</td>
<td>No</td>
</tr>
<tr>
<td>Isabel Pascual</td>
<td>2008</td>
<td>Rat</td>
<td>Regular anastomosis</td>
<td>Colon</td>
<td>Coated with biosutures</td>
<td>Histopathological features, adhesion formation and anastomotic strength</td>
<td>Lower adhesion index. No significant differences in anastomotic healing.</td>
<td>No</td>
</tr>
<tr>
<td>Isabel Pascual</td>
<td>2010</td>
<td>Rat</td>
<td>anastomosis within adhesion-free environment</td>
<td>Colon</td>
<td>Coated with biosutures + icodextrin</td>
<td>AL, adhesion formation, and anastomotic strength</td>
<td>No differences in AL, adhesion index; Higher bursting pressure</td>
<td>No</td>
</tr>
<tr>
<td>Dirk Van de Putte</td>
<td>2017</td>
<td>Rat</td>
<td>Anastomosis after irradiation</td>
<td>Colon</td>
<td>Local injection + IV</td>
<td>Ulcer size, mucosal vascular density, hemorrhage, inflammatory process</td>
<td>Reduces ulcer size, increases mucosal vascular density, limited hemorrhage and inflammatory process</td>
<td>3 deaths. No SCs related</td>
</tr>
<tr>
<td>Panithi Sukho</td>
<td>2017</td>
<td>Rat</td>
<td>Insufficient suturing anastomosis</td>
<td>Colon</td>
<td>Stem cell sheets wrapping anastomosis</td>
<td>AL, abscess formation, adhesion, formation, anastomosis burst pressure (ABP), and histology</td>
<td>Reduced AL, increased CD3+ T-cells and CD163+ anti-inflammatory macrophages. No difference in ABP, vessel density and collagen deposition</td>
<td>No</td>
</tr>
<tr>
<td>Hong Pan</td>
<td>2020</td>
<td>Porcine</td>
<td>Tissue fusion anastomosis</td>
<td>Small bowel</td>
<td>Local injection</td>
<td>Postoperative complications, ABP and histology</td>
<td>No differences in postoperative complications and ABP. Higher number of proliferating cell nuclear antigen- (PCNA-) positive cells</td>
<td>1 death in SCs (ileus)</td>
</tr>
<tr>
<td>Yasuhiro Maruya</td>
<td>2017</td>
<td>Porcine</td>
<td>Ischemic anastomosis</td>
<td>Small bowel</td>
<td>Stem cell sheets wrapping anastomosis</td>
<td>Bursting pressure, hydroxyproline content, and mRNA expression of collagen-1 α1 and collagen-3α1</td>
<td>Increased bursting pressure, increased hydroxyproline content, increased mRNA expression of collagen-1 α1 and collagen-3α1</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2 (continued)
<table>
<thead>
<tr>
<th>Author</th>
<th>Publish Year</th>
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<th>Result</th>
<th>Security concerns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marta</td>
<td>2020</td>
<td>Rat</td>
<td>Small bowel</td>
<td>Small bowel</td>
<td>Local transplantation</td>
<td>Rejection risk, cytokine concentration</td>
<td>Lower risk of rejection, increased Treg cells and TGFβ1 levels</td>
<td>1 ileus, 3 high fever. No SCs related</td>
</tr>
<tr>
<td>Liu</td>
<td>2015</td>
<td>Rat</td>
<td>Closed perforations</td>
<td>Stomach</td>
<td>Local injection or coated with fibrin glue</td>
<td>ABP, wound adhesions, AL and histology</td>
<td>Higher ABP; Reduced adhesions and AL. Reduced inflammation, and increased granulation and re-epithelialization</td>
<td>No</td>
</tr>
<tr>
<td>Sosuke Komiyama</td>
<td>2013</td>
<td>Rat</td>
<td>Regular anastomosis</td>
<td>Stomach</td>
<td>Local transplantation</td>
<td>Angiogenesis, collagen deposition, bursting pressure</td>
<td>Enhanced angiogenesis and collagen deposition after 7 d, reduced collagen deposition after 28 d. Higher bursting pressure</td>
<td>No</td>
</tr>
<tr>
<td>Takanobu Hara</td>
<td>2019</td>
<td>Porcine</td>
<td>Regular anastomosis</td>
<td>Bile duct</td>
<td>Stem cell sheets wrapping anastomosis</td>
<td>Macroscopic changes, infiltration of inflammatory cells, and collagen content</td>
<td>Decreased diameter and cross-sectional area of the bile duct wall, less inflammatory cells and collagen fibers</td>
<td>No</td>
</tr>
<tr>
<td>Yi Zhang</td>
<td>2019</td>
<td>Porcine</td>
<td>Regular anastomosis</td>
<td>Bile duct</td>
<td>Coated with plastic stents or topical MSCs suspension</td>
<td>Fibrosis and angiogenesis</td>
<td>Decreased fibrosis and increased angiogenesis</td>
<td>1 death (SCs + mesh)</td>
</tr>
</tbody>
</table>

ADMSCs, adipose derived mesenchymal stem cells; SCs, stem cells; AL, Anastomotic leak; VEGF, vascular endothelial growth factor; ABP, anastomotic bursting pressure.
collagen formation were identified in the bile duct wall. To conclude, topical extraluminal immersion with MSCs seems more efficient than MSC-coated stents. More animal studies comparing the effectiveness of different MSC administration methods are needed in the future.

Despite the promising results in animal studies for enhancing anastomotic healing, several critical disadvantages of stem-cell-based treatment have been noticed, including safety issues, few cell survival, immunological rejection, inconsistency among preclinical studies and low cost-effectiveness (90). Given that transplanted ADMSCs are viable multipotent cells, direct transplantation may lead to undesired differentiation of MSCs like ossification, calcification, and tumor formation (91,92). Although there are many potential security concerns of MSCs, the most concerning hazards is tumor formation involved in the transportation of stem cells (93). Of the 14 studies reviewed, only four of them (28.6%) mentioned adverse events of MSC but none of them declare carcinogenesis concerns in their study. No conclusions can be drawn as to whether MSC transplantation led to tumorigenesis due to their relatively short follow-up time. Previous studies investigating the relationship between MSC and tumors are contradictory. MSC have been reported as a role to promote malignance phenotype and progression (97,98) but also served as an anti-cancer therapeutic strategy (99). MSC are well known for their immunosuppressive effect, which is considered as the main mechanism for promoting tumor progression. When MSCs were co-culturing with tumor cells, a promotion role was reported in some studies (100,101), but in other studies with co-culture, tumor growth was suppressed (102,103). Although some in vitro experiments reported that long-term culture of MSCs can lead to tumor formation (104), it may be caused by tumor cell contamination in the culture dishes. Moreover, the cellular microenvironments in vivo and in vitro are totally different, and in vitro tests results provided relatively weak evidence. To conclude, there is still no consensus on the pro-tumor or anti-tumor properties of MSC, more research and more long-term follow-up are necessary before drawing a conclusion.

As for other adverse effects, most clinical studies declared no severe adverse effects related to MSC administration (105-107). The first clinical study reported severe adverse effects was published in 2007. Three patients suffered from severe adverse effects such as bilateral totally visual loss, detached retina and vitreous bleeding after they received intravitreal administration of autologous “ADMSCs” (in fact it was stromal vascular fraction) (108). A similar case was also reported in 2007 (109). These cases illustrated the necessary for caution in the application of ADMSCs in topical ocular therapy.

As for the future direction, firstly, there is an urgent need to perform more research in a big animal model, which is more similar to the human body compare with small animal model. Currently, as shown in Table 2, only 4 studies (two in small bowel and two in bile duct anastomosis) were conducted on a big animal model and none of the studies on colorectal anastomosis were conducted in large animals. Thereby, more large animal studies are needed to confirm the effectiveness of ADMSCs on promoting anastomotic healing before there is sufficient evidence to conduct clinical trials in humans. Secondary, each of the current used MSCs delivery methods has its own shortcomings, and more advanced materials need to be developed in the future to implant MSCs at the anastomosis with higher efficiency. Stem cell sheet was considered as a more effective delivery method compared with injection and coating with biomaterials (80,110). However, because the limited number of studies introducing stem cell sheets, more future studies are needed to clarify if this approach could be better than injections and other methods. Last but not least, it is reported that the therapeutic ability of MSCs has been attributed to their secretory components through cell-to-cell communication, more exactly, paracrine activity. The primary mediators of paracrine activity are EVs, which play a critical role in cellular interaction by transporting a variety of biologically active molecules. In the future, studies will investigate the role of ADMSCs-derived EVs in promoting gastrointestinal anastomotic healing.

To finalize, there are several limitations in our review. The main limitation of our review is the nature of included studies: the different sample size, study groups, experimental models (normal, ischemia or radiation, etc.), methods of stem cells administration, postoperative parameters monitored, and the lack of a given confidence interval. They are too heterogeneous for us to perform a systematic review or meta-analysis and here we only presented a descriptive review. Only research published in English were included can be another limitation. Considering these limitations, the results presented should be interpreted with caution.

Conclusions

Several traditional strategies have been developed to prevent anastomotic leak in last few decades, including the
administration of perioperative drugs and interoperative local reinforcement strategies. However, perioperative drugs are restricted by their adverse effects while the effectiveness of local reinforcement strategies for preventing AL has not been confirmed. Ischemia is considered as one of the most important risk factors for AL and studies have also shown that ADMSCs promote angiogenesis via increasing the expression of VEGF. Currently, ADMSCs have been applied to prevent gastrointestinal system anastomotic leak, including colonic, gastric, small intestinal and biliary anastomoses in the animal model. The effectiveness of ADMSCs in preventing AL has been proven by some studies, while the results among different studies were inconsistent and another limitation is that ADMSCs have not been studied in humans. In the future, much more pre-clinical studies are needed to verify the effectiveness of ADMSCs on the prevention of gastrointestinal system anastomotic leak.

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References


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