Application of acellular dermal matrix for intestinal elongation in animal models

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AIM: To investigate the efficacy of acellular dermal matrix (ADM) for intestinal elongation in animal models.

METHODS: Japanese white big-ear rabbits (n = 9) and Wuzhishan miniature pigs (n = 5) were used in the study. Home-made and commercial ADM materials were used as grafts, respectively. A 3-cm long graft was interposed in continuity with the small bowel and a side-to-side anastomosis, distal to the graft about 3-4 cm, was performed. The animals were sacrificed at 2 wk, 4 wk, 8 wk and 3 mo after surgery and the histological changes were evaluated under light microscope and electron microscope.

RESULTS: The animals survived after the operation with no evidence of peritonitis and sepsis. Severe adhesions were found between the graft and surrounding intestine. The grafts were completely absorbed within postoperative two or three months except one. Histological observation showed inflammation in the grafts with fibrinoid necroses, infiltration of a large amount of neutrophils and leukomonocytes, and the degree varied in different stages. The neointestine with well-formed structures was not observed in the study.

CONCLUSION: It is not suitable to use acellular dermal matrix alone as a scaffold for the intestinal elongation in animal models.

INTRODUCTION

Short bowel syndrome (SBS) is defined as a spectrum of small intestinal dysfunction for a major portion of small intestines being resected and the patients need chronic total parenteral nutrition (TPN) in lifetime. However, a long-term use of TPN is associated with significant
morbidity and mortality\cite{1-3}, such as system infection and progressive cholestatic liver disease and many patients could not afford the high cost of the nutrition support. Several bowel elongation procedures were successful in increasing the absorptive surface area, but these methods were not widely applied for serious complications. Small intestinal transplantation may be a valid option, but the result is unsatisfactory for immunologic problems.

Recently, acellular biomaterials have received extensive attentions, including acellular dermal matrix (ADM). ADM provides a biologic scaffold that allows native cellular ingrowth and tissue remodeling. In this study, we applied the ADM as scaffold for the mucosal growth and intestinal elongation in animal models.

**MATERIALS AND METHODS**

**Animal**

Japanese white big-ear rabbits (\(n = 9\), body weight 2.0-2.2 kg) and Wuzhishan miniature pigs (\(n = 5\), body weight 41-44 kg) were used in the study. Animal care and use complied with the institutional regulations established and approved by the Animal Care and Use Committee of the Capital Medical University for Animal Research Program. All the animals were housed in individual cages under standard laboratory conditions and fed animal chow and water ad libitum.

**Preparation of ADM**

A Japanese white big-ear rabbit and a Wuzhishan miniature pig were used as the donors, respectively. Segments of the small intestine were harvested and cut into 5-cm lengths. The grafts were sterilized in 75% ethanol, rinsed in saline solution, and stored at 4°C in a Penicillin-Streptomycin solution until use. The dermis was acellularized in accordance with the method reported previously by Takami et al\cite{4}. In brief, the segment of the small intestine was treated with 0.25% Trypsin (Roche corp.) at 4°C for 24 h to remove the epidermis and other cellular components from the dermal matrix. Subsequently, the dermal matrix was incubated in buffered 0.5% Triton X-100 (Sigma-Aldrich Corp.) for 24 h at 37°C. An oscillator was used during these procedures. Dispase-Triton ADM was then extensively washed with phosphate buffered solution (PBS) and stored in PBS at 4°C until use (Figure 1). All solutions were treated with filter sterilization.

**Surgical procedures**

Surgical procedures were performed under general anesthesia with 3% Pentobarbital (25 mg/kg). All the Japanese white big-ear rabbits (group A) and two Wuzhishan miniature pigs (group B) were transplanted with the allograft materials respectively, and the other two pigs (group C) with the human acellular dermal matrix (HADM, Ruimu\textsuperscript{®}, Qingyuanweiye Bio-Tissue Engineering Ltd, Beijing, China).

A laparotomy in the right lower abdominal quadrant was performed, and the small bowel was transected in the middle portion. A graft (3 cm long) was interposed in continuity with the small bowel using interrupted end-to-end two layers anastomosis (muscularis-serosa layer and full-thickness layer) with 1-0 nonabsorbable sutures. Then a side-to-side anastomosis, distal to the graft about 3-4 cm (Figure 2), was performed. The defect of the mesentary was repaired to avoid enterocele. All the viscera were laid back to the abdominal cavity. The abdominal wall was closed with running suture, while the skin with interrupted suture. In the group of HADM, the graft was sewn at the edges with sutures to construct a tube, and then the same operation was repeated as mentioned above.

All the animals were maintained on a liquid diet and water for 48 h and then on a full diet. Two rabbits were sacrificed at 2, 4 and 8 wk, respectively after surgery. The study endpoint was 3 mo after the operation. Pathologic specimens including the anastomotic site were fixed in formalin, embedded in paraffin, and processed routinely for light microscopy. Histological changes were evaluated under light microscope and transmission electron microscope.

**RESULTS**

The animals survived after the operation with no evidence of peritonitis or sepsis. All the rabbits had gradual weight loss from median 2.1 to 1.8 kg and the survival after the operation was no longer than 8 wk, while the body weight of pigs increased from median 42 to 51 kg which were executed at 3 mo after the surgery according to the scheme.

In group A, severe adhesions were found between the graft and surrounding intestine. There was almost no blood supply in the grafts and the lumens were full of dry stool or pus at 2 and 4 wk after surgery. At the 8th postoperative week, the grafts totally disappeared, similar to group B and one pig in group C and there were only two blind-ended pouches. The other one in group C seemed to be the most successful one considering the small intestinal continuity was restorative. But the graft shrank from 3 to 0.2 cm and became a narrow-ring without flexibility (Figure 3).

Histological observation showed acute inflammation in the tissue during the first two weeks. In the first week, there were focal fibrinoid necroses and infiltration of a large amount of neutrophils and leukomonocytes (Figure 4A and B). In the second week, there was a further development of the inflammation. The fibrinoid necroses and cell infiltration became dispersed and inflammatory exudates of neutrophils and pus cells could be seen (Figure 4C and D). At the same time, a lot of microangi and fibroblasts appeared in the scaffold. By the 4th postoperative week, the infiltration of leukocytes and leukomonocytes mitigated. No well-formed structure could be seen and all the layers were contorted in the narrow-ring of group C (Figure 5). The thickening serosa and infiltration of a small amount of leukocytes and leukomonocytes could be discovered. Immunocytochemical stain with smooth muscle actin showed smooth muscle cells in the narrow-ring of ADM with disordered structure.
DISCUSSION

Several surgical procedures have been used to improve the SBS\[^{5,6}\]: (1) to decrease transit time of the bowel content by creation of intestinal valves or interposition of bowel segments; (2) to increase mucosal surface area; and (3) to increase the bowel length or to transplant the bowel. Objective assessment of these surgical techniques was deficient due to a relatively small number of patients. The small intestine transplantation is becoming a less morbid therapy in recent years, but it remains complicated and is limited due to the donor availability.

ADM is a dermal biomaterial in which all of the cellular elements have been removed. The biologic properties of ADM, including its ability to support tissue regeneration repopulation with fibroblasts, revascularization, new collagen deposition and eventual absorption and replacement with native tissue permit its use in tissue reconstruc-

Figure 1 Acellular dermal matrix. A: Segments of the small intestine; B: Segments of acellular dermal matrix; C, D: Hematoxylin and eosin-stained histological photomicrography showed that all the cellular elements of the small intestine have been removed (C: ×100, D: ×400).

Figure 2 Surgical procedures. a: ADM; arrow: Side-to-side anastomosis.

Figure 3 Postoperative gross specimen. A: ADM has been absorbed completely (a: Blind-ended pouch); B: The graft was shrinking (arrow: The ADM material).

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reconstruction, contaminated abdominal wall defects and fistula.

We supposed that the ADM can be used as a scaffold for tissue regeneration with the normal morphology of the mucosa, submucosa, muscle and serosa layers. An in vivo study in dog models showed that an extracellular matrix (ECM) plus autologous muscle could facilitate the reconstruction of the esophagus with near normal biomechanical properties and structure\cite{12}. Pahari et al\cite{13} used the acellular human dermis in the form of tubular scaffolds for intestinal elongation. Intact mucosa with well-formed crypts was evident at 6 mo after operation\cite{13}.

But it did not develop like what we had designed in the study. The grafts were almost completely absorbed...
in two or three months. Pus could be seen in the lumens of ADM and histological examination showed the inflammatory exudates of neutrophils and pus cells. Thus, the ADM might be a source of infection in this study, though we have tested that the ADM can be used in contaminated wound for its resistance to infection. Inadequate blood supply and the rapid absorption were the most likely reasons. All the ADM depends on the blood infiltrating from the two anastomotic ends and the supply was very limited comparing with the granulation tissue of the wound surface. Immunoenzytochemical stain with smooth muscle actin showed smooth muscle cells in the narrow-ring at 3 mo after surgery, which is an exciting event and consistent with our previous study.

The biggest challenge of bowel tissue engineering is the recovery of peristaltic activity of the regenerated bowel, which needs both smooth muscle and reinnervation of the regenerated bowel. It seemed that there was no evidence of innervations of the neointestine to date. Even the neointestine had well-formed structures, the dismotile segment may become dilated, resulting in fecal stasis, bacterial overgrowth and malabsorption.

Our conclusion is different from some of the previous studies. We have made several reviews of the whole procedure, but no obvious errors could be found. Even if there were some problems in the process of preparation of the ADM, which was more elaborate, group C (HADM, Ruinuo) had the same result. So we believe that the result is reliable. Though ADM has been successfully used to repair and reconstruct the tissue defects in many fields, it seemed that the single use of ADM is not suitable to the intestinal elongation in the present study and there is a long way to go before the ADM can be used for intestinal elongation.

COMMENTS

Background

Short bowel syndrome (SBS) has puzzled surgeons for a long time. Though some treatments have been reported, the effects are unsatisfactory. In recent years, acellular dermal matrix (ADM) has been used in many fields to provide a biologic scaffold for tissue regeneration or scarless healing, and few studies for SBS have been performed, and the results are uncertain.

Research frontiers

There are only few studies about ADM used for intestinal elongation. This study was designed to use both the home-made and commercial ADM materials. At the same time, different species were transplanted with the allograft materials. All of these can improve the credibility of this research.

Innovations and breakthroughs

This study was designed according to the previous work, but it showed different results. The authors gave some possible reasons, which were worthy of in-depth studies.

Applications

ADM may be used for intestinal elongation, but some reforms are necessary in its future application.

Terminology

ADM is a new material that all of the cellular elements have been removed. The kind of material has special biologic properties, including support of tissue regeneration with concomitant revascularization and neouploration with fibroblasts, new collagen deposition, absorption and replacement with native tissues.

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