Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Association for Academic Surgery

Bioscaffold-mediated mucosal remodeling following short-segment colonic mucosal resection

Jenna L. Dziki, PhD,[a,b,1] *Timothy J. Keane, PhD,*[c,1] *Shahab Shaffiey, MD,*[d] *Dan Cognetti, BS,*[a] *Neill Turner, PhD,*[a,e] *Deborah Nagle, MD,*[f] *David Hackam, MD, PhD,*[g] *and Stephen Badylak, DVM, PhD, MD*[a,b,e,*]

[a] *McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania*
[b] *Department of Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania*
[c] *Department of Materials, Imperial College London, London, UK*
[d] *Division of Pediatric Surgery, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania*
[e] *Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania*
[f] *Division of Colon and Rectal Surgery, Beth Israel Deaconess Medical Center, Boston, Massachusetts*
[g] *Department of Surgery, Johns Hopkins University, Baltimore, Maryland*

ARTICLE INFO

Article history:
Received 17 February 2017
Received in revised form
13 June 2017
Accepted 19 June 2017
Available online xxx

Keywords:
Extracellular matrix
Colonic mucosal resection
Bioscaffold
Constructive tissue remodeling

ABSTRACT

Precancerous or cancerous lesions of the gastrointestinal tract often require surgical resection via endomucosal resection. Although excision of the colonic mucosa is an effective cancer treatment, removal of large lesions is associated with high morbidity and complications including bleeding, perforation, fistula formation, and/or stricture, contributing to high clinical and economic costs and negatively impacting patient quality of life. The present study investigates the use of a biologic scaffold derived from extracellular matrix (ECM) to promote restoration of the colonic mucosa following short segment mucosal resection. Six healthy dogs were assigned to ECM-treated (tubular ECM scaffold) and mucosectomy only control groups following transanal full circumferential mucosal resection (4 cm in length). The temporal remodeling response was monitored using colonoscopy and biopsy collection. Animals were sacrificed at 6 and 10 wk, and explants were stained with hematoxylin and eosin (H&E), Alcian blue, and proliferating cell nuclear antigen (PCNA) to determine the temporal remodeling response. Both control animals developed stricture and bowel obstruction with no signs of neomucosal coverage after resection. ECM-treated animals showed an early mononuclear cell infiltrate (2 weeks post-surgery) which progressed to columnar epithelium and complex crypt structures nearly indistinguishable from normal colonic architecture by 6 weeks after surgery. ECM scaffold treatment restored colonic mucosa with appropriately located PCNA+ cells and goblet cells. The study shows that ECM scaffolds may represent a viable clinical option to prevent complications associated with endomucosal resection of cancerous lesions in the colon.

* *Corresponding author*. University of Pittsburgh, McGowan Institute for Regenerative Medicine, Pittsburgh, PA 15219. Tel.: +14126245253; fax: +14126245256.
E-mail address: badylaks@upmc.edu (S. Badylak).
[1] These authors contributed equally.
0022-4804/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jss.2017.06.066

Introduction

Disorders of the lower gastrointestinal tract affect more than six million patients worldwide.[1-3] Diseases such as inflammatory bowel disease and colorectal cancer are treated by medical and/or surgical methods. Endomucosal resection (EMR) is often required for severe colonic pathologies and particularly, cancer resection. Recent work has shown that neoplasia confined to the epithelium (T1) can be treated with transanal excision of the colonic mucosa,[4] but removal of large lesions can be associated with complications including bleeding, perforation, fistula formation, and/or stricture.[5-7] The incidence of such complications is correlated well with the size of mucosal resections. Therefore, larger lesions are often too risky for local excision and require colectomy, which though an effective solution, is associated with increased morbidity and a significant impact on quality of life.[8-10]

A biomaterial-based strategy involving endoscopic deployment of a biologic scaffold composed of extracellular matrix (ECM) has shown promising results following excision of large areas of neoplasia confined to the mucosa of the esophagus.[11,12] To date, 13 patients with esophageal adenocarcinoma (T1a) confined to the mucosa or Barrett's disease with high-grade dysplasia have been treated with ECM bioscaffolds following long-segment mucosal resection.[12-14] All patients showed rapid mucosal restoration, and none of the patients experienced recalcitrant stricture formation or cancer recurrence. These promising outcomes suggest that ECM bioscaffolds may enable aggressive endomucosal resection (EMR) in the lower gastrointestinal tract by promoting mucosal healing and mitigating stricture formation. The objective of the present study was to determine the ability of small intestinal submucosa (SIS) ECM to promote mucosal healing and prevent stricture following short segment colonic mucosal resection in a canine pilot study.

Materials and methods

Overview of study design

All animal procedures were conducted in accordance with the University of Pittsburgh's Institutional Animal Care and Use Committee. The ability of SIS-ECM to promote mucosal remodeling was evaluated in a dog model of rectal mucosectomy. Six mongrel dogs were divided into two experimental groups. Four animals were treated with multilaminate SIS-ECM sheets, and two animals served as mucosectomy only controls. Biopsies were taken biweekly to evaluate histologic changes following surgery. Colonoscopies were conducted biweekly postoperatively to examine gross changes in the colon. Animals were euthanized at 6 or 10 wk after surgery.

Production of bioscaffold materials

SIS-ECM was produced as previously described.[15] Briefly, the small intestine was isolated from market weight pigs (240-260 lbs, Tissue Source, Lafayette, IN). The intestine was mechanically abraded to remove the tunica muscularis externa and the majority of the tunica mucosa. The remaining tunica submucosa and basilar portion of the tunica mucosa was then disinfected and decellularized in a 0.1% peracetic acid solution followed by two rinses in phosphate-buffered saline solution and deionized water. A tubular scaffold device was created by vacuum pressing eight, sequentially wrapped sheets of SIS-ECM around a mandrel (outer diameter = 30 mm). Dry, multilaminate tubular scaffolds were terminally sterilized by exposure to ethylene oxide.

Surgical procedure

All procedures were performed in accordance with the Institutional Animal Care and Use Committee of the University of Pittsburgh guidelines. Six mongrel dogs (18-25 kg) were subjected to short segment (4 cm) rectal mucosectomy using a transanal approach to remove the mucosa of the rectum and distal sigmoid colon. After bowel preparation, each dog was placed in the supine position under general anesthesia and a Lone Star Retractor was used for transanal access (Fig. 1A). A mucosectomy was performed by circumferential sharp incision at the dentate line for a distance of 4 cm (Fig. 1B), and electrocautery was used to achieve hemostasis. Control animals ($n = 2$) were subjected to mucosectomy without the implantation of a scaffold. In the experimental group ($n = 4$), size-matched tubular SIS-ECM bioscaffolds were implanted into the mucosal defect using vicryl sutures, with four sutures placed at wound margins at 12-, 3-, 6-, and 9-o'clock positions (Fig. 1C-F). The dogs were closely monitored daily for weight loss, bloody stools, and rectal bleeding. Colonoscopy was performed biweekly postoperatively and included imaging and biopsy evaluation of the mucosa from the distal anus to the most proximal suture line of the defect. Blood was collected for a complete blood count preoperatively and at 24 h, 72 h, and 1 wk postoperatively (Table 1). Animals were sacrificed at 6 or 10 wk postoperatively, and the distal colon was resected *en bloc* using nonabsorbable proximal and distal marker sutures to identify the defect site. The tissue was imaged grossly, fixed in neutral buffered formalin, and then serially sectioned for histologic evaluation.

Biopsy collection and histology

The tissue remodeling response was monitored via biweekly biopsy with endoscopic guidance under light sedation and via water enema. A 2-3 mm diameter specimen was collected from the scaffold implant site at distal, middle, and proximal sites and was fixed in neutral buffered formalin. Hematoxylin and eosin staining, Alcian blue staining, and proliferating cell nuclear antigen (PCNA) immunolabeling were conducted to evaluate the remodeling response. Semiquantitative histomorphologic scoring was completed by two blinded scorers according to previously established criteria for hyperplasia, goblet cell loss, crypt changes, and villous blunting as shown in Table 2. Cumulative scores were averaged between two blinded scorers. Proliferating cells and mucin-expressing cells were quantified with a CellProfiler Image analysis software pipeline.