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RESEARCH ARTICLE

Anti-leakage Effect of Bacterial Cellulose Cover Used in Colonic Anastomosis in Rats

Kenan BINNETOGLU [1,a (*)] Ahmet MIDI [2,b]

[1] Kafkas University, Faculty of Medicine, Department of General Surgery, TR-36000 Kars - TURKEY
[2] Bahcesehir University, Faculty of Medicine, Department of Pathology, TR-34000 Istanbul - TURKEY
ORCIDs: [a] 0000-0001-7517-5970; [b] 0000-0002-6197-7654

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Abstract

In surgical operations, anastomoses are often preferred for the functionality of the gastrointestinal tract. It has been well known that anastomotic leakages are one of the most serious complications causing high mortality and morbidity. In this experimental study, bacterial cellulose was used to prevent leakage after colon anastomosis as a wound healing agent. The preventive effects of bacterial cellulose, which is often preferred in surgical interventions, on colon leakage were investigated. In our study, colonic anastomosis was performed on 20 Sprague-Dawley rats. In the control group, primary anastomosis was performed on 10 animals. In the experimental group, primary anastomosis followed by cellulose cover along the anastomosis line was performed on 10 animals. After 7 days, anastomotic bursting pressure was measured and histopathological examinations were performed. According to the findings of our study, the bursting pressure increased with bacterial cellulose application. On the other hand, histopathological examinations, bacterial cellulose group showed a significant increase in the fibroblast density, eosinophil density, and collagen density. As a result, bacterial cellulose has been shown to accelerate wound healing mechanically and histopathologically. In our study, it has been demonstrated that bacterial cellulose can be used as a physical barrier with biological properties to prevent leakage after colon anastomosis. Besides, our study will shed light on the advanced clinical studies to be conducted on the effect of bacterial cellulose preventing anastomotic leakage, and we claim that bacterial cellulose may be used for this purpose in the near future.

Keywords: *Anastomosis, Bacterial cellulose, Colonic leakage, Rat*

Ratlarda Kolon Anastomozunda Bakteriyel Selüloz Örtüsünün Kaçak Önleyici Etkisi

Öz

Cerrahi uygulamalarda mide barsak sistemin işlevselliği için anastomozlar cerrahlar tarafından sık olarak tercih edilmektedir. Bunun yanı sıra anastomoz kaçakları yüksek mortalite ve morbiditeye neden olan en ciddi komplikasyonlardan biri olduğu bilinmektedir. Bu çalışmada biyolojik yara iyileştirici olarak artan şekilde popülerliğe sahip olan bakteriyel selüloz, kolon anastomozu sonrası kaçağı önleyici olarak kullanılmıştır. Özellikle cerrahi müdahalelerde sıklıkla tercih edilen bakteriyel selülozun kolon kaçağı üzerine koruyucu etkileri bu çalışmada araştırılmıştır. Materyal ve metot; Yaptığımız bu çalışmada deney hayvanı olarak 20 adet Spraque-Dawley cinsi rat kullanılmıştır. Kontrol grubunda 10 adet deney hayvanına primer anastomoz uygulaması yapılmıştır. Deney grubunda ise 10 adet deney hayvanına primer anastomoz uygulaması yapılarak sütur hattına bakteriyel selüloz örtüsü serilmiştir. Anastomoz uygulamasından 7 gün sonra anastomoz patlama basıncı ölçülerek histopatolojik analizler yapılmıştır. Çalışmamızın sonuçlarına göre bakteriyel selüloz uygulamasının patlama basıncını arttırdığı görülmüştür. Diğer yandan histopatolojik analiz sonuçlarına göre ise bakteriyel selüloz grubunda fibroblast yoğunluğunun, eizinofil yoğunluğunun ve kollojen yoğunluğunun önemli derecede arttığı görülmüştür. Sonuç olarak bakteriyel selülozun mekanik ve histolojik olarak yara iyileştirmesinin hızlandırdığı gösterilmiştir. Bakteriyel selülozun anastomoz sonrası kolon kaçağını önlemek amacı ile biyolojik özellikte fiziksel bir bariyer olarak kullanılabileceği bizim çalışmamızda ortaya konmuştur. Buna ilaveten çalışmamız bakteriyel selülozun anastomoz kaçağını engelleyici etkisi ile ilgili yapılacak olan ileri klinik araştırmalara ışık tutarak, yakın zamanda bakteriyel selüozun bu amaçla klinik kullanıma girebileceğini düşünmekteyiz.

Anahtar sözcükler: *Anastomoz, Bakteriyel selüloz, Kolon kaçağı, Rat*

How to cite this article?

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(*) Corresponding Author

Tel: +90 464 6127317
E-mail: kenanbinnet@hotmail.com (K. Binnetoğlu)

Introduction

Anastomotic separation is a serious postoperative complication and the risk of anastomotic leakage is higher in colon surgery than in other gastrointestinal tract (GIT) anastomoses [1]. Anastomotic leakages are one of the most serious complications causing high mortality and morbidity. It is reported that colorectal surgery cases have more frequent mortality and morbidity rates than other anastomoses in the GIT [2]. The size of an anastomotic leakage varies from a tiny point opening to wide clefts and to complete separation [3]. Moreover, it is known that there are leakages due to technical problems [4].

Anastomotic leakages and resulting morbidity/mortality increase towards the distal. Leakage rates vary in the literature. Parameters affecting the leakage rate can be listed as emergency or elective treatment of the patient, definition of leakage and its diagnosis. It is known that the rate is between 3-5% after elective surgery and between 10-15% after emergency surgery [3]. Based on certain reasons such as perforation and mechanical intestinal obstruction, applying emergency surgery on cases with colorectal cancer is still controversial today. Opting for resection and primary anastomosis in such cases ends up with a 10% risk of mortality, and it is assumed that 4-6% of this rate is due to anastomotic leakage [5]. In preventing anastomotic leakages, certain methods are applied today such as applying different anastomotic techniques, antibiotic prophylaxis, pre-operative intestinal cleansing, and fecal diversion with proximal ostomies [6].

The basic producers of cellulose are the plants; as per bacterial cellulose (BC), it is the form of cellulose that is generated by microorganisms. Although BC has the same chemical formula as vegetable cellulose, it stands out with its different physical properties and purity, as well as its potential, particularly in the medical field thanks to the improvements in the biomedical and cell-tissue culture techniques in recent years [7,8]. It has been known for many years that cellulose, which forms the basic structural matrix of the cell walls of almost all plants, some fungi and algae species, is synthesized by *Acetobacter xylinum* [9]. BC has a high biocompatibility owing to its resemblance to extra cellular matrix and its non-toxic structure. It is largely non-immunogenic in that it has a carbohydrate structure, not a protein structure [10]. BC is widely used in various treatments of human and animals. Owing to its high biocompatibility and low inflammatory response, it is applied in humans, especially in the treatment of ulcers, burns and dental implants [11,12]. Today, studies are being carried out for new areas of application for BC, which has a use expanding each passing day. BC has been previously used with satisfactory results in different areas of experimental surgery such as in the scarring of cutaneous wounds, as well as in the grafting used in vascular surgery. BC's biocompatibility has also been well demonstrated in experimental and clinical studies [13]. In this context, research on biological materials is very important to prevent anastomotic leakages. Therefore, we aimed at investigating possible effects of BC on anastomotic leakage.

Material and Methods

Animals and Ethics Approval

The study was carried out on 20 Albino Wistar female rats weighing 250-350 grams. Ethical approval (045.2016.mar) was obtained on 04.04.2016 from the Experimental Animal Ethics Committee of Marmara University. The animals were fed with dry food without any nutrient restriction, each in separate steel cages, in an environment where the temperature was fixed at 23°C degrees. A 12-h light/12-h dark cycle was maintained.

Experimental Groups

The rats were randomly divided into 2 groups as experimental and control groups. In the control group, primary anastomosis was performed on 10 animals. In the experimental group primary anastomosis followed by cellulose cover along the anastomosis line (10 animals) was performed.

Applying BC to Anastomosis Line

Bacterial cellulose was produced in the form of a cover in the laboratories of Immunology Department using *Acetobacter xylinum*. Sterilization was made with ethylene oxide [14].

In the research, anesthesia of the experimental animals was conducted through anesthetic 100 mg/kg ketamine and 30-50 mg/kg chlorpromazine. The abdomens of all rats to be used in the study were shaved and laid on the surgical table in supine position. Following a 4 cm incision on the mid-line of the abdomen, a 4 cm colon segment was resected from 3 cm above the peritoneal reflex *(Fig. 1)*. Subsequently, anastomosis was performed with 5/0 prolene using a single-layer continuous suture technique. The abdomens of the experimental animals were closed in the primary anastomosis group, while BC was laid on the suture line of the other group and the abdomens were closed with 4-0 silk *(Fig. 2)*. Normal saline (0.5 mL) was administered subcutaneously to all animals during surgery as fluid resuscitation. The mice were placed on heating pads to keep them warm during and after surgery and protect animals from hypothermia. At the end of the 7th day following the anastomosis intervention, the rats were euthanized with anesthetic overdose.

Mechanical Measurement of Anastomosis Recovery

Mechanical measurement is often used to assess anastomosis recovery [15]. In our research, the bursting pressure was measured in the postoperative 7th day. In order to measure the bursting pressure, a catheter was advanced 3-4 cm

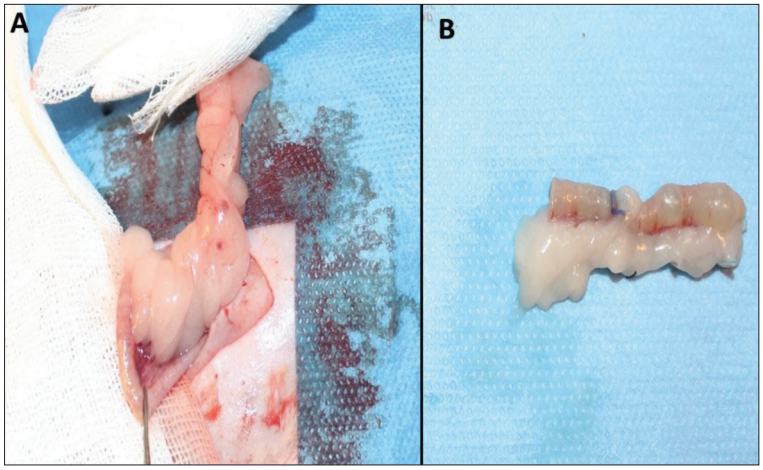


Fig 1. A: Taking the colon out of the abdomen, **B:** Resection of the colon segment

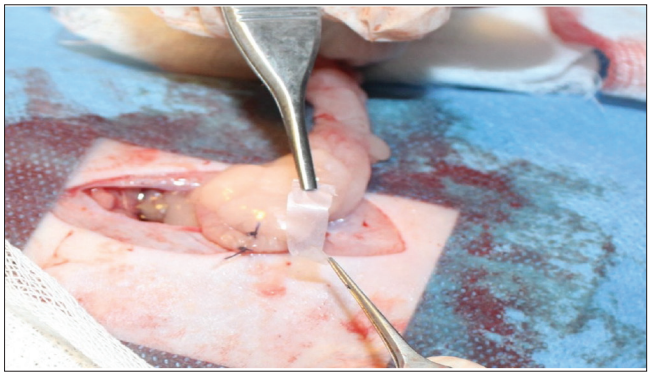


Fig 2. Fixating the bacterial cellulose that covers the anastomosis line

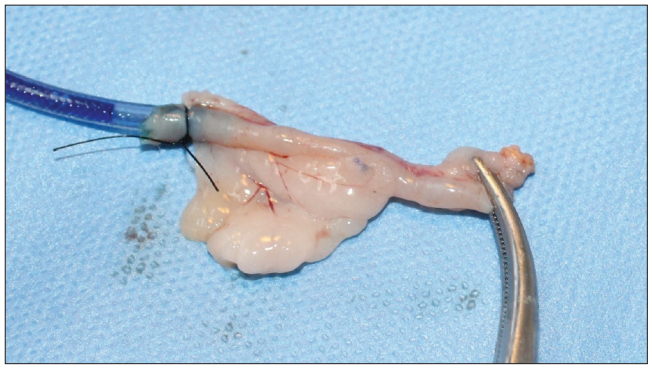


Fig 3. Measuring the burst pressure by injecting the methylene blue

through the anus, and was placed so that its tip came closest to the anastomosis mid-line. After filling the abdomen with saline, 2 cm below and 2 cm above the colon anastomosis were ligated with 2/0 silk. The colon segment connected to the two ends with an inserted catheter was inflated with isotonic colored methylene blue *(Fig. 3)* using an infusion pump at an infusion rate of 4 mL/min; during the inflation process, the pressure values were monitored with the help of a pressure transducer and the bursting pressure was measured. Subsequently, the colon anastomosis line was excised and histopathological examinations were started.

Histopathologic Evaluation

Materials were fixed for five days in 10% formaldehyde. After fixation, the samples were sampled to receive the anastomosi suture line and placed in the cassettes for tissue follow-up. The samples were labeled according to the number sent to the laboratory without knowing which group they belonged to. Then, automatic tissue tracking device (Shandenexelsior ES] was followed for 13 h. In this procedure, tissues were exposed to 2 times 30 min formaldehyde, 6 times 60 min alcohol, 3 times 60 min xylene, once 60 min and two times 80 min paraffin respectively. After the follow-up, 2-micron thick sections were taken from the paraffin-embedded tissues and stained with Hematoxylin & Eosin (H&E). Investigation of the sections was performed by a pathologist in a blind manner under the light microscope (The Olympus Bx 50, Olympus Optical). Active and chronic inflammation, angiogenesis, microabscess, fibrosis, collogen and eosinophil density were the parameters to assess the degree of healing. Histopathological staging of the anastomosis line was performed according to the Ehrlich Hunt model [16,17]. Criteria for staging is as follows 1: There is a small amount but scattered, 2: There is a small amount in every area, 3: Large amount, scattered, 4: Large amount, every area.

Statistical Analysis

In statistical evaluation of the data obtained in the study, IBM 20.00 SPSS package program was used. A nonparametric test was used in examining two different groups in terms of certain variables, the student t-test was used for bursting pressure, and Mann Whitney U test was used for examining the group as the source of difference in histopathological analyses. $P<0.05$ was accepted as the statistical significance limit.

Results

During and after the operation phase, one animal from the control group and one animal from the BC group died. Died animals were excluded from the study. During the

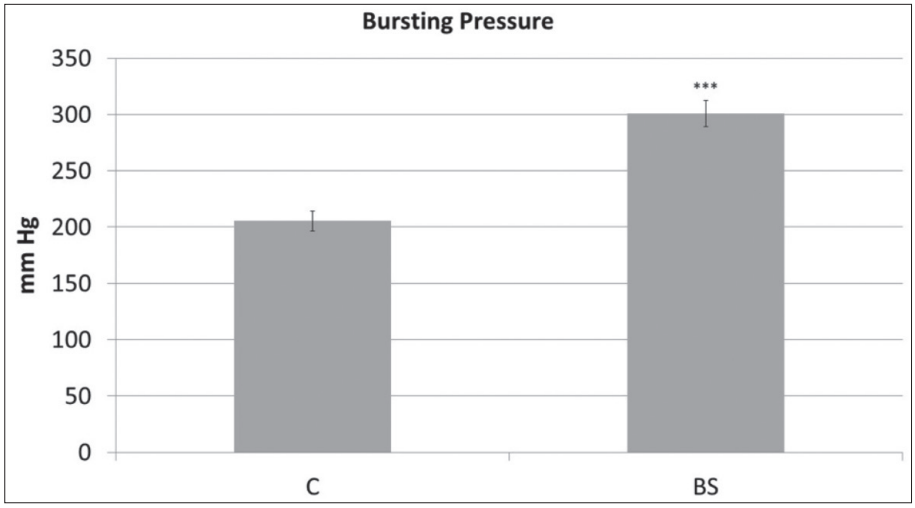
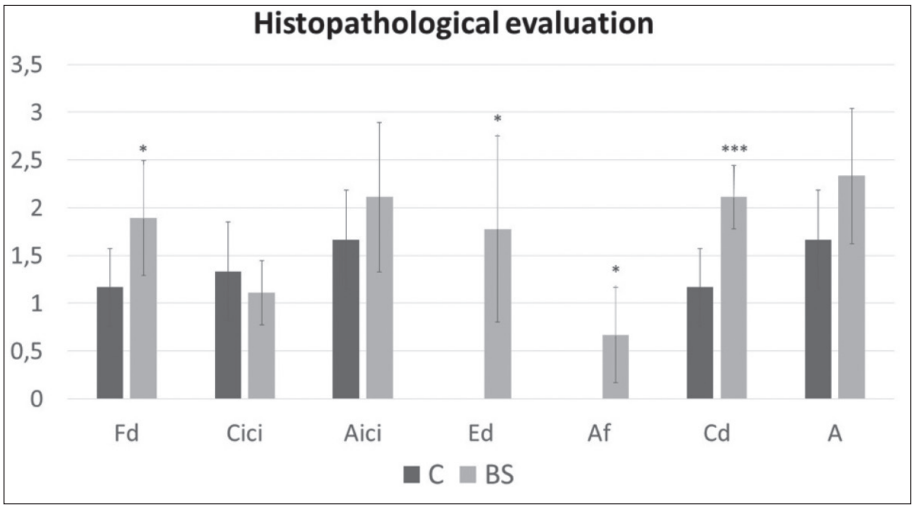


Fig 4. Colon Bursting Pressure (mmHg) ** P<0.05

Fig 5. Histopathologic findings according to Ehrlich-Hunt model. **C:** Control, **BC:** Bacterial cellulose, **Fd:** Fibroblast density, **Cici:** Chronic inflammatory cell infiltration, **Aici:** Acute inflammatory cell infiltration, **Ed:** Eosinophil density, **Af:** Abscess formation, **Cd:** Collagen Density, **A:** Angiogenesis. * P<0.05, *** P<0.005



measurement of anastomosis pressure, the anastomoses of the subjects in all groups exploded, and the average bursting pressures were found to be 205.5±8.77 mm Hg in the control group and 300.92±11.8 mm Hg in the BC group *(Fig. 4)*. Based on the anastomosis bursting pressure values obtained from the groups, the application of BC increased the bursting pressure, creating a statistically significant difference compared to the control group (P<0.05).

In the histopathological staging conducted in line with the Ehrlich-Hunt model to evaluate the regeneration of the anastomosis line, it was observed that all parameters in the control group were mild (0-1-2), while particularly acute inflammatory cell infiltration was intense (2-3) in the BC group. Moreover, fibroblast density, which is an indicator of wound healing rate, increased with the application of BC, demonstrating a statistically significant difference compared to the control group (P=0.02). However, no statistically significant change was observed in the acute inflammatory cell infiltration and chronic inflammatory cell infiltration. It was also evaluated that eosinophil density demonstrated a statistically significant increase compared to the control group (P=0.05). The abscess formation was evaluated to be increased with a statistically significant difference (P=0.05). Collagen density values demonstrated the most significant increase in the BC group compared to the control group (P=0.003). On the other hand, angiogenesis parameters increased with the application of BC in comparison to the control group; however, this increase was not statistically significant *(Fig. 5)*. Microscopic images of the histopathological examination are shown in *Fig. 6*.

Discussion

Anastomotic leakage, a significant complication in colorectal surgery, occurs due to problems in the colon healing process [18]. When anastomotic leak occurs in the colon, the postoperative hospital stay increases two times. In order to prevent leakage in colon anastomoses, there should be a healthy bowel, tightness in the anastomosis line and sufficient blood flow Apart from that, anastomosis healing starts with hemostasis and then an inflammatory response occurs. The inflammatory response continues with the release of mediators that increase collagen production and mucosal reepithelization. One of the best indicators of anastomosis healing is increased angiogenesis. If the blood

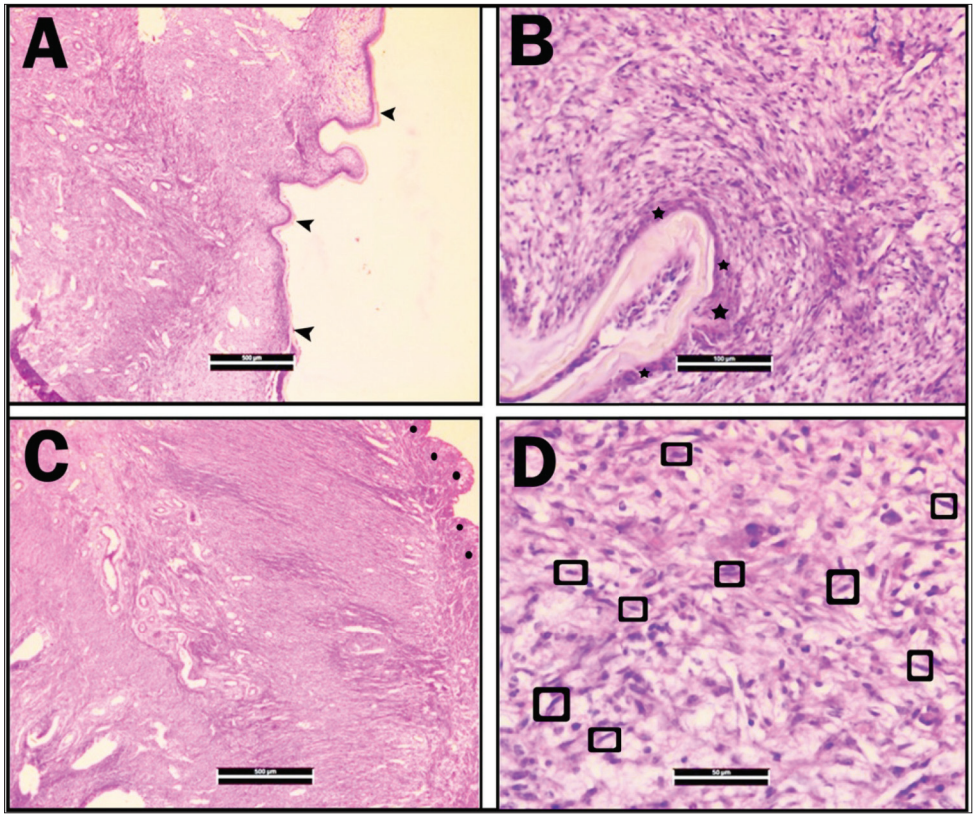


Fig 6. Microscopic images of the histopathological examination of colon tissue. **A:** Complete integration in the serosal surface in the cellulose group *(Arrow)* H&E X40, **B:** Dense fibroblast proliferation around the cellulose material to the cellulose group *(Star)* H&E X100, **C:** Healed appearance of the defect area with thick fibrous tissue in the primary group *(Round)* H&E X40, **D:** Intensive fibroblast proliferation in the primary group *(Square)* H&E X400

supply is good, anastomosis recovery will be faster [19]. In order to prevent colonic anastomotic leakages and to support the anastomosis mechanically, a wide variety of substances have been used such as sheep's intestines, cartilage plaques, goose trachea, and raw hide. Additionally, various drugs, surgical techniques, prosthetics, and adhesives have also been tried [20]. There is an ongoing quest for the most appropriate technique in order to reduce mortality and morbidity rates in anastomoses. Examining the literature, it is observed that numerous experimental research studies have been conducted based on the idea to support with prosthetic materials (patch supporting anastomosis, sponges) for the perfect manual single-layer colonic anastomosis [6].

After a surgery in the GIT, a healing wound demonstrates different characteristics. There are many different characteristics such as a large pool of microorganisms contained in the lumen, the effect of serosa on closing the suture line, vascular nutrition specific to the GIT, whose perfusion decreases in the case of hypovolemia. Under normal conditions and unlike the skin wounds, in bowel wounds smooth muscle cells synthesize collagen in addition to fibroblasts. This synthesis is regulated by different mechanisms. GIT wound healing mainly involves the stages of inflammation, proliferation, fibroplasia and maturation. It is the most important source of tensile strength in the intact mucosa and it is the principal layer on which the sutures that connect the anastomotic tips cling. The accumulation of collagen in the layer determines the mechanical resistance of the wound and its capacity to carry sutures. The tensile strength of the healed suture line reflects the level of recovery in quality and quantity [18]. In our study, with the application of BC in changes that were histopathalogically detected, a significant increase was observed in the amount of collagen. This increase created a statistically significant difference. Studies on bowel anastomoses reported a significant decrease in bowel anastomosis strength within the first 3-4 days. It was thought that this condition was due to increased collagenase activity in the wound area, however, it was observed that there was no decrease in the amount of collagen in reality. Thus, it was admitted that the decrease in anastomosis strength was due to a deficiency in the enzymatic structure of the collagen fibers. In a different study, it was suggested that proteases and free oxygen radicals released from neutrophils temporarily arriving at the wound area can cause a decrease in tensile strength by altering the extracellular matrix. After the 4th day, collagen production and accumulation in the wound area begins to become apparent, and an increase in anastomosis strength occurs with an increase in the amount of collagen. Accordingly, based on our study findings, it can be said that there is an increase in anastomosis strength with the application of BC. Numerous methods and material applications are being tried to obtain a stronger anastomosis line in GIT anastomoses [21,22]. In recent years, the number of studies has been increasing that are recommending the use of surgical tissue adhesive to improve the safety of anastomosis. The most important

examples of these adhesives are fibrin tissue adhesive, bioglue and cyanoacrylate [23].

In determining the quantitative degree of anastomosis recovery, histological, mechanical and biochemical parameters are used, but the value of each one alone is limited. Mechanical parameters are the tools that most reliably reflect anastomosis recovery. Therefore, anastomosis bursting pressure and tensile strength are often applied. Anastomosis bursting pressure is a method of evaluating anastomosis recovery at an early stage after surgery [24]. Since the anastomosis bursting pressure is lowest in the postoperative 3rd day and increases in the 7th, it is usually accepted as the appropriate time to measure the bursting pressure in the 7th postoperative day [15]. According to the results of mechanical measurements in our study, it is observed that BC strengthens the anastomosis line and increases the bursting pressure. Another method that can be used to evaluate the recovery in the anastomosis line caused by cellulose is the histological examination. Among the most frequently applied methods is the examination of cellular infiltration and fibroblastic activity in the anastomosis line with light microscopy [16]. In our study, in evaluating the degree of recovery, active and chronic inflammatory status around the anastomosis, angiogenesis and fibrosis were parametrically evaluated [17]. According to our histopathological findings, it was determined that there were statistically significant increases in the fibroblast density, acute inflammation and eosinophil density with the BC application compared to the control group. There were also significant increases in the chronic inflammation, abscess formation, collagen density and angiogenesis with the application of BC; however, these increases were not statistically significant. Unlike plant cellulose, BC bears several unique characteristics such as high level crystallinity, high purity, high water retention, high mechanical strength, and enhanced biocompatibility. With these exceptional properties, BC can serve as the ideal biomass for the improvement of various industrial products [25]. Both *in vivo* and *in vitro* studies have been conducted to investigate biocompatibility. As an example for its biocompatibility, in an in vivo study, Cai et al. prepared BC-collagen composites by immersing a BC membrane in collagen solution. Fibroblast cells were kept in incubation for 48 hours on a planted BC surface, after which the cells showed healthy adherence and proliferation [26]. In another study, it was observed that human vascular endothelial cells incubated in the BC structure grew in both horizontal and vertical directions and performed cell migration for neovascularization. However, due to the gradient difference in oxygen distribution, it was found that cell penetration and adhesion were weakened on BC membranes with a thickness of 5 mm [27]. In another study, in which BC film was used as a graft for bile duct repair, it was asserted that it was a bio-compatible material that provides a complete healing process and bile flow continuity [28]. BC can be sterilized, it is tissue-compatible, porous, elastic, and easy to be held by hand, it contains moisture to an extent since it absorbs water which ensures a faster recovery for the wounds, and additionally, it prevents the development of secondary infections in the injured area, reduces pain by absorbing the heat in the burnt area and inhibits the growth of the wound in the tissue [29,30]. BC has been used in the treatment of skin injuries such as carcinoma/skin graft with basal cells, severe body burns, facial peeling, sutures, dermal abrasion, skin lesions, chronic ulcers, and skin grafts in both donor and recipient areas [12]. In the recent reports, it has been stated that applying wound grafts made of cellulose in the treatment of chronic wounds has superior effects compared to other existing wound-healing materials. Kucharzewski et al.[30], compared BC with Unna hydrocolloid to treat chronic venous ulcers, and demonstrated that BC was more effective. There are many studies on the wound-healing effect of BC. When the literature was searched, it was found that BC was not investigated in colon anastomosis before. In our study, it is observed that BC accelerates wound healing with both histopathological and mechanical measurements. In a study conducted to determine the effectiveness of BC in closing the pharyngocutaneous fistula, fibroblast and inflammatory cell storage significantly increased, but no significant difference was observed in angiogenesis [14]. Promising results were also obtained in another study examining the effects of BC on facial nerve regeneration [31]. Moon Hwa Kwaka confirmed the effectiveness of BC for 15 days on healing burn wounds. At the end of the 15 days, when the skin regeneration was evaluated and the relationship of angiogenesis was examined, it was demonstrated that BC accelerated the wound healing process on the skin of burn injuries of rats through angiogenesis and regulation of connective tissue formation without stimulating any special toxicity to the liver and kidney. In this study, the thickness of the epidermis and dermis and the number of blood vessels were higher in the group treated with BC, while the skin healing severity score was lower in the group treated with BC compared to the group covered with gause patch [32]. In our study, it is observed that histopathologically angiogenesis had a small increase in the application of BC, but this was not a statistically significant difference. In another study, Helenius et al.[33], implanted subcutaneous BC on rats for 1, 4, and 12 weeks, and they evaluated through chronic inflammation, foreign reaction, cell growth and angiogenesis, histology, immunohistochemistry and electron microscopy. In the study, it did not cause any acute or chronic inflammatory reactions. No signs of microscopic or macroscopic inflammation were observed. Angiogenesis was observed at all periods and it was also seen that new blood vessels grew in the implanted cellulose. In our study, parallel to that study, there was an increase in angiogenesis, however, it was not statistically significant. Additionally, in our study, fibroblast density was observed to increase to a level that would create a statistically significant difference. Moreover, an increase was not observed in chronic

inflammatory cell infiltration, while a small increase was recorded in acute inflammatory cell infiltration. As a conclusion, our study demonstrated that in rat colon anastomosis, the application of BC in the anastomosis line was better than in the primary anastomosis group in mechanical evaluation. Although fibroblast density, eosinophil density and collagen density were significantly higher in the BC group than in the primary suture. Eosinophils are known to be distributed frequently in the lamina propria within the GIS and to a lesser extent in the epithelium. The density of increased eosinophils tissue is not entirely defined. Eosinophils that coalesce in the epithelium or exhibit intense degranulation are always considered abnormal. In this context, eosinophilic inflammation is usually defined as a hypersensitivity reaction. Moreover, eosinophil density increases in some infections, gastroesophageal reflux disease, autoimmune gastritis, specific drug reactions, and inflammatory bowel disease and radiation enteritis. This study histopathologically examined eosinophil density as an indicator of hypersensitivity reaction to BC [34]. However statistical results on fibroblast density showed similar results with the findings in the literature about the effect of BC on wound healing, and an increased collagen formation was observed. It was observed that BC, which is highly biocompatible and can be used as a tissue graft and accelerates wound healing, could be used in preventing anastomotic leakage, which is one of the most important complications of anastomosis. In our study, it has been demonstrated that bacterial cellulose can be used as a physical barrier with biological properties to prevent colon leakage after anastomosis. Besides, our experimental study will shed light on the advanced clinical studies to be conducted on the effect of bacterial cellulose preventing anastomotic leakage.

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Conflict of Interest

The authors declared no conflict of interest.

Author Contributions

KB- Study design, data collections, data analysis, writing. AM- Study design, data collections, data analysis

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