

Answering reviewers:

Dear reviewers:

First of all, thank you for your first decision, consideration for acceptance and suggestion of revision. Actually, your suggestions will take very important role to our research.

According to reviewer's (NO 2445717) suggestions, we added the necessary conclusion in the end of the discussion. The reference 14 was corrected. The directly related papers (A Newly Designed Anal Fistula Plug: Clinicopathological Study in an Experimental Iatrogenic Fistula Model. *Int Surg.* 2013 Apr-Jun;98(2): 122-8), (Histologic Analysis of Acellular Dermal Matrix in the Treatment of Anal Fistula in an Animal Model. *Journal of the American College of Surgeons*, Volume 208, Issue 6, June 2009, Pages 1099–1106) and (Experimental model of anal fistula in rats. *Journal of Coloproctology*. Volume 33, Issue 3, July–September 2013, Pages 135-138) were included and discussed in the text.

Secondly, According to reviewer's (NO 2542015) suggestions, inconsistency of the "perioperative management" section and "postoperative intervention" section was corrected. (On the 28th day after surgery, the rubber bands were taken out of the experimental group. The two groups of animals received clinical magnetic resonance imaging and histopathological evaluation on the 38th day and 48th day after surgery respectively). Essential figure legend was given, and all figures were separated. We separated the figure 1-3, and Essential figure legend included Corresponding to each figures were completed.

Answering to Reviewer's (NO 2542015) professional question from 1 to 3:

Answer to Question 1: We concluded that the anal fistulas established with a rubber band ligation method could simulate the clinical anal fistula in pathophysiology. Compared with construction of animal model using BLAKE silicone drains of Masayasu A et al, rubber band model creation process was easily observed in clinical. The imaging and pathophysiological manifestation of rubber band created model was very similar to human. We searched most of the animal anal fistula model research article using pubmed, web of science database from all the professional publication. However, we collected only 4 related research. Researchers in these studies confirmed the construction of fistula just according to the clinical manifestation only. They did not detect how the animal model was established or observed by any measurement method such as pathophysiology, magnetic resonance imaging or histopathology. In our research, we detected the rubber band was soft and reliable using magnetic resonance imaging or histopathology. Although we could not conclude the very exact pathophysiologic evidence of model creation, but we believed that imaging assessment and histopathology were more practical and more similar to human fistula in ano. In addition, pathophysiologic detection of fistula model was not easily obtained. We think that the anal fistulas established with a rubber band ligation method could simulate the clinical anal fistula in pathophysiology. We will perform this part of the study in order to obtain pathophysiologic evidence of rubber band ligation in the near future work.

Answer to Question 2: The core process of fistula model creation is epithelialization of the artificial fistula tract. Therefore, we concluded that diameter size of rubber band does not affect the success rate of modeling.

However, considering excessive foreign body reaction, we selected the rubber band with diameter of no more than 0.5cm.

Answer to Question 3: Created fistulas assessed histopathologically had a lumen, and abundant surrounding granulation tissue similar to that seen in human fistula-in-ano. Epithelialization was the key evident in fistula track. Fistulas were visualized as high signal tracks using magnetic resonance imaging. It is also very similar to that seen in human fistula-in-ano using MRI. On the 28th day after surgery, the rubber bands were taken out of the experimental group. The epithelization time of created fistula tract is at least three weeks according to the previous research(A Newly Designed Anal Fistula Plug: Clinicopathological Study in an Experimental Iatrogenic Fistula Model. Int Surg. 2013 Apr-Jun;98(2): 122-8). After three weeks, the created fistula will not be closed. The typical histopathologic canal epithelization will be find at least one month later after surgery according to the research of Han Jia Gang(Histologic Analysis of Acellular Dermal Matrix in the Treatment of Anal Fistula in an Animal Model. Journal of the American College of Surgeons,Volume 208, Issue 6, June 2009, Pages 1099–1106). Therefore ,the two groups of animals received clinical magnetic resonance imaging and histopathological evaluation time was selected two important time : on the 38th day and 48th day after surgery respectively. The meaning of these mesurment is to detect the stability and reliability of created fistula.

Dear reviewers:

After discussion about your questions or suggestions, we have informed necessary information to our animal experiment. Therefore, we thank you very much for your peer review process and your work. In the same time, thank you again for your first decision, consideration for acceptance and necessary suggestion of revision to our research. We will perform additional

part of the study to obtain more evidence of model creation and therapeutic findings in the future work.