Experimental Research

Active drain system with reticulated open-pore foam-surface dressing for postoperative pancreatic fistula in a rat model

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ABSTRACT

Background: Postoperative pancreatic fistula (POPF) is one of the most harmful complications after pancreatic resection. Efficient drainage affects the clinical outcome of POPF. Inefficient drain of the fluid collection should contribute greatly to the need of additional interventional drainage, secondary morbid complications, and death.
Methods: A rat model of POPF was established by distal pancreatosplenectomy. A novel active drain system (ADS) for POPF was developed by wrapping polyvinyl alcohol sponges (PVA) to an end of the drainage tube. Passive drain system (PDS), ADS and ADS with PVA were used for POPF in rat models. The volume and amylase of ascites were measured. CT scan was applied to assess abdominal fluid collection. Rats pancreatic transection stumps were stained by hematoxylin and eosin (H&E).
Results: The volume of drainage of ADS with PVA group was less than that of PDS group and ADS group at late stage. CT scan showed obvious abdominal fluid collections in 2/8, 2/8 and 0/8 rats in PDS, ADS and ADS with PVA group separately. Macrofindings showed significant intra-abdominal adhesions and inflammation in PDS and ADS group but not in ADS with PVA group. H&E staining showed less inflammatory cells and destroyed pancreatic glands in ADS with PVA group.
Conclusion: ADS with PVA drained ascites effectively in the rat model of POPF. The effective drainage of pancreatic juice reduced the inflammation of abdominal organs and pancreatic resection stumps, and might promote the healing of POPF.

1. Introduction

Postoperative pancreatic fistula (POPF) is one of the most harmful complications after pancreatic resection [1,2]. Despite all the advances and technical modifications developed during recent years to prevent POPF, the incidence of this complication still ranges between 3 and 45% of pancreatic operations at high volume centers [3,4].

Digestive enzyme activation outside the pancreas may lead to secondary pathophysiological changes including infection, bleeding, and systemic inflammatory response syndrome [5]. POPF is thought to contribute to the most morbid complications of the operation such as erosion of retroperitoneal vessels and hemorrhage, intra-abdominal abscess, sepsis, multisystem organ failure, and death [6]. Efficient drainage affects the clinical outcome of POPF. Inefficient drain of the fluid collection should contribute greatly to the need of additional

interventional drainage, secondary morbid complications, and death [7]. Pancreaticoduodenectomy is a procedure with risk of POPF. In a multicenter RCT involving 137 patients randomized to no drainage versus drainage, pancreaticoduodenectomy without drainage was associated with greater morbidity (3% vs 12%), which suggested the importance of drainage for POPF [6]. Routine placement of intraperitoneal drainage after pancreatic operations has traditionally been considered mandatory and supported by clinical consensus [8], which was very different from other operations such as splenectomy, hepatectomy, gastrectomy, and colorectal resection.

The influence of the type of drain system to the POPF has not yet to be robustly investigated. Drain systems can either be passive that drain fluid by gravity, or active that drain fluid by negative pressure generating from collapsed reservoir. There is controversy as to whether one of these two systems is superior [9]. Active drain system generate

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high-pressure gradient and may promote the development of a POPF, while it has also been postulated to improve drainage and decrease the severity of the POPF if it does occur [10,11]. Only one RCT compared the differences between the different drain systems, in which ADS was reported with reduction in severity of complications associated with POPF, but there was no differences in morbidity or mortality at 30 days between them [12]. Negative-pressure wound therapy has emerged as a major advancement in the treatment of complex and chronic wounds as well as in the prevention of wound breakdown when applied over a closed incision [13]. Different form ADS, it contains a resilient, reticulated open-pore foam-surface dressing which decreases dead spaces of the wound [14]. Thus, we proposed that the addition of these foam-surface dressing may have the potential to increase the efficacy of ADS for POPF, occupy the irregular spaces after pancreatic resection, and decrease the severity of POPF.

In the present study, the differences of passive drain system (PDS), active drain system (ADS) and ADS with polyvinyl alcohol sponges (PVA) were evaluated on a rat model of POPF. The ADS with PVA showed superiors on drainage efficacy, pancreatic stump healing and abdominal inflammation.

2. Methods

2.1. Development of ADS with PVA

The animal model was conducted in accordance with the ARRIVE guidelines [15]. As shown in Fig. 1A and B, we created a novel drain system. An end of the drainage tube was wrapped with PVA, and a pinched rubber suction bulb was used to provide persistent negative pressure about 3.5 KPa for postoperative drainage, as exhibited in Fig. 1C.

2.2. Rat model of POPF and grouping

All rat experiments were conducted in accordance with the guidelines approved by the local ethic committee (Tongji Medical College, HUST, China). SD-Rats about 8 weeks old males weighing approximately 250 g were obtained from Vitalriver (Beijing, China) and housed access to water and food. Rats were anesthetized using isoflurane and placed in the supine position. The POPF was induced by the distal pancreatosplenectomy (Fig. 1D). A drainage tube was placed near pancreatic transection stump to drain the fluid collection. Twenty-four rats were randomized into three groups. In PDS group, a drainage tube with a plump rubber suction bulb attached to its end was used; in ADS group, a drainage tube with a pinched rubber suction bulb attached to its end was used; in ADS with PVA group, A PVA-wrap drainage tube with a pinched rubber suction bulb attached to its end was used. In addition, four rats underwent sham surgery acted as control. All rats were sacrificed seven days after the distal pancreatosplenectomy or sham surgery. The macrofinding adhesion was scored as: 0 = no adhesion; 1 = adhesion that could be bluntly separated. 2 = adhesion that could be sharply separated. 3 = adhesion that could not be separated [16].

2.3. Analysis of ascitic samples

Ascites samples were collected for 7 days after the distal pancreatosplenectomy. The volume was recorded, and amylase concentrations were measured using an amylase activity kit (Abcam, Cambridge, UK) followed the manufacture instructions. All data are expressed as the mean $\pm$ SD and were analyzed using GraphPad Prism 6 software (GraphPad Inc., La Jolla, CA,USA).

2.4. CT scan

Inliview-3000 B small-animal PET/SPECT/CT (Novel Medical,
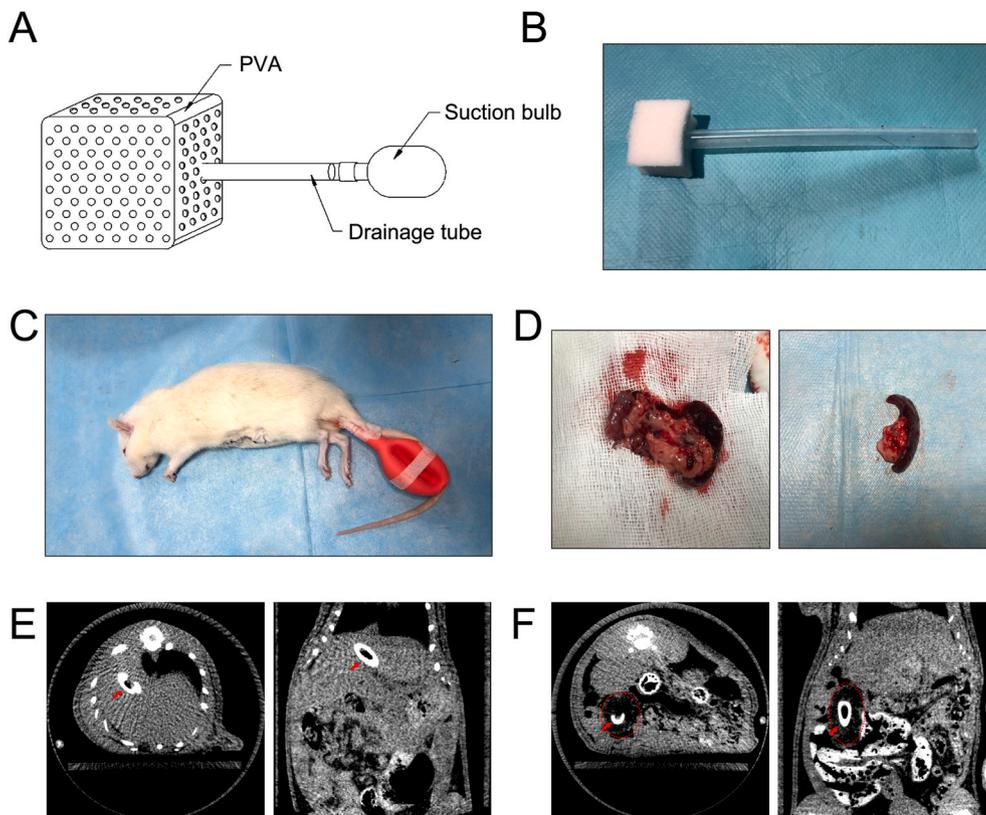

Fig. 1. Establishment of the POPF rat model and the ADS with PVA. (A) Schematic representation of the ADS with PVA. (B) Image of the drainage tube wrapped with PVA. (C) the POPF rat model using ADS with PVA. A pinched rubber suction bulb was used to provide negative pressure. (D) Resected distal pancreas and spleen. (E). CT image of the rat of ADS group. (F) CT image of rat of ADS with PVA group. Red arrow showed drainage tube in CT image; red dotted circle showed PVA in CT image. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Beijing, China) were used to visualize phantoms. Rats were anesthetized and maintained with 2.0% isoflurane in 100% oxygen. They were placed on the examining table in the prone position and underwent CT imaging.

2.5. Histology

The pancreatic transection stump specimens were collected and fixed in neutral buffered formalin and embedded in paraffin. Specimens were stained with hematoxylin and eosin (H&E). The inflammatory was scored as: 0 = no inflammation; 1 = < 5% neutrophilic or lymphoplasmacytic inflammation; 2 = < 50% neutrophilic or lymphoplasmacytic inflammation; 3 = > 50% neutrophilic or lymphoplasmacytic inflammation; 4 = necrosis of pancreatic gland [16].

3. Results

3.1. Drainage after pancreatic resection

As shown in Fig. 2A, the total volume of drainage during the observation period of the three groups was comparable without significant difference. However, if the observation period was divided into early stage (day 1 – day 3) and late stage (day 4 – day 5), there is a trend that the volume of drainage of ADS with PVA group was less than that of the other two groups at late stage without significant difference. Drainage was observed in 6 of 8, 6 of 8 and 3 of 8 rats in PDS, ADS and ADS with PVA group at day 4 separately, and Drainage was observed in 3 of 8, 0 of 8 and 0 of 8 rats in PDS, ADS and ADS with PVA group at day 5 separately. The volume of drainage at late stage in ADS with PVA group was 112.50 ± 145.77 μL compared with 662.50 ± 763.33 μL in PDS group and 350.00 ± 297.61 μL in ADS group (Fig. 2B–D, P = 0.10). This maybe due to the earlier healing of POPF in ADS with PVA group. Drainage was observed in none of the three groups from day 6.

3.2. Ascitic amylase

As shown in Fig. 3, ascitic amylase level decreased fast during the observation period in all the three groups. Neither significant difference on ascitic amylase level nor the trend of ascitic amylase level change was observed among the three groups.

3.3. Abdominal fluid collection

CT scan was performed on all rats at day 6 after pancreatic resection. 2 of 8, 2 of 8 and 0 of 8 rats in PDS, ADS and ADS with PVA group were with obvious abdominal fluid collection. Fig. 4A showed a rat with abdominal fluid collection in PDS group, Fig. 4B showed a rat with abdominal fluid collection in ADS group, Fig. 4C showed a rat in ADS with PVA group. The CT scan findings demonstrated that ADS with PVA removed abdominal fluid collection effectively.

3.4. Abdominal inflammation

Severe abdominal inflammation was observed in both rats with and without abdominal fluid collection on CT scan in PDS group and ADS group. Fig. 5B and C showed rats without abdominal fluid collection in PDS and ADS group separately. Significant intra-abdominal adhesions and inflammation can be seen in macrofindings. Different from that in PDS and ADS group, rats in ADS with PVA group showed mild intra-abdominal adhesions and inflammation in macrofindings (Fig. 5D). The inflammatory score in ADS with PVA group was 0.375 ± 0.52 compared with 1.25 ± 1.16 in PDS group and 1.375 ± 1.19 in ADS group (Fig. 5E). The intra-abdominal adhesions and inflammation of rats in ADS with PVA group was comparable with that of rats underwent sham surgery (Fig. 5A).

3.5. Histological findings

Histologically, a large number of inflammatory cells were identified and many pancreatic glands were destroyed in the pancreatic transection stumps of the rats in PDS and ADS group (Fig. 6A and B). In contrast, fewer inflammatory cells were observed and pancreatic glands were almost unchanged in the pancreatic transection stumps of the rats in ADS with PVA group (Fig. 6C). The inflammatory score was 1 ± 0.76 in ADS with PVA group compared with 2.13 ± 1.13 in PDS group and 2.25 ± 1.28 in ADS group (Fig. 6D).

4. Discussion

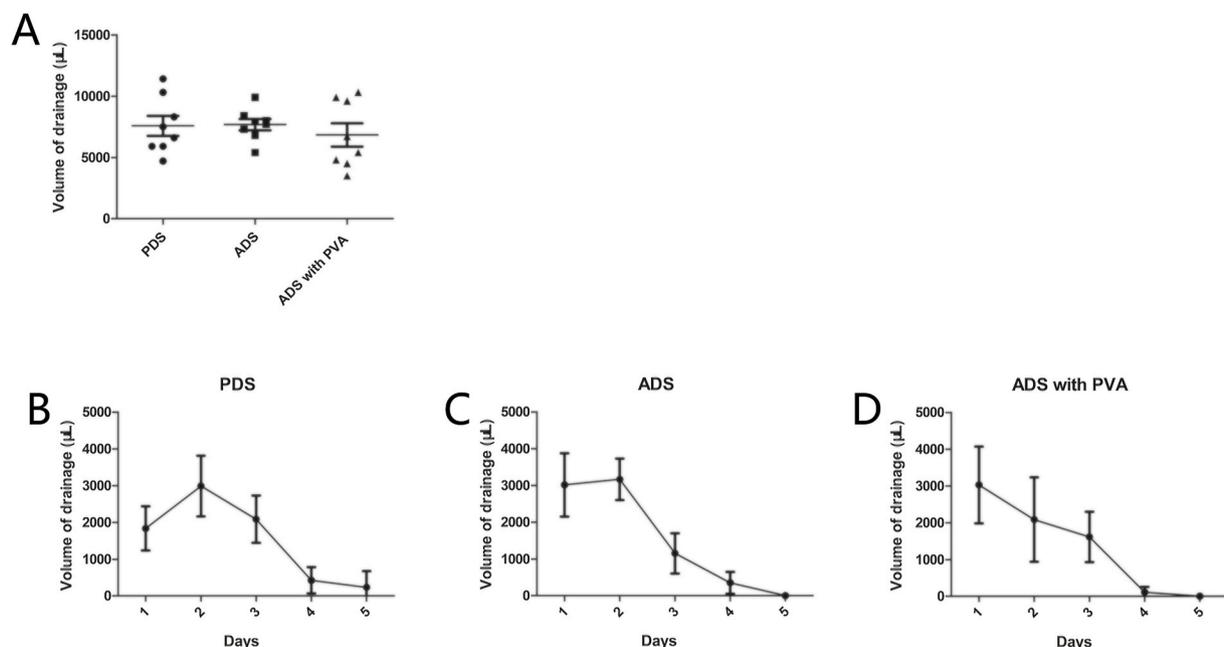This is a study that compared differences of PDS, ADS and ADS with

Fig. 2. Drainage volume after pancreatic resection. (A) The total volume of the drainage during the observation period of the three groups. (B) The volume of drainage in PDS group. (C) The volume of drainage in ADS group. (D) The volume of drainage in ADS with PVA group.
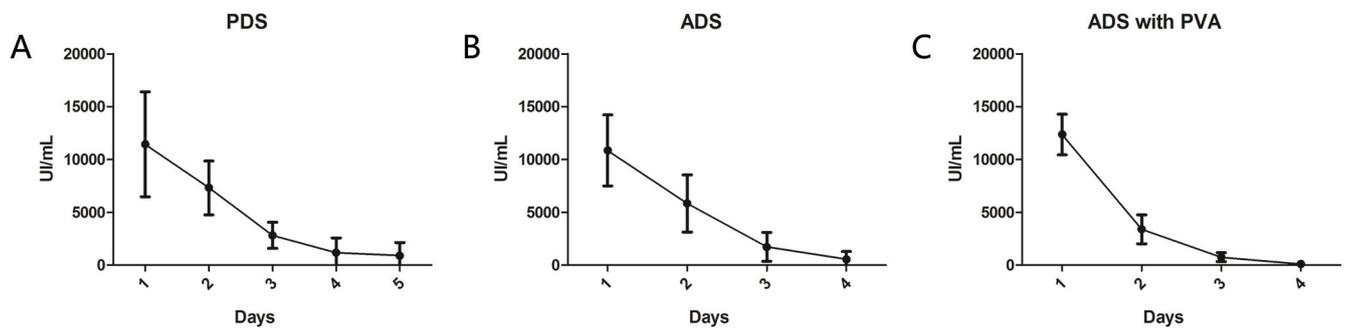
Fig. 3. Ascitic amylase level after pancreatic resection. (A) The ascitic amylase level in PDS group. (B) The ascitic amylase level in ADS group. (C) The ascitic amylase level in ADS with PVA group.
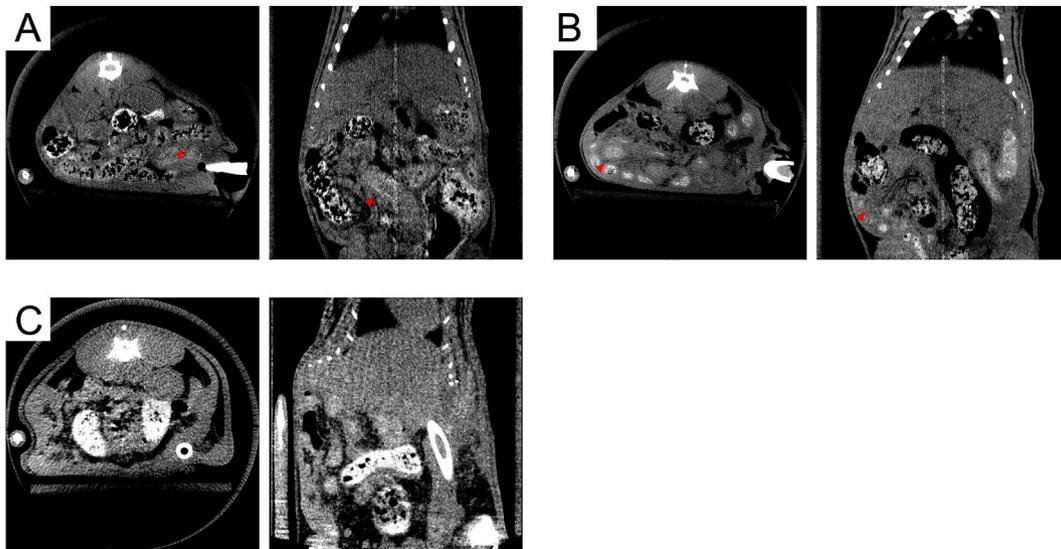

Fig. 4. CT scan findings. CT scan was performed on day 6 after pancreatic resection. (A) CT scan in PDS group. (B) CT scan in ADS group. (C) CT scan in ADS with PVA group. Red triangle showed abdominal fluid collection. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PVA in treatment of POPF using a rat model. Results showed that ADS with PVA drained ascites effectively. The effective drainage of pancreatic juice reduced the inflammation of abdominal organ and pancreatic resection stumps, and may promote the healing of POPF.

Intraperitoneal drainage after pancreatic operations has been the common practice for pancreatic surgeons, which is understandable given the frequency of POPF and its associated complications. The rationale behind placement of intraperitoneal drainage after pancreatic operations may contribute to effective evacuation of fluid including bile, pancreatic juice, and chyle that may accumulate within abdomen postoperatively. The evacuation of fluid might decrease the risk of secondary infection and hemorrhage, and the drainage might also serve as a warning sign of the infection and hemorrhage [17,18].

The routine practice of intraperitoneal drainage after pancreatic operations was ever questioned. The drainage can lead to retrograde infection of ascitic fluid, and bacterial contamination in ascitic fluid is associated with the development of clinically relevant pancreatic fistula [19–21]. But a randomized prospective multicenter trial of pancreatic resection with and without routine intraperitoneal drainage was terminated early due to increased severity and rate of morbidity as well as a nearly four-fold increase of mortality in the no drainage group [6]. To date, although the controversy was not totally eliminated, most pancreatic surgeons preferred practice of prophylactic drainage after pancreatic resection [3].

Although the prophylactic drainage after pancreatic resection was

proposed by pancreatic surgeons, it is still unknown that which type of drain system is more suitable [9,22]. ADS was expected to remove collected bile, chyle and pancreatic juice within the peritoneal cavity effectively and reduce the incidence of infection and subsequent hemorrhage due to enzymatic erosion of intraabdominal tissue. However, the efficacy of the ADS for irregular spaces was uncertain, and high-pressure gradient generated by the ADS was be worried to promote the development of a POPF. In the only RCT involving 160 patients randomized to ADS versus passive system, Jiang et al. found a trend of reduction in severity of complications associated with POPF in the group of ADS, but no differences in morbidity or mortality at 30 days between the two groups [12].

Different from ADS for POPF, ADS for non-healing wounds, which was known as negative-pressure wound therapy (NPWT) system, contains a resilient, reticulated open-pore foam-surface dressing. The open cell foam that allows egression of fluids at the same time enables equal distribution of the negative pressure over the entire wound surface, irrespective of any surface irregularities [23]. Thus, we tentatively put forward that the addition of open cell foam to the ADS for pancreatic operation should drain the fluid more effectively and promote the healing of POPF. None foam type has been reported to be used in ADS for POPF. PVA usually have smaller and denser pores developed for the coverage of more delicate structures such as tendons and blood vessels in NPWT system. This foam type additionally restricts granulation formation and is thought to be remove form tissue. In the present study, we
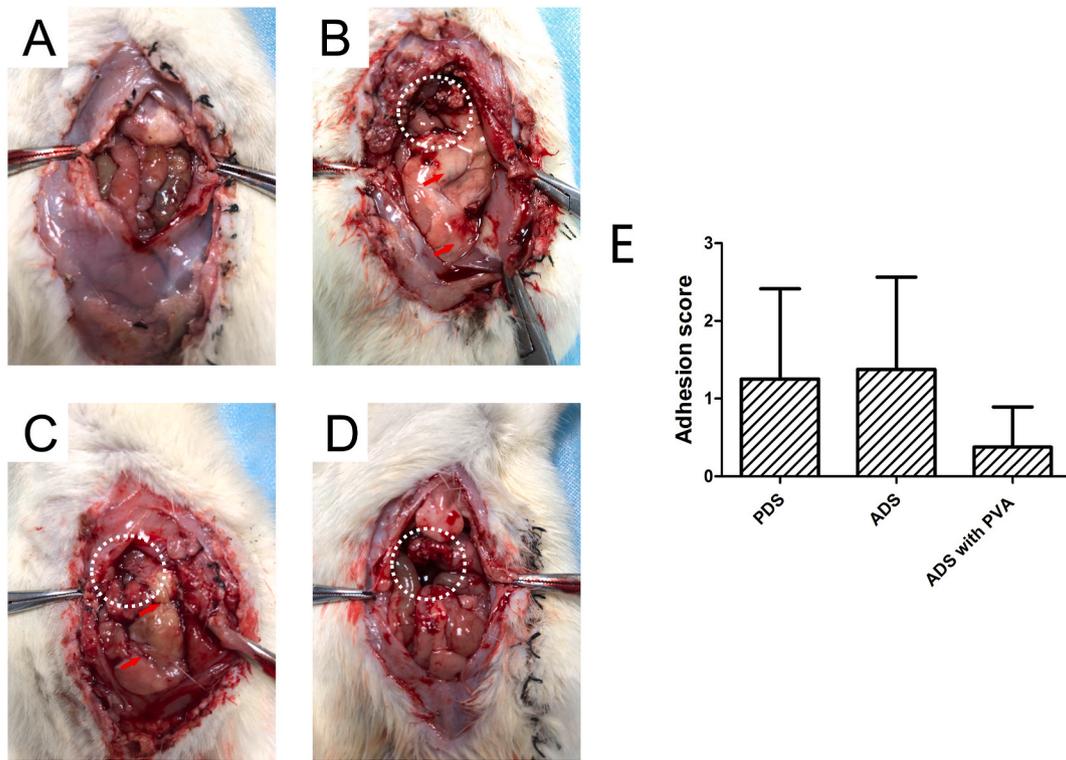
Fig. 5. Macrofindings. Rats were sacrificed on day 7 after pancreatic resection. (A) Macrofindings in rat underwent sham surgery. (B) Macrofindings in PDS group. (C) Macrofindings in ADS group. (D) Macrofindings in ADS with PVA group. (E) Adhesion scores. White circle showed area of operation, red arrow showed severe inflammation in the abdomen. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
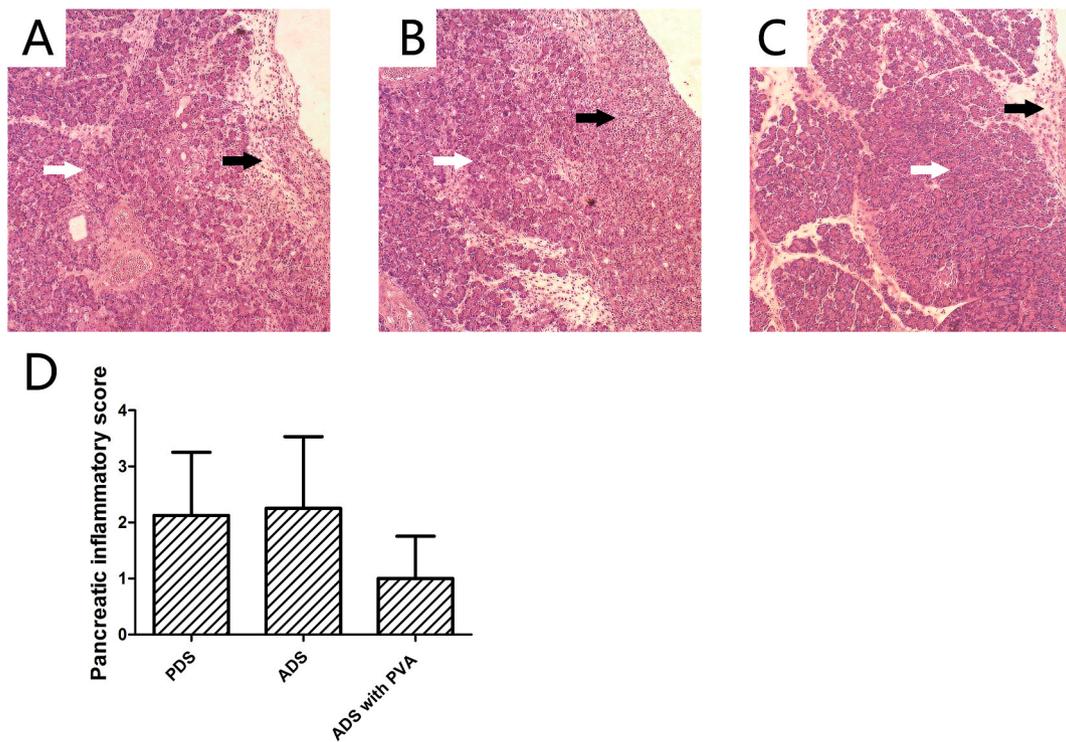

Fig. 6. Histological findings. Rats were sacrificed on day 7 after pancreatic resection, and pancreatic transection stumps were stained by hematoxylin and eosin. (A) Histological findings in PDS group. (B) Histological findings in ADS group. (C) Histological findings in ADS with PVA group. (D) Pancreatic inflammatory scores. White arrow showed pancreatic glands with generally normal appearance, black arrow showed invaded inflammatory cells and destroyed pancreatic glands.

used PVA as the open cell foam. The results verified our hypothesis. A distal pancreatosplenectomy model was used in the present study. For pancreaticoduodenectomy, the PVA can be designed to totally package the pancreatic anastomosis, thus the ADS with PVA should also work for pancreaticoduodenectomy. Up to now, a separate line was placed in the reticulated open-pore foam-surface dressing of NPWT system to add saline or antiseptic solution to the wound bed. It can set a variable 'dwell time' and remove the solution automatically after the preset period of time. It was considered to be able to remove wound debris and the reduction of microbial load and thus promote wound healing [24]. Whether similar structure improve the ADS for pancreatic operation need further investigation.

A limitation of the present study is the insufficiency on mechanism. Only Immunohistochemistry was performed, and results showed that ADS with PVA reduced inflammation of pancreatic transection stumps. Many different theories have been established on mechanisms of NPWT system previously. NPWT system was reported to promote wound healing by reduction of edema formation, reduction of bacterial burden, improvement of local blood circulation, stimulation of growth factor expression, Increase of collagen organization, increase of fibroblasts migration, reduction of inflammatory response and reduction of biofilm [25–29]. The ADS for pancreatic operation may share similar mechanism of NPWT system on promotion of wound healing. Another limitation was that a pancreatic resection without pancreatic anastomosis. POPF after pancreatic resection with pancreatic anastomosis is usually more complicated and severe than that without pancreatic anastomosis. The drainage may not only contain pancreatic juice but may also contain intestinal juice and bile. The third limitation was many issues need to be addressed before clinical application. In the present study, PVA act as the reticulated open-pore foam-surface dressing for the novel ADS. The proper material, pore diameter and pressure for clinical use needs further study. In addition, materials in the prophylactic ADS should be easy to be removed when the POPF was excluded or cured without residue for clinical use. A long reticulated open-pore foam-surface dressing with similar diameter of drainage tube that wrapped around the drainage tube may make the system be easy to remove (Supplementary Fig. 1). Absorbable foam interfaces could be another solution of the issue.

In conclusion, the study on rat model of POPF showed that the ADS with PVA was superior to PDS and ADS without PVA on drainage efficacy and pancreatic stump healing. The abdominal inflammation in the group of ADS with PVA was not as severe as that in the other two groups, which maybe due to its more effectively drain of pancreatic juice. Addition of reticulated open-pore foam-surface dressing to ADS for pancreatic operation has the potential on promotion of POPF healing and decrease of severity POPF.

Provenance and peer review

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Consent for publication

Not applicable.

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Ethical Approval

The study was approved by the Ethics Committee at Tongji Medical College, HUST, China

Research Registration Unique Identifying Number (UIN)

Name of the registry:
Unique Identifying number or registration ID:
Hyperlink to your specific registration (must be publicly accessible and will be checked):

Author contribution

Yang Li, Ying Sun: performed the experiments; Zhiqiang Liu, Yongfeng Li: collected the data; Shanmiao Gou: wrote the paper and analyzed the data. All authors read and approved the final manuscript

Guarantor

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.102559.

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