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Original Article

Histological characteristics of collagen denaturation and injuries in bipolar radiofrequency-induced colonic anastomoses

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ABSTRACT

Bipolar radiofrequency-induced thermo-fusion has been explored as an advanced surgical method for intestinal anastomoses; however, the histological characteristics of collagen denaturation and injuries arising from this process remain unclear. The aim of this study was to investigate the microcosmic changes and tissue damage of fusion regions with various parameters of injury. *Ex vivo* colons of pigs were fused serosa-serosa on two carrier rings, which were installed on a homemade anastomotic device. Five levels of compressive pressure from 171 to 313 kPa were applied for 5 s to fuse the colons under radiofrequency power of 160 W, and then the collagen denaturation of the fused region was examined by transmission electron microscopy. Light microscopy was utilized to observe histological slices that were stained with picrosirius red in order to visualize the tissue injuries under two levels of radiofrequency power (120 *vs.* 140 W) and operation time (5 *vs.* 10 s). Transmission electron micrographs showed that increased compressive pressure led to thicker denatured collagen fibrils and wider gaps between each collagen fibril. Serosa adhesion regions appeared abundant in collagen. No histological differences were observed when 120 W of power was applied for 5 and 10 s. Significant muscle cracking occurred when colons were fused using 140 W for 5 s. When the operation time was extended to 10 s, 140 W led to tight fusion and less splitting on muscles. These results suggest that higher compressive pressure results in more severe collagen unfolding and also reduces collagen crosslinking in fused colons. Improved radiofrequency power along with operation time could avoid tissue injury upon radiofrequency-induced colonic anastomoses.

Introduction

Depending on the radiofrequency (RF) of interaction with biological tissue, electrosurgery can achieve dissection, cautery, or ligation both for laparoscopic and open surgeries [1]. To evaluate the safety and reliability of electrosurgery, studies to assess the histological characteristics of injuries caused by different RF instruments with various parameters, including compressive pressure (CP), RF power (P) and operation time (T) [2,3], have been carried out on animals and humans. In particular, the proposition of using RF-induced thermo-fusion to create colonic anastomoses [4–8] represents a potentially important advance [8] but also poses a new challenge.

In comparison with techniques that seal vessels by LigaSure [9–12] or ultrasound [13,14], colonic anastomoses by thermo-fusion is a more complex process. New collagen in the submucosa and re-epithelialization near the fusion region occur after RF-induced "side-side" intestinal anastomoses *in vivo* [8]. The surrounding tissue of the fused region must heal within a period of time so that colon reconstruction is achieved.

Furthermore, collagen denaturation in fused tissue, which is demonstrated by uncoiling into random peptide chains upon RF

Abbreviations: CP, compressive pressure; P, radiofrequency power; RF, radiofrequency; T, operation time.

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and laser vessel sealing [15,16], may affect fusion strength. Changes in collagen after laser-induced vessel sealing [17–19] have been well-studied. Also scanning electron microscopy and transmission electron microscopy (TEM) have been used to detect collagen denaturation in ultrasound vessel sealing [13]. However, only a few studies provide evidence of collagen denaturation upon RF-induced tissue thermo-fusion [20]. Thus, the histological characteristics of colonic wall injuries after fusion are worthy of investigation.

In this study, we used TEM to investigate the impact of CP on collagen denaturation in RF-induced colonic anastomoses. Moreover, we used light microscopy to detect the histological characteristics of fused regions after RF-associated damage from different *P* and *T* in RF-induced colonic anastomoses.

Materials and methods

Preparation of colon samples

Fresh colons of young pigs (females, 20 kg) were obtained from the slaughterhouse immediately after they were euthanized. The colons were separated into 50–60-mm segments, washed twice to remove feces, immediately immersed in chilled phosphate-buffered saline (pH 7.2) and delivered to the laboratory.

Experimental settings

Colonic anastomoses were conducted on a homemade device (Fig. 1a). Copper electrodes were placed on insulated heat-resistant ring carriers, which were supplied constant force by a press module (Fig. 1b). The precision was 0.1 Newton (N). Compressive pressure per unit area of fusion tissue was calculated using the following formula: Compressive pressure (kPa) = 10^{-3} × pressure (N)/($\pi R^2 - \pi r^2$) (mm^2), where *R* and *r* stand for external and internal radii of the ring electrode, respectively. LigaSure (Valleylab, Covidien, USA) was used to provide RF energy at 120, 140 or 160 W.

To study the effect of CP on collagen denaturation, five levels of CP were applied to colon samples while *P* and *T* remained constant. The experimental parameters were defined in Table 1, I–V. Fused areas were examined by TEM to provide a representative assessment of the microscopic effects on collagen. Samples were prepared by a senior technician according to a standard protocol and were viewed with a HITACHI Transmission Electron Microscope (TEM H-600, HITACHI High-Tech, Japan).

Samples with CP of 277 kPa were further sub-divided into four subgroups to investigate the effect of *P* and *T* on histological characteristics of thermal injuries. The detailed parameters were defined in Table 1, IV-1–IV-4. Fused areas were collected and fixed immediately in 10% formalin. The specimens were dehydrated, made transparent, immersed in paraffin wax, and then cut into 6-μm thick transverse slices to the fused area, which were stained with picrosirius red. All experimental conditions were repeated at least five times.

Table 1
Group setting of the colonic anastomoses by radiofrequency undergoing distinct parameters.

Group	CP (kPa)	*T* (s)	*P* (W)
I	171		
II	206		
III	242	5	160
IV	277		
V	313		
IV-1		5	120
IV-2	277	10	
IV-3		5	140
IV-4		10	

Results

Effects of CP on collagen denaturation as assessed by TEM

Control specimen is normal colon without CP or RF, which showed typical periodic light and dark streaks on fibrils. The borders of collagen fibrils appeared as sharp regular circles and the background was clean (Fig. 2a). By comparison, specimens that underwent RF treatment under distinct CPs appeared as broken collagen fibrils that were randomly diffused in the extracellular matrix. The borders of the collagen fibrils were fuzzy, and the fibrils were darker and thicker (Fig. 2b–f). At 171 kPa, collagen fibrils in the longitude and transverse directions were slightly denatured, while the structure of bundles still existed and no significant cracking occurred (Fig. 2b). Increasing the CP to higher levels led to more denaturation. At a CP of 206 kPa, collagen fibrils in the longitudinal direction appeared as a mass of short segments (Fig. 2c). At a CP of 242 kPa, denatured collagen fibrils in the transverse direction appeared as corrugate waves (Fig. 2d). The effect on the collagen fibrils was most dramatic at CPs of 277 and 313 kPa, which produced thicker rope-like bundles. At 277 kPa, collagen fibrils assembled so tightly that the borders of the bundles were completely fuzzy. Some floccules appeared next to the bundles (Fig. 2e). However, the gaps between each collagen fibril at a CP of 313 kPa were obviously wider than those of other experimental groups (Fig. 2f).

Effects of T and P on histology as assessed by light microscopy

The normal colonic wall showed abundant collagen that was distributed mainly in the serosa and longitudinal muscle, partly in the muscularis mucosa and circular muscle (Fig. 3).

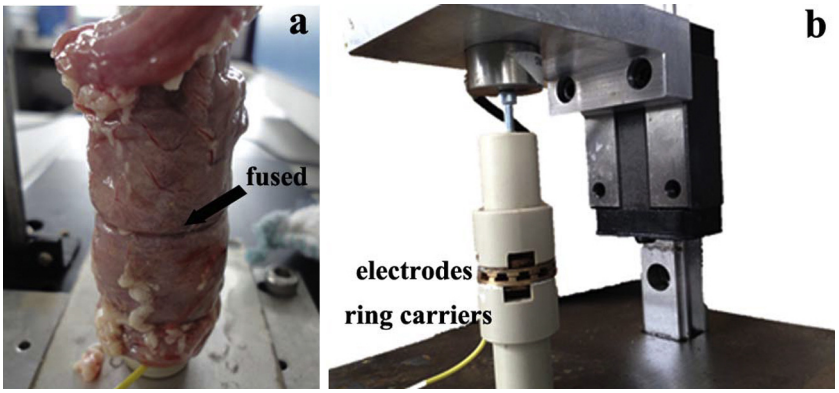

Fig. 1. Anastomotic device developed for creating colonic anastomoses upon radiofrequency-induced thermo-fusion. (a) Colon samples were fused (arrow) serosa-to-serosa on ring carriers. (b) Ring carriers were pressed by a press module. The device has a pressure sensor to measure compressive force.